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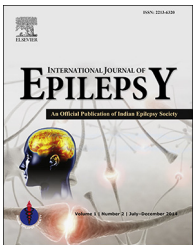

Original Article

Effect of aqueous extract of *Moringa oleifera* leaves on pharmacological models of epilepsy and anxiety in mice

Suvarna P. Ingale *, *Foram P. Gandhi*

SCES's Indira College of Pharmacy, Pune 411033, India

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ABSTRACT

Objective: Among the psychiatric co-morbidities in epilepsy, anxiety disorders are highly frequent and have profound influence on the quality of life of epilepsy patients. *Moringa oleifera* Lam. (Moringaceae) is used in traditional medicine to treat various ailments including anxiety and epilepsy. However, no scientific evidence exists to support its use. We studied antiepileptic and anxiolytic activities of aqueous extract of *Moringa oleifera* Lam. leaves (AEMO).

Methods: Antiepileptic activity was evaluated using pentylenetetrazole (PTZ) induced seizure and maximum electroshock (MES) induced seizure test and anxiolytic activity was evaluated using elevated plus maze, light/dark box and hole board test.

Results: In present study, AEMO (250, 375 and 500 mg/kg, i.p.) demonstrated significant antiepileptic and anxiolytic effects. To study involvement of GABA in anxiolytic and antiepileptic activity of AEMO, we also evaluated effect of AEMO on Baclofen induced catatonia, a GABA mediated behavior, wherein AEMO significantly potentiated (preponed) baclofen induced catatonia, which is suggestive of its GABA mimetic action.

Conclusion: Thus, it may be concluded that aqueous extract of *M. oleifera* possess anxiolytic and antiepileptic effects possibly mediated via of GABA mimetic action and these findings authenticate the traditional claims about use of *Moringa oleifera* in treatment of epilepsy and anxiety.

1. Introduction

Epilepsy is a group of chronic neurological disorders characterized by seizures, which are the result of abnormal, excessive or hypersynchronous neuronal activity in the brain. Epilepsy is a worldwide problem that affects between 2% and 3% of the population and accounts for 0.5% of the global burden of disease. According to WHO, epilepsy is one of the most common neurological diseases globally, as approximately 50

* *Corresponding author at*: Department of Pharmacology, SCES's Indira College of Pharmacy, New Pune–Mumbai Highway, Tathawade, Pune 411033, India. Tel.: +91 9423219990.
E-mail address: suvarnaingale@gmail.com (S.P. Ingale).

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million people worldwide have epilepsy. Epidemiological studies show that psychiatric disorders are more prevalent among people with epilepsy than in the general population. The existence of comorbid psychiatric disorders has a significant impact on the treatment of epilepsy.[1] Among the psychiatric co-morbidities in epilepsy, anxiety disorders are highly frequent and have a profound influence on the quality of life of epilepsy patients. Several studies have reported elevated rates of panic attacks, panic disorder, obsessive compulsive disorder (OCD), social anxiety disorder and generalized anxiety disorder (GAD) in adult patients with epilepsy as compared with the general population.[2] Anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat.[3] Anxiety affects 1/8th population worldwide and has become an important research area in – the field of psychopharmacology.[4]

There is a complex relationship between anxiety and epilepsy like – anxiety as a reaction to a diagnosis of epilepsy, anxiety as a response to social and family stigma due to epilepsy, anxiety as an epileptic aura, ictal anxiety, anxiety (agitation), which occurs during epileptic psychosis, real phobic anxiety related to the attack and anxiety that precipitates an attack.[5] Thus, there is need to come up with the drug therapy useful in the treatment of epilepsy as well as anxiety, which will improve quality of life in epileptic patients.

Moringa oleifera Lam. (Family: Moringaceae) is commonly known as drumstick tree or Shevga. Different parts of these plants are being employed for the treatment of various ailments in the indigenous system of medicine. Several bioactive compounds were recognized in the leaves of *M. oleifera* such as vitamins, carotenoids, polyphenol, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins and oxalates and phytates. The leaves are rich in Vitamin A and C. It also contains Niazirin, Niazirinin, three mustard oil glycosides, 4-[(4′-O acetylalpha-L-rhamnosyloxy) benzyl]isothiocyanate, Pyrrole alkaloid (pyrrolemarumine 400-O-a-L-rhamnopyranoside), and 40-hydroxyphenylethanamide (marumosides A and B), α and γ-tocopherol.

It possess antitumor, antipyretic, anti inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, antidiabetic and hepatoprotective activities.[6,7] Traditional claims about *M. oleifera* Lam. suggest its potential in treatment of epilepsy and anxiety.[8,9] There was no scientific reports presented on the antiepileptic and anxiolytic activity of aqueous extract of *Moringa oleifera* Lam. leaves (AEMO). Hence, the present study is designed to validate the uses of *M. oleifera* Lam. leaves in epilepsy and anxiety.

2. Materials and methods

2.1. Plant material

The fresh leaves of *M. oleifera* Lam. were collected in the month of March and authenticated by Dr. J. Jayanthi, Botanical Survey of India, Pune-411001. The voucher specimen of the same was deposited in Botanical Survey of India, Pune. (Voucher Specimen No: FOGMO3).

2.2. Preparation of extract

Fresh leaves of *M. oleifera* Lam. were shade dried. Shade dried powdered leaves (1 kg) mixed with boiling water and macerated for 2 h. Process is continued by gradually adding boiling water until extraction process was completed. The aqueous extract was filtered, concentrated by evaporation on hot water bath. The finally obtained extract was weighed; and stored in cool place (Yield = 22.7%). After qualitative and quantitative chemical investigation, the aqueous extract of *Moringa oleifera* leaves (AEMO) was used for pharmacological studies (Rathi et al., 2006).

2.3. Drugs and chemicals

Baclofen, Diazepam, and Phenytoin were obtained from Alkem Laboratories Ltd, Nacharam, Hyderabad.

2.4. Animals

Swiss Albino mice weighing 20–25 g of either sex were used. The animals were maintained under standard laboratory conditions at temperature $24 \pm 2\ ^{\circ}C$ and relative humidity (30–70%) with a 12:12 h light:dark cycle throughout all the experiment. The animals were fed with standard pellet diet and free access of water. The animals were shifted to the laboratory one hour prior to the experiment. All the experimental procedures and protocols used in this study were approved by the Institutional Animal Ethics Committee (IAEC).

2.5. Qualitative phytochemical analysis

Aqueous extract of *Moringa oleifera* leaves (AEMO) was subjected to qualitative phytochemical screening for presence of phytoconstituent in accordance with the standard protocol.[10]

2.6. Quantitative phytochemical estimation

2.6.1. Total phenolic content

The total phenolic content was determined by using Folin–Ciocalteu assay. An aliquot (1 ml) of extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l) was added to 25 ml volumetric flask, containing 9 ml of distilled water.1 ml of Folin–Ciocalteu's phenol reagent was added to mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to 25 ml with distilled water and mixed. After incubation for 90 min, absorbance against prepared reagent blank was determined at 750 nm with UV–Vis spectrophotometer. All samples were analyzed in triplicates.[11]

2.6.2. Total flavonoid content

Total flavonoid content was measured by the aluminum chloride colorimetric assay. An aliquot (1 ml) of extracts (10 mg/100 ml of alkaline methanol) or standard solution of chrysin (20, 40, 60, 80 and 100 mg/l) was added to 10 ml volumetric flask containing 4 ml of dd H_2O. To the flask was added 0.3 ml 5% $NaNO_2$. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At 6th min, 2 ml 1 M NaOH was added, and the total volume was made up to 10 ml with dd H_2O. The solution was mixed well, and the absorbance was measured against