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ANTIEPILEPTIC ACTIVITY OF THE WHOLE PLANT EXTRACTOF *MELISSA OFFICINALIS* IN SWISS ALBINO MICE

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ABSTRACT

Epilepsy is a neurological disorder characterized by unprovoked, recurring seizures that disrupt the nervous system and can cause mental and physical dysfunction. Based on the ethnopharmacological information of the plant, the methanol and aqueous extract of the whole plant of *MELISSA OFFICINALIS* was evaluated for its antiepileptic activity in Swiss Albino Mice .Antiepileptic activity was assessed by using MES and PTZ induced models (250 and 500 mg/kg). Body weight doses were used for the present study. In the MES model the methanol and aqueous extracts showed a dose dependent reduction in the duration of hind limb extensor phase. In pentylenetetrazole induced model methanol and aqueous extracts at dose level of 500mg/kg body weight showed significant reduction in the tonic convulsions induced by PTZ when compared with control group. The results suggest a possible anticonvulsant effect of the methanol and aqueous extracts of *Melissa officinalis* in Swiss Albino Mice.

INTRODUCTION: Seizure is a characteristic feature in epilepsy and is associated with disordered and rhythmic high frequency discharge of impulses by a group of neurons in the brain [1]. Around 0.5-1% of the world's population is affected with epilepsy and 30,000 people develop epilepsy every year [2, 3].

According to NINDS (National Institute of Neurological Disorders and Stroke) about half of all the seizures have no known cause but may result from either brain damage or diseases. As per many researches, the cell membrane surrounding the neuron, which is crucial in generating electrical or nerve impulses, plays an important role in epilepsy [2].

Now-a-days several antiepileptic drugs (AEDs) are available to treat epilepsy. By using these antiepileptic drugs, it may leads to many side effects like chronic toxicity, teratogenic effects [3]. Plants may serve as the alternative sources for the development of new anti-convulsant agents due to their biological activities.

Several plants used for the treatment of epilepsy in different system of traditional medicine have shown anti-epileptic activity when tested on animal models with less side effects [4]. Therefore, it demands for the development of cheap, effective and safe antiepileptic agents from plants and other natural sources. During the course of this investigation *Melissa officinalis,* had been chosen for the present study.

Lemon balm (*Melissa officinalis*) is an herb with a lemon scent native to southern Europe [5]. Historically, lemon balm has been said to possess sedative/tranquilizing, anti- gas, fever- reducing, antibacterial, spasmolytic, hypotensive (blood pressure lowering), memory-enhancing, menstrual- inducing, and thyroid-related effects and has been proposed by some to be an herbal cure-all [6].

Laboratory data suggest that lemon balm may contain high concentrations of antioxidants [7]. The German Commission E recommends lemon balm for nervous sleep disorders and functional gastrointestinal complaints [8]. The European Scientific Cooperative on Phytotherapy (ESCOP) recommends its use for tenseness, restlessness, and irritability. There is no scientific evidence for the antiepileptic activity of the whole plant of MO. In the present study, to our knowledge for the first time an attempt has been made to prove the antiepileptic activity of the plant by using MES and PTZ induced models.

MATERIALS AND METHODS:

Plant Material: The plant Badranjboya (*Melissa officinalis)* was purchased from Shamsi Dawakhana, Ballimaran, Delhi-110006, India. The authenticity and identity was confirmed on the basis of classical description in unani literature at department of Ilmul Advia F/O Medicine (u), Jamia Hamdard, New Delhi and modern Botanical information was established by matching with the specimens available at the National Institute of Science Communications. The wealth of Indian division, Dr K. Krishnan Marg, New Delhi, 100012. Reference no.2010-11/1380/157.Voucher deposited in D/O Ilmul Advia F/O Medicine, Jamia Hamadard, New Delhi-110062.

Preparation of Extracts: The whole plant was collected, washed and dried at room temperature. After complete drying, it waspowdered and passed through a 60 mesh sieve and storedin air tight container. Dried powdered drug was used to prepare extract. 200g of the powdered whole plant drug was taken and extracted with methanol in soxhlet apparatus for 72 hrs. The extracts were evaporated to dryness in a rotary flash evaporator at a temperature not exceeding 60^{o}C. Aqueous extract was prepared bymacerating the whole plant power in double distilledwater. The extract was concentrated in water bath and stored in desiccators

Animals: Swiss Albino Mice of either sex were used for the study of the crudeextracts. Institution Animal Ethics Committee has approved the project (Reg. No. 733/CPCSEA). The animals were kept at 27±2 ^{o}C, relative humidity 44-56% and light and dark cycles of 10 and 14 hr, respectively, for 1 week before and during the experiments. Animalswere provided with water ad libitum and standard diet (Lipton, India) and the food was withdrawn 18-24 hr before the start of the experiment. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals.

Acute Toxicity Study: Acute toxicity study was performed according to the OECD guidelines on Swiss Albino Mice and the animal were kept fasting for overnight providing water ad libitum, after which the extracts were administered orally 2000 mg/kg b.wt. and observed the mortality of animals.

Maximal Electroshock induced Seizures: The seizure was induced by maximal electroshock in Swiss Albino Mice with the help of electroconvulsiometer by passing current of 45 mA for 0.2 second using ear clip electrodes. The animals were divided into six groups each containing 6 animals (n = 6). The test samples were given 1 hr prior to induction of convulsions.

- Group I (Control): Received normal saline (1 ml/kg b.wt., po).
- Group II (Standard): Received diazepam (5mg/kg b.wt., po).
- Group III and IV: Received AEMO (250 and 500 mg/kg b.wt., po).
- Group Vand VI: Received MEMO (250 and 500 mg/kg b.wt., po).

The animals were observed for the extensor phase as well as its duration. The abolition of extensor (tonic phase) in groups treated with extracts and diazepam were considered as criteria for anticonvulsant activity when compared with the control group.

PTZ induced Seizures: The animals were divided into six groups each containing 6 animals (n = 6).

- Group I (Control): Received normal saline (1 ml/kg b.wt., po).
- Group II (Standard): Received diazepam (5 mg/kg b.wt., po).
- Group III and IV: Received AEMO (250 and 500 mg/kg b.wt., po).
- Group Vand VI: Received MEMO (250 and 500 mg/kg b.wt., po).

After 30 minutes of the dosing all the groups were injected with the convulsing agent pentylenetetrazole (60 mg/kg b.wt.) and animals were kept in individual plastic cages to observe convulsions for 1 hr.

Statistical Significance: The results of the study were expressed as mean ± SEM, n = 6. ANOVA was used to analyze anand compare the data, followed by Tukey's multiple Comparisons.

RESULTS: There was no mortality amongst the graded dose groups of animals and they did not show any toxicity or behavioral changes at a dose level of 2000 (mg/kg b.wt.). This finding suggests that aqueous extract of *Melissa officinalis* (AEMO) and methanolic extract of *Melissa officinalis* (MEMO) were safe in or non-toxic to rats up to 2000 (mg/kg b.wt.). Hence, the doses of 250 and 500 (mg/kg b.wt.) were selected for the antiepileptic activity. Antiepileptic activity In the case of MES, it was observed that the MEMO 250 and 500 (mg/kg b.wt.) were showed 66.75% and 80.56% inhibition of convulsion, respectively. AEMO at the doses of 250 (mg/kg b.wt.) and 500 (mg/kg b.wt.) were exhibited the 46.59% and 64.61% inhibition of convulsion produced by MES, respectively. The Diazepam inhibited 91.25% of convulsion (**Table 1**).

TABLE 1. ANTICONVULSANT ACTIVITY BY MAXIMAL ELECTROSHOCKS (MES)

Group	Flexor	Extensor	Clonic	Stupor	Death OR Recovery	% Protection
Normal	1.50 ± 0.14	24.25 ± 0.36			Death	91.25
Diazepam	1.25 ± 0.75	12.12 ± 0.78*	7.35 ± 0.48	22.80 ± 0.45	Recovery	46.59
AEMO(250 mg/kg)	2.78 ± 0.59	12.95 ± 0.55*	7.35 ± 0.48	56.59 ± 0.66	Recovery	64.61
AEMO500 mg/kg)	2.55 ± 0.66	18.58 ± 0.33	12.55 ± 0.66	38.66 ± 0.78	Recovery	66.75
EEMO(250mg/kg)	2.38 ± 0.98	17.82 ± 0.32*	10.28 ± 0.84	52.58 ± 0.86	Recovery	80.56
EEMO(500mg/kg)	2.50 ± 0.35	14.47 ± 0.55*	12.26 ± 0.32	26.28 ± 0.62	Recovery	84.66

Values are expressed as mean ±SEM, (n = 6) (compared to control group) by using One Way Analysis of Variance (ANOVA) followed by Tukey's Multiple Comparison Test P<0.001

In the model of PTZ induced seizures, it was observed that MEMO showed 12.22% and 25.53% protection from seizures at the dose of 250 and 500 (mg/kg b.wt.), respectively while the AEMO showed 29.59% and 62.24% protection from seizures at the doses of 250 and 500 (mg/kg b.wt.), respectively. The Diazepam showed 85% protection from seizures (**Table 2**).

TABLE 2: ANTICONVULSANT ACTIVITY BY PTZ INDUCED SEIZURES

Group	No. of seizures	% Protection
Normal	98	-
Diazepam	84	85.0
AEMO(250 mg/kg)	29	29.59
AEMO500 mg/kg)	61	62.24
EEMO(250mg/kg)	11	12.22
EEMO(500mg/kg)	26	25.53

DISCUSSION AND CONCLUSION: Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is the second most common chronic neurological condition seen by neurologists. Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing country is 100 per 100,000 [9]. India is home to about 10 million people with epilepsy (prevalence of about 1%) [10].

The number of Epilepsy Specialists and Neurologists being very small in India, most people with epilepsy are being diagnosed and treated by non-specialists at both primary and secondary care levels. It is a disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness [11]. Several different types of human epilepsies have been characterized based on the classification ofInternational League against Epilepsy (ILAE).

According to thisclassification, epilepsy has been divided into partial epilepsy (simple and complex), generalized symptomatic epilepsy and unclassified epilepsy.An imbalance between the excitatory and inhibitory neurotransmitters are responsible for seizures. At neuronal level, seizures activity often occurs when glutamatergic excitatory neuro-transmitters overrides gammaaminobutyric acid (GABA) mediated inhibition. In the assessment of antiepileptic study, several models have been developed. Many drugs that increase the brain contents of GABA have exhibited the antiepileptic against seizures induced by MES induced seizures and PTZ induced seizures [12].

The MES is probably the best validated method for assessment of antiepileptic drugs in generalized tonic-clonic seizures. At the highest tested dose (500 mg/kg b.wt.), methanolic extract was found to significantly decrease the duration of the hind limb tonic extensor phase whereas the lower dose (250 mg/kg b.wt.) shown less effect against seizures. The extracts of *Melissa officinalis* exhibited anticonvulsant activity by delayingthe onset of PTZ induced seizures and protecting treated mice frommortality induced by seizures.

Drugs protecting against tonic-clonic seizures induced by PTZ are considered as useful in controlling myoclonic and absence seizures in humans [12]. The phytochemical study of extracts revealed the presence of alkaloids, tannins, triterpene and steroids. The phytochemicals such as tannins, triterpene and steroids were reported as active substances for anticonvulsant activity [12]. Hence, these phytochemicals might be contributing to the anticonvulsant activity of AEMO and MEMO. Further study is necessary to determine the mechanism of action and isolation of active principle(s) from aqueous and methanol extracts of MO for antiepileptic activity.

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