International Journal of Pharmacology and Clinical Research (IJPCR)
IJPCR |Vol.8 | Issue 3 | Jul - Sept -2024
www.ijpcr.com
DOI : https://doi.org/10.61096/ijpcr.v8.iss3.2024.258-267
ISSN: 2349-5448

Research

Evaluation of anti-epileptic activity of tuberous barks of *morinda reticulata gamble* by maximal electroshock and isoniazid induced convulsion in wistar rats

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Published on: 26 Jul 2024

Published by:
Dr Sriram Publications

Abstract

The aim of the present study is to evaluate the antiepileptic activity of ethanolic extract of tuberous barks of *Morinda reticulata gamble* in mice. The antiepileptic activity of ethanolic extract of *M. reticulata* at the doses of 200 and 400 mg/kg, p.o. was evaluated by maximum electroshock (MES), pentylenetetrazole (PTZ) and isoniazid (INH)-induced convulsions in mice. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett's test. In MES method, the chloroform extract significantly protected the mice from convulsions induced by electroshock method in a dose-dependent manner and exhibited more activity at the dose of 400 mg/kg when compared with Phenytoin (25mg/kg p.o) treated animals. In PTZ method, the extract inhibited convulsions in mice potent than standard drug. In INH method, it delayed the latency of convulsions in mice in a dose-dependent manner. The ethanolic extract exhibited significant and dose-dependent antiepileptic activity, which may be due to the presence of antioxidant principles like flavonoids.

Keywords: *Morinda reticulata gamble*, Phenytoin, Epilepsy, Isoniazid, Pentylenetetrazole.

INTRODUCTION

Epilepsy, a disorder of brain function which is defined clinically as a syndrome of two or more unprovoked or recurrent seizures on more than one occasion [1]. Epilepsy accounts for 0.5% of global health burden. Approximately 80% of incidence is reported from developing countries [2]. It is accounted that one out of 21 men and one out of 28 women will develop epilepsy during their lifetime [3]. The incidence is high in the pediatric age group, decreasing through adulthood until approximately 60 years and the incidence again increases [4]. The unpredictable nature of epilepsy causes psychological stress to the individual even if the disease is well controlled. People with epilepsy may have poor health, suffer from unemployment, inability to work and higher mortality compares to non-epileptics [5]. It has been reported that there is a higher risk of suicide in epileptic individuals [6]. A low socioeconomic status has been shown to be a risk factor for developing epilepsy [7,8].

Currently, the treatment of epilepsy is by pharmacological agents such as phenytoin, sodium valproate, carbamazepine, which control the excess abnormal electrical activity of brain neurons. These agents act by blocking sodium/calcium channels and balancing the inhibitory and excitatory neurotransmitter system in central nervous system. Research is going on identifying new targets and new molecules. The limitation of the use of available drugs is that they can only offer remission but not cure of epilepsy. In addition, the long term side effects and cost of medication especially recently introduced drugs such as levetiracetam and compliance to lifelong therapy imposes the necessity to develop new drugs. None of the antiepileptic drugs, including those that act on newly identified targets, can be considered as an ideal drug that reliably cures epilepsy [9]. Temkin NR, reviewed many clinical trials on epilepsy and found that the use of antiepileptic drugs suppressed seizures in the short-term, but not long-term [10]. The outcome of antiepileptic treatment is also not the same in all individuals which could be due to genetic variation which has led to personalized therapy [11]. Hence in this scenario, there is a need to search for alternative drugs with lesser adverse effects and equal efficacy.

Morinda reticulata belongs to the family of Rubiaceae, It is large woody climbing shrubs. Leaves 6-12 x 2-4.5 cm, oblanceolate to linear-lanceolate, attenuate at base, caudate acuminate at apex, waxy shining above, lateral nerves 10-12 pairs; petioles to 6 mm long; stipules acute, connate. Flowers white in terminal umbellate heads; peduncle 1-2 cm. Calyx truncate, limb forming a ring. Corolla rotate; tube c. 1.5 mm long, very hairy within; lobes 4, oblong, recurred. Stamens 4, included. Stigma 2-fid. Syncarpium irregularly lobed, 0.5-1 cm diam., with prominent scars of calyx ring, orange; pyrenes many, bony, pyriform, triquetrous in viscous pulp. Habit: Climber, Flowering & Fruiting: March-September, It was found in Choodal, Kallar, Kulathupuzha, Kottayali, on way to Nilamel, Boneccord, Kottur R.F., Merchiston, Thenmalai, Bonaccord, Karamanayar region [12]. In traditional Japanese, Korean and Chinese medicine, *Morinda reticulata* is considered to be an herb with biological properties, However until today, there were no reports to justify its ethnobotanical claim. Hence the present work was designed to evaluate the antiepileptic activity of ethanolic extract of tuberous barks of *M. reticulate* against the convulsions induced by maximum electroshock (MES), pentylenetetrazole (PTZ) and isoniazid (INH) in mice.

MATERIALS & METHOD

Plant collection & Preparation of Extract

The tuberous barks of *Morinda reticulata* was collected from local market and they were sliced into thin pieces and dried in shade and made to a coarse dry powder. And they were subjected to extraction. A weighed quantity of the powder were extracted with 70 % ethanol at room temperature for 5 days using Soxhlet's apparatus. The filtrates were collected and then evaporated under reduced pressure to give a viscous mass, which gave a brownish golden colour residue. The extract was stored at 0-4°C.

Preliminary phytochemical analysis of EEMR: [13,14]

The *Morinda reticulata Gamble*. was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents.

Experimental animals

Wistar rats of weighing 100 -150 gm were used for this study. The inbred animals were procured from the animal house of SSM College of Pharmacy, Erode. They were housed three per cage under standard laboratory conditions at a room temperature at 22±2°C with 12 hour light/dark cycle. The animals were acclimatized to laboratory conditions for one week provided with standard pellet chow and water *ad libitum*. Ethical committee approval was obtained from IAEC of CPCSEA.

Maximal Electroshock (MES) Induced Epilepsy In Wistar Rats

MES model is one of the physical methods to evaluate the anti-epileptic activity of drug. This model is used to screen drugs which are effective for generalized tonic-clonic (grandmal) and focal seizures whereas anti-absence seizure drugs cannot be tested. Rats or mice are commonly used for this method. A stimulating apparatus with corneal or ear electrodes supplying a constant current 50 mA for mice and 150 mA for rats at a frequency of 50-60 Hz is applied for a duration of 0.2 seconds. The animals are observed for a period of 2 minutes after application of electrical stimulus. The seizure which occurs passes through various phases: phase of tonic limb flexion, phase of tonic limb extension and a variable short clonic interval. Efficacy of new antiepileptic drugs is measured by the suppression of tonic hind limb extension.

The animals were divided into 5 groups consisting of 6 each. Group I was normal control. Group II Seizure was induced by MES 60 Hz alternating current of 150 mA intensity for 0.2 sec on 21st day. Group III Seizure was induced by MES 60 Hz alternating current of 150 mA intensity for 0.2 sec after the last dose of standard drug (Phenytoin 25mg/kg p.o) on 21st day. Group IV Lower dose of (EEMR 200mg/kg p. o) administered for 21 days, on 21st day seizure was induced by MES 60 Hz alternating current of 150 mA intensity for 0.2 sec

(after the last dose). Group V Higher dose of (EEMR 400mg/kg p. o) administered for 21 days, on 21st day seizure was induced by MES 60 Hz alternating current of 150 mA intensity for 0.2 sec (after the last dose). The various stages of epilepsy which was listed below were observed: Flexion, Extensor, Clonus, Stupor, Recovery, The percentage protection of animals was estimated. [15-20]

Isoniazid (INH) Induced Epilepsy In Wistar Rats

Isoniazid model is one of the chemically induced methods to evaluate anti-epileptic activity of the drug. Isoniazid can precipitate convulsions in patients with seizure disorders. Isoniazid is regarded as a GABA-synthesis inhibitor, it is an anti-tuberculosis drug, induces epilepsy by depleting brain level of Gamma-Aminobutyric Acid (GABA), a major inhibitory transmitter substance in the mammalian brain, through inhibition of pyridoxal-5-phosphate- dependent Glutamic Acid Decarboxylase (GAD). Pyridoxal-5-phosphate is the active form of pyridoxine, a cofactor for GAD, and an enzyme required for GABA synthesis. The decrease in GABA levels results in recurrent seizures that characterized epilepsy. The animals were divided into 5 groups consisting of 6 each. Group I was normal control. Group II Seizure was induced by Isoniazid (300mg/kg i.p) on 21st day. Group III Seizure was induced by Isoniazid (300mg/kg i.p) after the last dose of standard drug (Diazepam 5mg/kg p.o) on 21st day. Group IV Lower dose of (EEMR 200mg/kg p. o) administered for 21 days, on 21st day seizure was induced by Isoniazid (300mg/kg i.p) after the last dose. Group V Higher dose of (EEMR 400mg/kg p. o) administered for 21 days, on 21st day seizure was induced by Isoniazid (300mg/kg i.p) after the last dose. Morbidity and Mortality status of the animals after 30min, 24 hour were observed. [15-20]

Histopathological Analysis Of Brain In MES Induced Epilepsy In Wistar Rats

The wistar rats from all the Groups (I, II, III, IV &V) were anaesthetized and sacrificed. The brains were carefully removed by opening the skull. The collected brains were washed with ice cold normal saline and fixed in 10% formalin saline. Paraffin embedded sections were taken 100µm thickness and processed in alcohol-xylene series and stained with Haematoxylin-Eosin dye. The sections were examined microscopically for histopathological changes in the cortex zone. [15-20]

Statistical Analysis [19]

The statistical analysis was carried by one way ANOVA followed by Dunnet's ―t test. P values <0.05 (95% confidence limit) was considered statistically significant, using Software Graph pad Prism 9.3.1.

RESULTS

Preliminary Phytochemical Analysis of Ethanolic Extract of *Morinda Reticulata Gamble*. (EEMR)

The result of preliminary phytochemical analysis of ethanolic extract of *Morinda reticulata gamble* showed presence of various phytochemical constituents such as Carbohydrates, phenols, Flavonoids, steroids, alkaloids, glycoside protein, tannins, terpenes and saponins with absence of sterol, gums and mucilage.

EFFECT OF EEMR ON MES INDUCED EPILEPSY IN WISTAR RATS

Flexion in MES induced epilepsy in wistar rats

The Flexion phase in Group II, IV ($p<0.0001$), V ($p< 0.05$) was significantly increased when compared with Group I (vehicle treated) and Group III was nonsignificant when compared with Group I. The Flexion phase in Group II was significantly increased when compared with group III, IV and V ($p<0.001$). The Flexion phase in Group III was significantly decreased when compared with Group IV ($p< 0.01$), and nonsignificant with Group V. [Table: 1]

Table 1: Effect of EEMR on Flexion in MES induced epilepsy in wistar rats.

GROUPS	FLEXION (SEC)
Group I	0
Group II	26.94 ± 3.48 a****
Group III	3.44 ± 1.53 a^{ns} b****
Group IV	10.61 ± 2.39 a**** b****C**
Group V	5.94 ± 1.42 a*b****C^{ns}

Values are expressed as mean ± SEM of 6 animals. Comparisons were made between the following: Group I compared with Group II, III, IV and V was considered as a, Group II compared with Group III, IV and V was considered as b, Group III compared with Group IV and V was considered as c, Statistical Significance test for comparison was done by one way ANOVA followed by Dunnets 't' test. Where * ($p< 0.05$), **($p< 0.01$), *** ($p<0.001$), ****($p<0.0001$) and ns- nonsignificant.

Extensor in MES induced epilepsy in wistar rats

The Extensor phase in Group II and IV ($p<0.0001$) was significantly increased when compared with Group I (vehicle treated) and Group III and V was nonsignificant when compared with Group I. The Extensor phase in Group II was significantly increased when compared with group III, V ($p<0.0001$) and IV ($p<0.001$). The Extensor phase in Group III was significantly decreased when compared with group IV ($p< 0.01$) and non-significant with group V. [Figure:1]

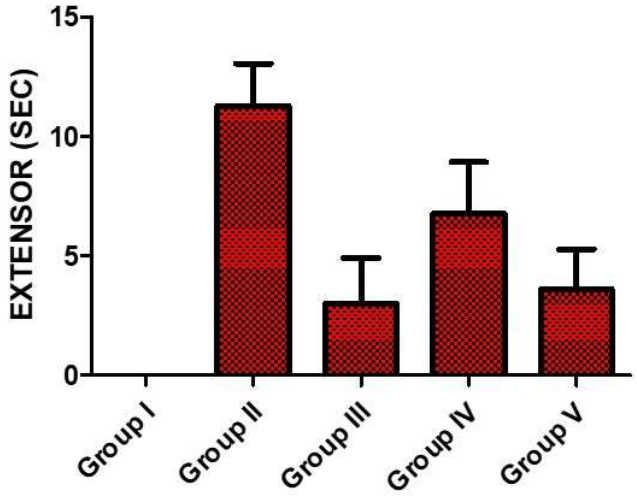

Fig 1: Effect of EEMR on Extensor in MES induced epilepsy in wistar rats.

Clonus in MES induced epilepsy in wistar rats

The Clonus phase in Group II, IV, V ($p<0.0001$) and III ($P<0.05$) was significantly increased when compared with Group I (vehicle treated) The Clonus phase in Group II was significantly increased when compared with group III, IV and V ($p<0.0001$). The Clonus phase in Group III was significantly decreased when compared with group IV and V ($p<0.0001$). [Figure:2]

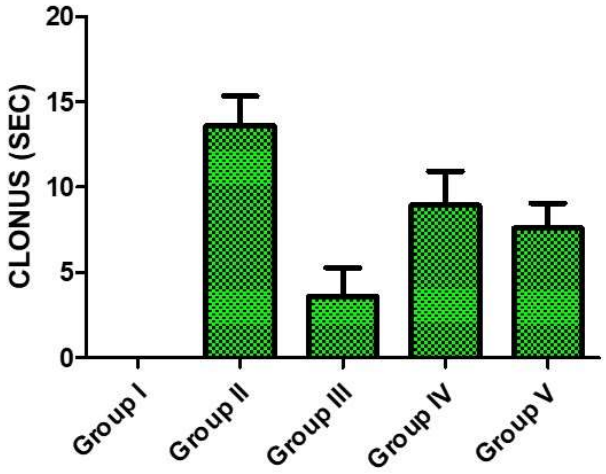

Fig 2: Effect of EEMR on Clonus in MES induced epilepsy in wistar rats.

Stupor in MES induced epilepsy in wistar rats

The Stupor phase in Group II ($p<0.0001$) and IV ($p<0.001$) was significantly increased when compared with Group I (vehicle treated). Group III and V was nonsignificant when compared with Group I. The Stupor phase in Group II was significantly increased when compared with Group III, V ($p<0.0001$) and IV

($p<0.001$). The Stupor phase in Group III was significantly decreased when compared with group IV ($p< 0.01$) and non-significant with group V. [Figure:3]

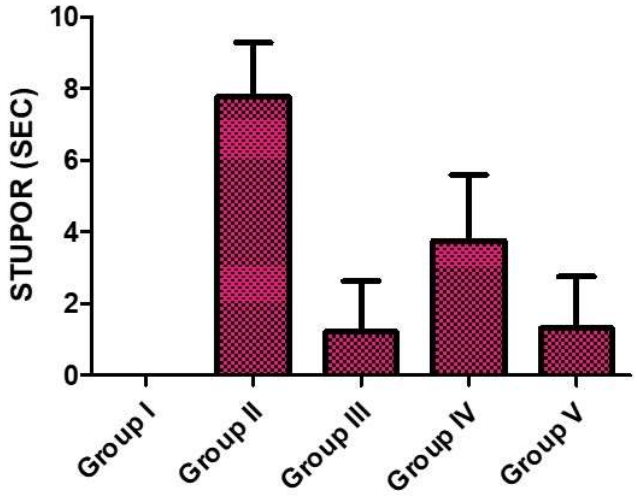

Fig 3: Effect of EEMR on Stupor in MES induced epilepsy in wistar rats

Recovery time in MES induced epilepsy in wistar rats

The Recovery time in Group II, IV &V ($p<0.0001$) was significantly increased when compared with Group I (vehicle treated). Group III was nonsignificant when compared with Group I. The Recovery time in Group II was significantly increased when compared with Group III, IV and V ($p<0.0001$). The Recovery time in Group III was significantly decreased when compared with Group IV ($p<0.001$) and V ($p< 0.01$). [Fig:4]

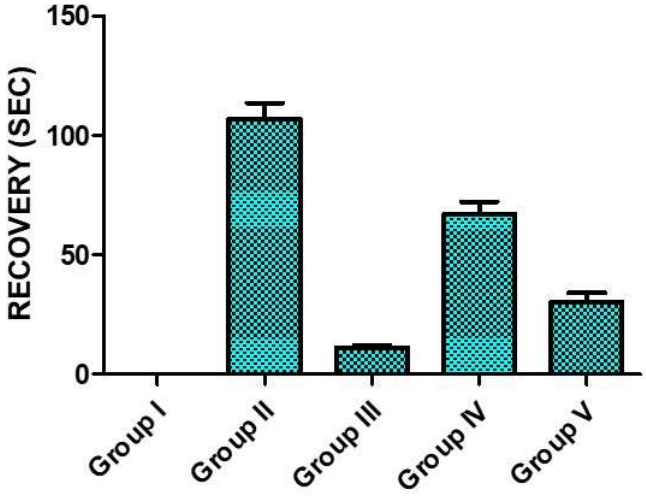

Fig 4: Effect of EEMR on Recovery in MES induced epilepsy in wistar rats.

Percentage protection in MES induced epilepsy in wistar rats.

The percentage protection in Group II was significantly abolished when compared with group III, IV and V. The percentage protection in Group III was significantly increased when compared with group IV and V. [Table:2]

Table 2: Effect of EEMR on Percentage protection in MES induced epilepsy in wistar rats.

GROUPS	PERCENTAGE PROTECTION
Group I	NIL
Group II	0%

Group III	100%
Group IV	82%
Group V	100%

Values are expressed as Percentage

Effect of eemr on isoniazid (inh) induced epilepsy in wistar rats.

Latency in INH induced epilepsy in wistar rats.

The Latency in Group II was significantly decreased compared with group III, IV and V ($p<0.0001$). The Latency in Group III was significantly abolished when compared with group IV and V ($p<0.0001$). [Table:3]

Table: 3 Effect of EEMR on Latency in INH induced epilepsy in wistar rats.

GROUPS	LATENCY (onset epileptic seizure in sec)
Group I	NIL
Group II	86 ± 4.23
Group III	NIL
Group IV	129 ± 3.56 a****b****
Group V	138 ± 2.39 a****b****

Values are expressed as mean ± SEM of 6 animals. Comparisons were made between the following: Group II compared with Group III, IV and V was considered as a. Group III compared with Group IV and V was considered as b. Statistical Significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test. Where * ($p< 0.05$), **($p< 0.01$), *** ($p<0.001$), ****($p<0.0001$) ns- nonsignificant.

Mortality in INH induced epilepsy in wistar rats at 30 min interval.

No mortality in Group I (vehicle treated), The Number of animals in Group II was significantly decreased when compared with group III, IV and V, The Number of animals in INH treated Group III was significantly increased when compared with Group IV and V. [Table:4]

Table: 4 Effect of EEMR on Mortality after 30 minutes in INH induced epilepsy in wistar rats.

GROUPS	Mortality after 30 min (No of animal alive)
Group I	NIL
Group II	3
Group III	5
Group IV	4
Group V	5

Values are expressed in Numbers

Mortality in INH induced epilepsy in wistar rats after 24 hours

No mortality in Group I (vehicle treated). The Number of animals in Group II was significantly decreased when compared with group III, IV and V. The Number of animals in Group III was significantly increased when compared with Group IV and V. [Figure:5]

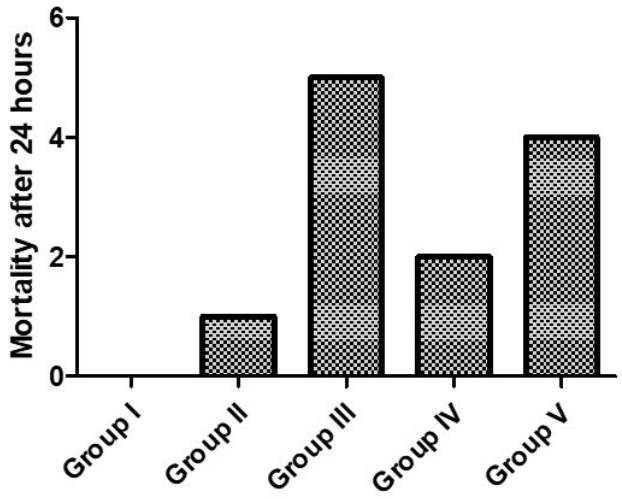

Fig 5: Effect of EEMR on Mortality after 24 Hours in INH induced epilepsy in wistar rats.

The percentage protection in INH induced epilepsy in wistar rats

The percentage protection in Group II was significantly decreased when compared with group III, IV and V. The percentage protection in Group III was significantly increased when compared with group IV and V. [Figure:6]

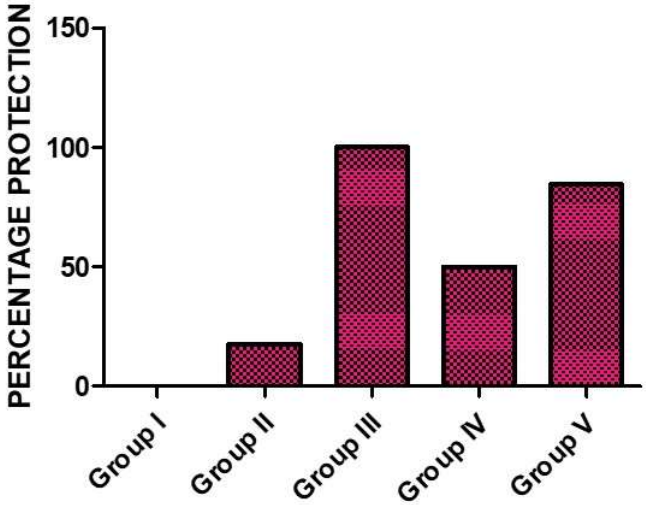

Fig 6: Effect of EEMR on Percentage protection in INH induced epilepsy in wistar rats.

Histopathological Analysis Of Brain In MES Induced Epilepsy In Wistar Rats

GROUP I showed normal brain tissue depicted intact cell, architecture with normal amount of neurotransmitters. GROUP II showed less neuron density. GROUP III showed no significant alterations observed in this group and tissues showed a normal picture or brain cells, less proliferation and more neuronal density at hippocampal region. GROUP IV showed no pathological damages and cellular architecture are intact with more neuronal density compared to the MES alone treated group. GROUP V showed increased neuron density when compared to the EEMR (200mg/kg/p.o).[Figure 7]

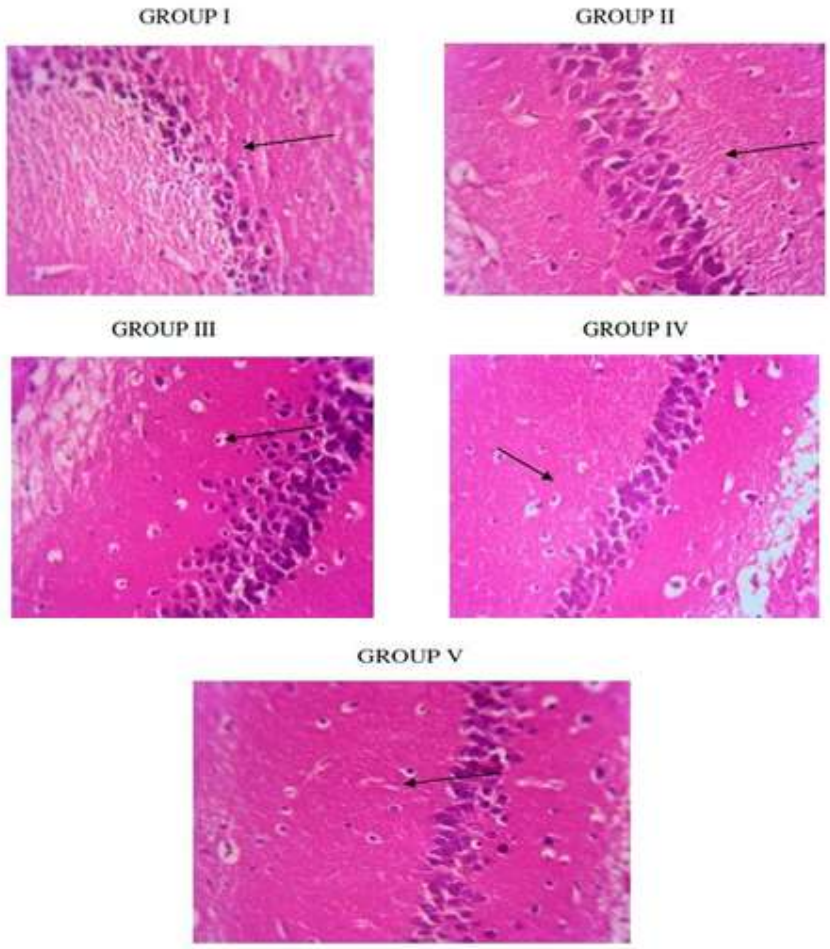

Fig 7: Histopathology of Brain in MES induced epilepsy in wistar rats

DISCUSSION

According to WHO, Epilepsy is a chronic non communicable disease of the brain that affects around 50 million people worldwide. It is characterized by recurrent seizures, which are brief episodes of involuntary movement that may involve a part of the body (partial) or the entire body (generalized) and are sometimes accompanied by loss of consciousness and control of bowel or bladder function. Seizure episodes are a result of excessive electrical discharges in a group of brain cells. Different parts of the brain can be the site of such discharges. Seizures can vary from the briefest lapses of attention or muscle jerks to severe and prolonged convulsions. Seizures can also vary in frequency, from less than 1 per year to several per day.

Hence herbal drugs as therapeutic agents are preferred to reduce severe adverse effects of the allopathy therapies. Therefore, scientists are on the hunt for newer alternatives, with lesser side effects, self-administrable, less expensive and with complete reversibility. Much of these properties are observed in drugs of natural plant origin. Globally traditional system of medicine has been used to treat various diseases throughout the human history. Many plants are reported to have anti-epileptic activity. [23]

The Maximal electroshock induced seizures produce repetitive stimulation of high frequency action potentials thus opening of Na^+ channels and increasing Ca^{2+} intracellularly leading depolarization of cell. It has been found out that treatment of Rats with *Morinda reticulata gamble* showed significant decrease in the hind limb extensor period. Animal models of seizures induced by electrical stimulation convey the advantage of reproducing epileptogenic features in the intact brain with low mortality and high reproducibility. Moreover, unlike chemical-induced seizures, postictal alterations from electrical stimulation can be investigated when the epileptogenic cause is no longer present. However, seizure modelling by electrical stimulation does not provide cell-type specificity in the brain. In addition, stimulation protocols can be costly and laborious when used for chronic studies. [24,25]

GABA is an inhibitory neurotransmitter and glutamate is an excitatory neurotransmitter which is responsible for the production of excitation of neurons thus plays an important role in the generation of seizures.

The Maximal Electroshock (MES) induced seizure showed significant decreased levels of GABA in brain, thus showing that GABA plays an important role in the inhibition of seizures i.e., The percentage protection in MES induced seizure, treated with EEMR is significantly increased when compared to MES alone induced seizure groups. GABA level in MES induced seizure, treated with EEMR is significantly increased when compared to MES alone induced seizure group.

The Isoniazid (INH) induced Epilepsy in wistar rat method, the EEMR and INH treated animals showed significant decrease in the onset and decreased duration of the seizure when compared with INH alone treated group animals. With that increased percentage protection from epilepsy in INH model shows that plant having anti- epileptic activity which can be used in TLE. The presence of Flavonoids may help in the anti-epileptic property of *Morinda reticulata gamble*. The Histopathological study of brain shows that there is increased neuronal density produced by the EEMR with MES induced seizure groups compared to the MES alone induced seizure group. The antioxidant activity of *Morinda reticulata*, helps to protect the brain from toxicity, which can help from seizures and other epilepsy conditions. [26-30]

CONCLUSION

The Tuberous barks of *Morinda reticulata gamble* showed reduction in the flexion, extensor, clonus and stupor duration in MES induced epilepsy model. Which shows the antiepileptic activity of EEMR. Hence the Ethanolic extract of *Morinda reticulata gamble* can be used in grandmal epilepsy. The Tuberous barks of *Morinda reticulata gamble* showed reduction in morbidity and mortality of animals in INH induced epilepsy model, which shows the antiepileptic activity of EEMR. Hence the Ethanolic extract of *Morinda reticulata gamble* can be used in TLE (Temporal Lobe Epilepsy) The Histopathology of brain showed normal architecture and there is increased neuronal density which is comparable with phenytoin. The Standard drug Phenytoin acts by GABA mediated mechanism. Hence it is used in the treatment of Epilepsy. As like Phenytoin, the EEMR also shows increase in GABA level as it contain the active constituent GABA in it. Hence it is used in treatment of epilepsy. Thus, it may be concluded that *Morinda reticulata gamble* produces significant Anti-Epileptic activity in both MES and INH induced epilepsy in Wistar Rats, which is comparable with that of Phenytoin. Further work is necessary to elucidate the mechanism of action involved in the antiepileptic activity of *Morinda reticulata gamble* with special reference to Phytochemical constituents.

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