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Original article

Anticonvulsant activity of Morusin isolated from *Morus alba*: Modulation of GABA receptor

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ABSTRACT

Aim of the study: Epilepsy is a complex neurological disorder affecting 50 million of world's total population. Number of medicinal plants has been used to treat the convulsion. In ancient time *Morus alba* was used to treat epilepsy and mental illness. In Chinese medicine also *M. alba* is used as neuroprotective herbs. The present study was designed to explore the effect of Morusin, a flavonoid glycoside isolated from *M. alba* as anticonvulsant activity along with biochemical mechanism.
Materials and methods: Morusin was isolated from *M. alba* and acute toxicity study was determined. Anticonvulsant activity of Morusin (5 and 10 mg/kg, i.p.) was studied by using isoniazid (INH) and maximal electroshock (MES)-induced convulsion models; diazepam (5 mg/kg) and phenytoin (20 mg/kg) were used as standards, respectively. Biochemical mechanism was investigated by estimating the GABA level in brain.
Results: The median lethal dose (LD_{50}) of Morusin was found up to 20 mg/kg. Treatment with Morusin (5 and 10 mg/kg) delayed onset of convulsion and tonic hind limb extension along with duration of tonic-clonic convulsions as well as it significantly reduced mortality in INH and MES-induced convulsion. Rats treated with Morusin (5 and 10 mg/kg) significantly increased level of brain GABA at both doses.
Conclusion: The findings of current study provide pharmacological credibility to anticonvulsant activity of Morusin. The protection against the convulsions and restoration of GABA level give a suggestion to its probable mechanism of action.

1. Introduction

Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing countries is 100 per 100,000. The entire currently available antiepileptic drugs are synthetic molecules associated with side effects and approximately 30% of the patients continue to have seizures with this therapy [1]. Thus, research for finding new drugs with less adverse effects and more efficacies, seems to be essential. As many of the herbal drugs have few adverse effects, assessment of herbal medications for their possible antiepileptic activity is worthwhile.

Mulberry, *Morus alba* L., as a non-toxic natural therapeutic agent, belongs with the family of Moraceae, and has been cultivated in many Asian countries such as China, India, Korea, Japan and Thailand where the leaves were used as food for silkworms [2], is a natural food additive having vitamins, carbohydrates, mineral, lipids, sugars, proteins, fibers, etc. in appropriate proportion [3]. It is a potent antioxidant commonly used as a dietary supplement [4,5]. Latest information shows that it can be used as a good pharmaceutical food [6,7].

Number of active phytochemical constituents like alkaloids [8], flavonoids [9], glycosides [10,11], terpenoids [12], steroids [13], volatile oils, tannins [14], etc has been reported from different parts of the plant. The plant has been reported to possess antidiabetic [15], hypolipidemic, antihypertensive [16], antimicrobial [17], antioxidant [18,19], anti-atherosclerotic [20], anticancer [21], neuroprotective [22], anxiolytic and antidepressant [23] etc. activities.

The anticonvulsant activity of Morusin, an isoprenylated flavone, isolated from *M. alba* was evaluated against maximal electroshock (MES)-induced and isoniazid (INH)-induced convulsions along with locomotor activity in experimental animal models.

2. Experimental animals

The animals were obtained from the animal house Siddhartha Institute of Pharmacy, Dehradun, India, maintained under standard conditions (12 h light/dark cycle; 25 ± 3 °C, 45–65% humidity), had

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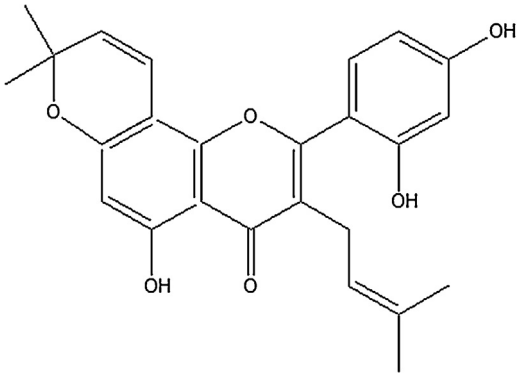

Fig. 1. Structure of Morusin.

free access to standard rat feed and water ad libitum and studies were performed as per CPCSEA, India.

3. Plant material

The *M. alba* L. plant material was collected from local area of Dehradun, (U.K), India, in April 2011, were authenticated by Dr. Imran Kazmi, Department of Pharmacognosy, Siddhartha Institute of Pharmacy.

4. Extraction and isolation of Morusin

The air-dried stem bark of *M. alba* (2 kg) was successively extracted with 70% ethanol. The extract was concentrated in vacuum to give a residue (451 g). One hundred grams of the extract was fractionated over cellulose column chromatography eluted with 100% MeOH to give flavonoids. Twenty-five grams of isolated fraction was subjected to silica gel column chromatography and eluted with a mixture of η-hexane and acetone, increasing the amount of acetone. Morusin was eluted on 3:2 fraction (2 g). Morusin was identified by the comparison with the authentic specimens [15]. The structure of Morusin is depicted in Fig. 1.

5. Chemicals

Diazepam (Calmpose Inj. Ranbaxy, India), Isoniazid (S.d.fine Chemicals) and Phenytoin (Zydus Neurosciences, India) were purchased. All other chemicals used were of analytical grade. Isoniazid was dissolved in normal saline and Morusin was suspended in Tween 80 (1%, v/v) in saline and used.

6. Acute toxicity study

Acute toxicity study was performed in mice according to OECD guidelines. The isolated Morusin was administered intraperitoneally (i.p) at doses of 1.75, 5.5, 17.5, 55 mg/kg. They were then observed for signs of toxicity, continuously for 2 h, and for mortality up to 24 h, after injection [24].

7. INH-induced convulsion model

Wistar albino rats (150–200 g) were divided into four groups ($n = 6$). Group I (solvent control), received 0.9% (w/v) of saline (1 mL/100 g), Group II (positive control), received Diazepam (5 mg/kg) Group III and IV received Morusin, 5.0 and 10.0 mg/kg suspended in Tween 80 (1%, v/v) in saline. All animals were treated intraperitonially 30 min prior to administration of INH (300 mg/kg).

Animals that did not convulse within 30 min were considered as protected and their results were expressed in terms of percentage. Each animal was observed for 60 min by placing in a separate cage for determination of percent protection. In unprotected animals, the latency to first convulsion and the durations of convulsions were recorded [25]. Forty-five minute after vehicle or Morusin and 30 min after diazepam, rats were sacrificed. The animals were sacrificed as soon as onset of convulsions occurs or 65 min after INH treatment. Brain tissue was isolated and transferred to homogenization tube, containing 10 mL of 0.01 M hydrochloric acid. Homogenate was transferred in a bottle with 16 mL of ice cold absolute alcohol. The samples were subjected to centrifugation at 16,000 rpm for 10 min to obtain the precipitate. Precipitate was washed thrice with 10 mL of 90% alcohol. Washed liquids were combined with supernatant. The combination was transferred to petri plate for evaporation and dried at 70 °C; 2 mL water and 4 mL of chloroform added to the dry mass and centrifuged at 2100 rpm. Upper phase containing GABA (4.0 mL) was separated and 20 μL of it was spotted on Whatman paper (No. 41). η-butanol (50 mL) acetic acid (12 mL) and water (60 mL) were selected as mobile phase. Ascending technique was adopted to develop the paper chromatogram. 0.5% ninhydrin solution in 95% ethanol was sprayed and it was dried for 1 h at 90 °C. Blue color spot was developed, cut and heated with 2 mL ninhydrin solution on water bath for 5 min. Water (10.0 mL) was added to solution and kept for 1 h. Supernatant (4.0 mL) was decanted and absorbance was measured at 570 nm [26].

8. MES-induced convulsion model

Maximal electroshock-induced convulsion model animals were divided as per the INH-induced seizure model. The positive control group animals were treated with phenytoin (20 mg/kg). After 30 min of treatments, the electroshock was induced in animals by passing the current of 150 mA for 0.2 second duration through auricular electrodes. The latency and incidence of tonic hind limb extension (THLE) and mortality rate was observed for 15 min.

9. Locomotor activity

Mice were acclimatized with environment and placed individually in an actophotometer (INCO, Ambala Pvt. Ltd., India) for 10 min and a basal activity score was obtained. Subsequently they were divided into four groups with six animals in each group and all the groups were treated as per INH-induced seizure model. After 30 min, the activity score was recorded [27]. The percentage reduction in locomotor activity was calculated.

10. Statistical evaluation

The data were expressed as mean ± SEM Statistical comparisons were performed by one-way ANOVA followed by Dunnett's-test using Graph Pad Prism version 5.0, USA. $P < 0.05$ was considered significant.

11. Result and discussion

Intraperitoneal administrations of stepwise, escalated doses of isolated Morusin in mice gave an LD_{50} value as 20 mg/kg.

In the present investigation, it is evident that Morusin was able to ameliorate the epileptic seizures induced by INH and MES along with along locomotor activity in laboratory animals. The various epileptogenic agents used in the investigation were INH and MES. It is understood from the literature that GABAergic neurotransmission is closely associated with the induction of epilepsy in the animals [28,29]. GABA is the major inhibitory neurotransmitter in the central nervous system and even slight deficiencies in