doi.org/10.36721/PJPS.2021.34.6.REG.2167-2171.1

# Dose dependent anticonvulsant activity of *Morus nigra* in strychnine induced seizures model

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**Abstract**: Herbal medicines have gained tremendous surge of interest in recent years. *M. nigra* leaves are a rich source of phenolics which are well-known for their antioxidant property. *Morus nigra* popularly known as black mulberry is considered to be the most significant species of genus *Morus*. This study was designed to evaluate its activity on seizure model in different doses. Five groups were made comprising of n=10 animals in each group respectively. Group I was on distilled water, Group II was administered with reference drug diazepam and Group III, IV and V were on 125mg/kg, 250mg/kg and 500mg/kg dose of *Morus nigra* for 15 days prior to experiment. On day 16$^{th}$ all animals were administered with strychnine after 30 minutes of respective treatments and three parameters were recorded i.e. duration, frequency and onset of seizures. *M. nigra* treatment showed significant seizure protection as noted by delayed latency of seizures (P≤0.05), decrease in frequency and jerk's duration (P≤0.05) in comparison to control and reference standard. Most significant (P≤0.05) anticonvulsant effects were observed with 500mg/kg dose. Anticonvulsant activity of *M. nigra* could be due to potentiation of both Gabaergic and glycinergic activities. Antiepileptic potential of extract could also be amplified due to its antioxidant activity. This could serve as a non-pharmacological treatment for seizure management.

**Keywords**: Anticonvulsant, diazepam, *Morus nigra*, seizures.

## INTRODUCTION

Epilepsy is a common neurological disorder that affects almost 65 million people worldwide. It is characterized by sudden recurrent abnormal electrical activity of brain called seizures. On the basis of brain part affected seizures have been classified into two broad groups partial and generalized (Porter and Medrum, 2012). Epilepsy is associated with chemical imbalance and may be due to hypoglycemia, hypoxia, hypocalcemia and hyponatremia etc. Seizures can be induced by any injury to brain or hippocampal lesions. Other causes may include high fever, head injuries, infections or birth trauma (Asif, 2015). Conventional antiepileptic therapy with drugs like phenobarbital, carbamazepine and phenytoin cannot effectively control seizures and in some patients their continued use may lead to several serious adverse effects including neurotoxicity (Kharibegashvili, 2020; St Louis, 2009). The purpose of the treatment of epilepsy should not only be to eliminate the incidence of seizures but also to enable the seizures patients to lead a self-sustained life (Giometto *et al*., 2021). Thus, there is a need to investigate for safer and effective antiepileptic therapy in order to reduce drug associated toxicity.

Herbal medicines have gained a tremendous surge of interest in recent years. Since prehistoric periods, plants have been utilized for medicinal use and the practice to use them to cure numerous ailments dates back to early civilization. In global health, these plants play an extensive role in human cultures and are important due to their therapeutic and medicinal values. World Health Organization (WHO) estimated that around 21,000 plant species have the potential for being used as medicinal plants which leads to the growing popularity of herbal treatment across the world. People are now predominantly relying on herbal medicines than allopathic in order to meet their primary health care needs particularly for treating chronic illnesses such as diabetes, inflammatory disorders, chronic pain and neuropsychiatric disorders (Pan *et al.*, 2013).

*Morus* is a genus of traditional angiosperm plants belonging to family Moraceae and is commonly known as mulberry. *Morus nigra* popularly known as black mulberry is considered to be the most significant and healthy species of genus *Morus*. It is a deciduous tree broadly cultivated in West Asia and Europe. The fruits of *M. nigra* contain greater quantity of flavonoids, total phenolic and anthocyanin contents than *M. alba*. The fruits of black mulberry have been consumed as a laxative, analgesic, sedative, antitussive as well as in the management of various ailments including inflammatory diseases (Dhiman *et al*., 2020; Souza *et al*., 2017; Padilha *et al.*, 2010). It is also effective in reducing blood pressure (Sabeen and Ahmad, 2009). This study was designed to evaluate the dose dependent anticonvulsant effects of *M. nigra* on seizure model.

## MATERIALS AND METHODS

### *Collection of fruits*

The fruits of *M. nigra* were collected and identified by Prof. Dr. Ghazala H. Rizwani, Hamdard University. The

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fruit specimens were deposited in Pharmacognosy Herbal Museum for reference in the future with voucher # A00162.

***Preparation of extract***

Fresh fruits of *M. nigra* (1kg) were washed thoroughly under tap water and soaked in 5 liters of methanol for 21 days with occasional shaking. The final extract was collected, filtered and concentrated under reduced pressure of 60°C in rotary evaporator and stored in airtight container at 4°C. The percentage yield obtained was 84.64%. The extract was liquefied in distilled water prior to administration (Galani and Panchal, 2014).

***Drug and chemicals***

Diazepam 10mg/2ml injections were purchased from Aga Khan University Hospital. All other chemicals used in this study were of analytical grade from Merck.

***Selection and housing of animals***

Healthy male albino wistar mice weighing 22-25gm were procured from animal house of Dow University of Health Science. All animals were kept at room temperature of 26 ±2°C in plastic cages under standardized environmental conditions (12h light and dark cycle; humidity: 52±2%). They were subjected to acclimatization for two days before dosing. All animals had free access to food and water.

***Ethical approval and animal handling***

The study was approved by the Advanced Studies and Research Board (ASRB), University of Karachi and given resolution No. 10 (P) 2. The animals were handled as per the specifications provided by Hubrecht and Kirkwood (2010).

***Grouping of animals***

All animals were randomly allocated into five groups having ten mice in each.

*Group I:* Disease Control (0.1 ml Distilled water)

*Group II:* 5mg/kg diazepam P.O (Twinomujuni *et al*., 2016).

*Group III, IV & V:* 125mg/kg, 250mg/kg and 500mg/kg of *M. nigra* fruit extract P.O. (Akhlaq *et al*., 2016; Deniz *et al*., 2017).

***Working methodology***

Anticonvulsant activity of black mulberry fruits were assessed against strychnine-induced seizures and their effects were compared with reference drug (diazepam). All mice were administered with respective dosage of extract and standard for 15 days prior to the day of experiment. On 16$^{th}$ day, all animals were administered with strychnine 1mg/kg intraperitoneal injection after 30 mins of respective treatment. They were assessed separately by placing in an individual transparent cage (20 cm × 15 cm). Three parameters were recorded i.e. duration, frequency and onset of seizures. Mice that survived after 30 min post convulsions were considered as protected (Nimbal *et al.,* 2011).

***Histopathology***

Mice were immediately sacrificed after study. Complete brain tissues were excised out from normal control, disease control, standard and treated animals by cervical dislocation and preserved in 10% buffered formalin solution. Morphological changes were also evaluated by gross examination. Consequently, in representative cassette sections from the region of hippocampus were submitted and processed in an automated "Medite TPC 15" tissue processor for 12h. Afterwards, tissues were embedded in paraffin using automated paraffin embedding station, "TED 99 Medite". Microtome "SLEE 4062" was used to cut embedded blocks of paraffin in 3 to 4µm thick tissue sections. Excised sections were then moved into water bath kept at 46-48°C and fixed on glass slides containing a definite number. Lastly, sections were mounted to haematoxylin and eosin stains (Gottle *et al*., 2014).

## STATISTICAL ANALYSIS

Values were presented as mean ± STD (n=10). Data was analyzed statistically by using IBM SPSS (version 24) one-way ANOVA followed by Post hoc Tukey's test for multiple comparison was performed. P≤0.05 was considered to be significant respectively.

## RESULTS

***Effect of Morus nigra on Onset of seizures***

Multiple comparison by post hoc analysis showed significant (p≤0.05) increase in time for onset of seizures by Group II and all groups of *M. nigra* in comparison to Group I. Significant (p≤0.05) rise in time for onset of seizures was noticed by Group III, IV and V in comparison to reference standard.

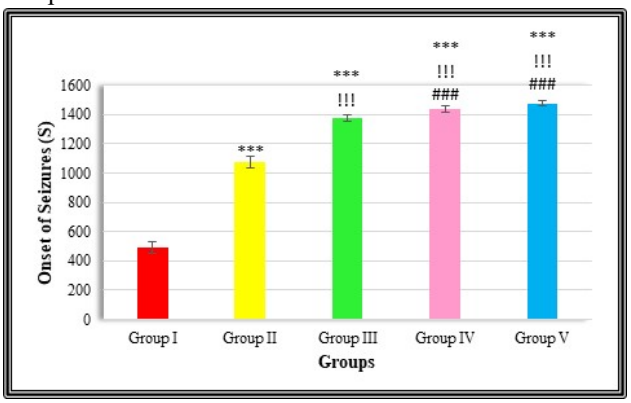


**Fig. 1**: Effect of methanol extract of *Morus nigra* on onset of seizures. Statistical significance at $^{***,!!!,###}$ p≤0.05 as significant in comparison to control, standard and among treated groups of *Morus nigra* respectively. One-way

ANOVA followed by Tukey's test for multiple comparison. Values are presented as mean ± STD (n=10).

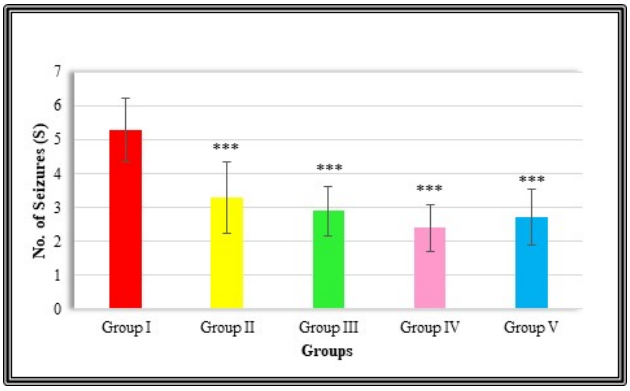


**Fig. 2**: Effect of methanol extract of *Morus nigra* on frequency of seizures. Statistical significance at $^{***}p\leq0.05$ as significant in comparison to control, $^{!!!}p\leq0.05$ as significant in comparison to standard and $^{###}p\leq0.05$ as significant when compared among treated groups of *Morus nigra*. One-way ANOVA followed by Tukey's test for multiple comparison. Values are presented as mean ± STD (n=10).

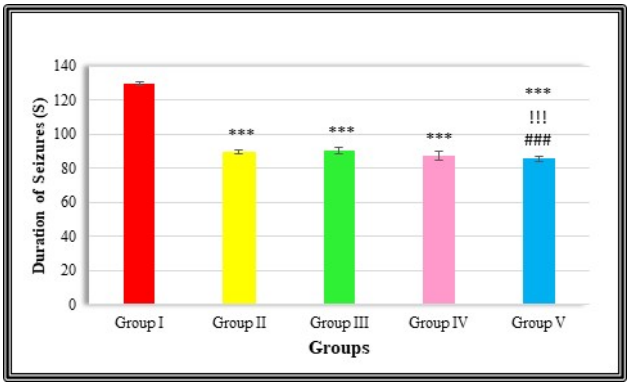


**Fig. 3**: Effect of methanol extract of *Morus nigra* on duration of seizures. Statistical significance at $^{***,!!!,###}p\leq0.05$ as significant in comparison to control, standard and treated groups of *Morus nigra*. One-way ANOVA followed by Tukey's test for multiple comparison. Values are presented as mean ± STD (n=10).

Among the treated groups of *M. nigra* significant rise ($p\leq 0.05$) in time for onset of seizures was noticed in Group IV and V when compared with Group III.

***Effect of Morus nigra on Seizures frequency***

Multiple comparison by post hoc analysis represented significant ($p\leq0.05$) decline in seizures frequency by Group II and all groups of *M. nigra* in comparison to Group I (disease control).

***Effect of Morus nigra on Duration of seizure***

Multiple comparison by post hoc analysis represented significant ($p\leq0.05$) decline in duration of seizures by Group II and all groups of *M. nigra* in comparison to disease control. Significant ($p\leq0.05$) decrease in duration was noted by Group V in comparison to Group II.

Among the treated groups of *M. nigra* multiple comparison by post hoc analysis represented significant ($p\leq0.05$) decline in duration of seizures by Group V in comparison to Group III.

Histopathology of mice brain revealed that animals treated with strychnine exhibited neuronal damages as observed in hippocampus that is revealed by neutrophil vacuolation with degenerating neurons and pyknotic nuclei (fig. IVb) and surrounding tissue shows gliosis, proliferating blood vessels and edema in comparison to normal control mice brain (fig. IVa).

Diazepam treated animals showed preserved architecture with well-developed neuron as observed in fig. (IVc). However, mice treated with *M. nigra* 125mg/kg as observed in fig. (IVd) showed hippocampal area with intact purkinje cell layer with large vesicular nuclei of neurons. Fig. (IVe) showed mostly preserved architecture with mild edema which was administered 250mg/kg *M. nigra*. The mice provided with 500mg/kg of *M. nigra* showed preserved architecture with intact hippocampus as observed in fig. (IVf).

## DISCUSSION

Food plays a key role in providing energy and nutritious support to sustain healthy life in living beings. It gives us all the essential nutrients needed by the body to carry out daily life activities. These essential nutrients generally comprise of carbohydrates, fats, proteins, vitamins, and minerals which possess different biological properties that make them therapeutically active (Dorni *et al*., 2018; Gupta and Prakash, 2014).

Seizure is a sign of uncontrolled neuronal activity. In the study, strychnine was used to induce seizures in an animal model. Strychnine is an alkaloid obtained from *Strychnos nux vomica* seeds. This neurotoxin stimulates the spinal cord by preventing the binding of glycine to glycine-gated chloride channel. Once it binds to this channel, it causes an increase influx of chloride ions leading to hyperpolarization of cells by inhibiting its ability to propagate nerve signals (Walter and Solomon, 1978). Strychnine is involved in the blockade of glycine effects which results in increased conduction of nerve impulses. However, strychnine intoxication lead to irregular muscle contractions, convulsions, tetany, which may result in death due to respiratory paralysis (Smith, 1990).

Our results showed delayed latency of seizures, decrease in frequency and jerks duration in mice pretreated with *M. nigra* in comparison to control and standard. *M.*

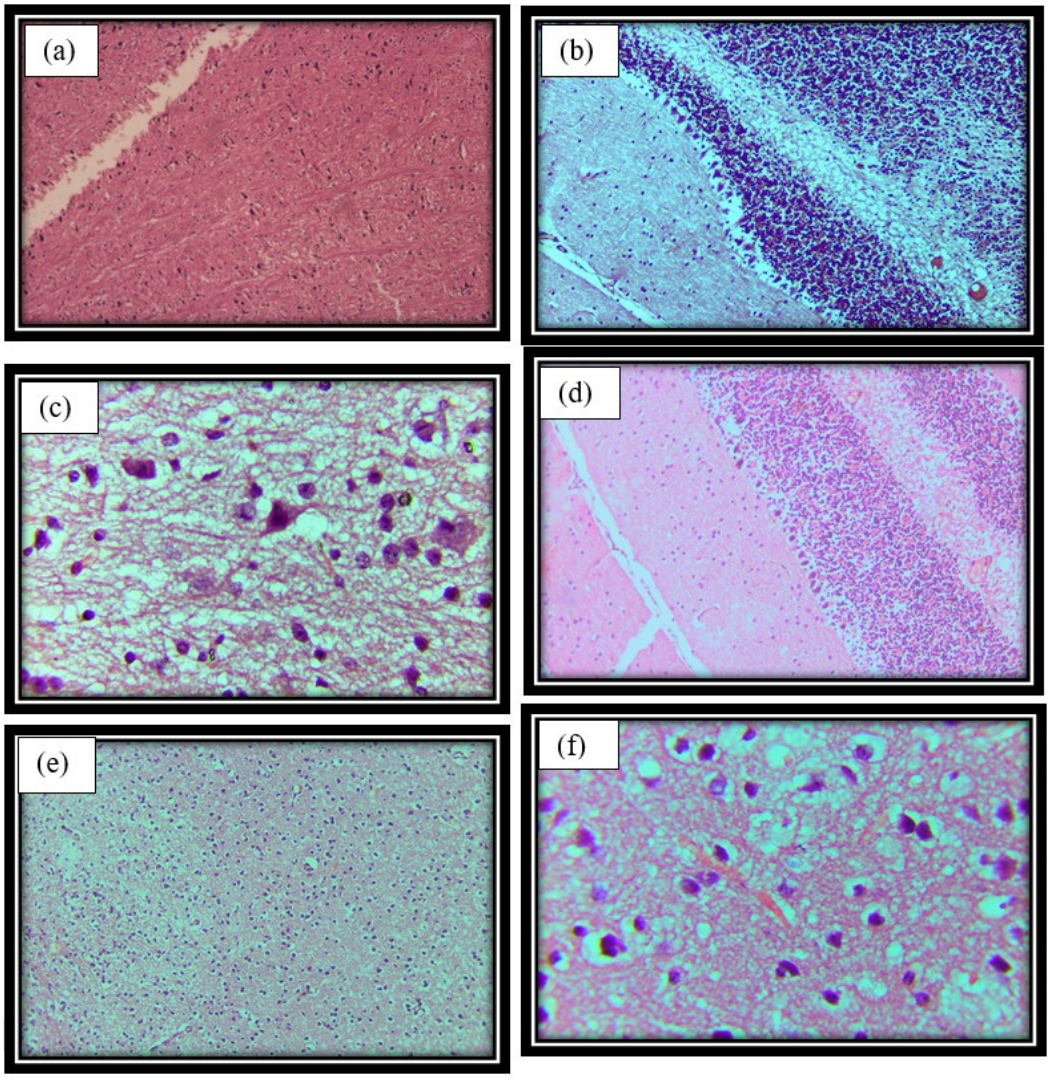


**Fig. 4**: (a) (b) (c) (d) (e) and (f) shows the histopathology of brain samples of normal control mice, disease control mice, diazepam treated mice and those treated with 125mg/kg, 250mg/kg and 500mg/kg *M.nigra* respectively.

*nigra* showed potent anticonvulsant effect. Morusin is an important prenyl flavonoid of *M. nigra*. Previous studies have revealed that morusin retains significant anticonvulsant activity against strychnine in addition to maximal electroshock and isoniazid induced seizures (Gupta *et al*., 2014). Morusin exerted a positive effect on GABA levels, hence it reduces seizures discharge within brain stem substrate by increasing the release of GABA, thus supporting GABAergic mechanism (Vergnes *et al*., 2000). Ascorbic acid, flavonoids and anthocyanin are already known to possess neuroprotective effects (Kelly *et al*., 2017). It is because of their powerful antioxidant, anti-inflammatory and anti-proliferative potential, that inhibits the occurrence of seizures by preventing the formation of free radicals (Tamara *et al*., 2015). 100gm of *M. nigra* contains 21.8 gm ascorbic acid and 1.88mg/g dry wt anthocyanin which provides potent antioxidant activity and inhibits damage of brain cells (Ercisli and Orhan, 2007; Sanchez *et al*., 2015). Observations of histopathological changes also support the neuroprotective ability of *M. nigra* indicating preserved neuronal architecture. Our results also indicate that the anti- seizure effects are dose related and this is a new finding which could be helpful in future researches.

These outcomes clearly support that black mulberry fruit might possess promising role in seizures and more preclinical and clinical researches are required to establish its true medicinal potential.

## CONCLUSION

It is concluded that methanolic fruits extract of *M. nigra* contain significant anti-seizure activity in dose dependent manner. Positive findings of this research will be helpful and can pave the way to support the clinical studies which should be conducted in future to evaluate the effectiveness of *M. nigra* in different doses.

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