

Anticonvulsant activity of *Morus alba* and its effect on brain gamma-aminobutyric acid level in rats

Sir,

Morus alba (locally known as Tut, commonly known as white mulberry, Family: *Moraceae*) has been domesticated over thousands of years and adapted to many continents of Asia, Europe, North and South America and Africa with tropical, subtropical and temperate zones. As per Ayurveda, all parts of *M. alba* are medicinally important. In folk medicine, the plant is reportedly used in treating epileptic convulsions, mental illness, insomnia and hemicranias.^[1] Number of active phytochemical constituents such as alkaloids,^[2] flavonoids,^[3] glycosides,^[4,5] terpenoids,^[6] steroids,^[7] volatile oils, tannins,^[8] has been reported from different parts of the plant. On the basis of reported traditional uses, we undertook the present study to determine the anticonvulsant activity of *M. alba* by using pentylenetetrazole (PTZ) and maximal electroshock (MES) - induced convulsion models in rats. *M. alba* leaves were collected at the Campus of Siddhartha Institute of Pharmacy, Dehradun. The plants were identified and authenticated by Dr. Imran Kazmi, Department of Pharmacognosy, Siddhartha Institute of Pharmacy, Dehradun. After collection, the plant leaves which were air-dried under shade were pulverized using a mechanical grinder. Methanol/water (1:1) solvent was used for the extraction of the air-dried pulverized leaves (100 g) to obtain methanolic extract of *M. alba* (MEMA).

An extract yield of 23.7% was obtained, when the extracts were dried with rotary evaporator in a vacuum at 40°C. Physiological saline solution (0.9%) was used as a vehicle to dissolve the extract. All the solutions, including standard, control and treatment were administered intraperitoneally except the PTZ which was administered subcutaneously (s.c.). Wistar albino rats (150-200 g) were divided into four groups ($n = 6$). Solvent control rats which received 0.9% (w/v) of saline (1 mL/100 g) was labeled as Group I while positive control rats which received diazepam (5 mg/kg) or phenytoin (20 mg/kg) was labeled as Group II. Groups III, IV and V received MEMA 25, 50 and 100 mg/kg respectively. Anticonvulsant activity of MEMA was established through PTZ and MES protocol - induced seizure.

All animals in the PTZ-induced seizure model group were treated with PTZ (80 mg/kg s.c.) 30 min after the administration of the standard or test drug. The convulsive behavior was observed for a period of 30 min. The parameters measured were onset of clonic convulsion and duration of convulsion. The ability of the test compound to prevent the above mentioned parameter or extend the latency was taken as an indication of anticonvulsant activity.^[9] After observation of the parameters for 30 min after the administration PTZ, all the rats were sacrificed. The isolated brain tissue from the sacrificed rats were homogenized, individually, with 5 mL of 0.01 M hydrochloric acid and was later left at 0°C for an hour in 8 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 using 5 mL of 75% alcohol. Washed liquids were combined with the supernatant and transferred to petri plate for evaporation and drying at 70°C. The solvents water/chloroform (1:2 ratio) were added to the dry mass and centrifuged at 2000 rpm. The gamma-aminobutyric acid (GABA) (2 mL) containing upper phase was separated and spotted (10 µL) on Whatman paper (No. 41). N-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. Ninhydrin solution (0.5%) in 95% ethanol was sprayed and was allowed to dry for 1 h at 90°C. A blue color spot developed on the chromatogram. The spot was isolated and heated, with water (5 mL) for 1 h, after addition of ninhydrin solution (2 mL) on water bath. The absorbance was measured at 570 nm with the decanted supernatant (2 mL) solution.^[10] The MEMA at 25 mg/kg delayed ($P < 0.05$) only the onset of clonic convulsion, but at 50 and 100 mg/kg delayed ($P < 0.01$ for both) the onset as well as reduced the duration of clonic convulsion ($P < 0.01$ for both) compared with control. The onset time of clonic convulsion was delayed by 100% with MEMA (100 mg/kg) and was comparable with diazepam 5 mg/kg. MEMA (25, 50 and 100 mg/kg) exerted a positive effect on GABA level with significant increase up to the dose of 100 mg/kg [Table 1]. The extract showed protection against PTZ-induced convulsions. PTZ is a GABA-A receptor complex antagonist and produces seizures by the GABA pathway inhibition in the central nervous system due to the imbalance in ionic concentrations of the membrane. It can be hypothesized that MEMA supports GABAergic mechanism by the enhancing the GABA release, thus reducing the epileptic seizures.

Latency and incidence of tonic hindlimb extension (THLE) were measured in MES-induced seizure model animals. The positive control group animals were treated with

Table 1: Effect of MEMA on PTZ-induced convulsion in rats

Treatment (dose, mg/kg, i.p.)	Onset of convulsion (s)	Duration of convulsion (s)	No. of animals convulsed/used	Percentage of delay in onset of clonic convulsion	GABA level (ng/g of brain tissue)
PTZ control (80 s.c.)	306.16±22.16	52.03±3.93	5/6	83.33	11.05±1.17
Diazepam (5)	683.02±24.72 ^c	2.10±0.11 ^c	0/6	0.00	29.15±1.82 ^c
MEMA (25)	452.17±20.91 ^a	49.82±1.41	3/6	50.00	17.04±1.48 ^b
MEMA (50)	491.42±29.07 ^b	13.36±1.18 ^b	1/6	16.66	21.52±1.27 ^c
MEMA (100)	699.10±31.28 ^c	3.09±1.00 ^c	0/6	0.00	27.91±0.19 ^c

Values are expressed in mean±SEM, where n=6. ^aP<0.05: Compared with vehicle treated group; ^bP<0.01: Compared with vehicle treated group; ^cP<0.001: Compared with vehicle treated group. PTZ: Pentylentetrazole; MEMA: Methanolic extract of *Morus alba*; SEM: Standard error mean; s.c.: Subcutaneously; GABA: Gamma-aminobutyric acid; i.p.: Intraperitoneally

Table 2: Effect of MEMA on MES-induced convulsion in rats

Treatment (dose, mg/kg, i.p.)	Duration of THLE (s)	No. of animals recovered/used	Protection against mortality (%)
MES control	11.04±2.16	3/6	50
Phenytoin (20)	Absence of extension	6/6	100
MAE (25)	5.83±1.36 ^a	5/6	83.33
MAE (50)	1.20±0.84 ^b	6/6	100
MAE (100)	Absence of extension	6/6	100

Values are expressed in mean±SEM, where n=6. ^aP<0.01: Compared with vehicle treated group; ^bP<0.001: Compared with vehicle treated group. MEMA: Methanolic extract of *Morus alba*; MES: Maximal electroshock; MAE: Mean absolute error; THLE: Tonic hind limb extension; i.p.: Intraperitoneally

phenytoin (20 mg/kg). After 30 min of treatment, the electroshock was induced in animals with 150 mA current for 0.2 s duration through auricular electrodes. The latency and incidence of THLE and mortality rate were observed for 15 min. MEMA exhibited significant ($P < 0.01$) reduction, at the 25 mg/kg dose, in THLE and 83.33% protection against mortality. Although, at the 50 and 100 mg/kg doses, MEMA exhibited significant ($P < 0.001$) reduction in THLE and 100% protection against mortality compared with phenytoin. Moreover, protection against THLE in MES-induced seizure indicates the capability of MEMA to either stop or to slow down the discharge of the seizure within the brain stem substrate.^[11] MEMA thus shows the anticonvulsant potential in the MES-induced seizures [Table 2]. Hence, the present study reveals that MEMA exhibits potential antiepileptic activity via facilitation of GABA transmission.

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