JOURNAL OF NATURAL REMEDIES

Antiepileptic and Antipsychotic Effects of *Ipomoea reniformis* (Convolvulaceae) in Experimental Animals

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Abstract

Ipomoea reniformis Chaos is claimed in Indian traditional medical practice to be useful in the treatment of epilepsy and neurological disorders. In the present study, pretreatment effect of methanolic extract of *Ipomoea reniformis* on epilepsy and psychosis was evaluated in rodents using standard procedures. Besides evaluating epileptic and behavioral parameters, neurotransmitters such as Gamma-Amino Butyric Acid (GABA) in epilepsy and in psychosis dopamine, noradrenaline and serotonin contents in the rodent brain were estimated. The extract pretreatment reduced maximal electro shock; Isoniazid (INH) and Pentylenetetrazole (PTZ) induced seizures and also significantly inhibited the attenuation of brain GABA levels by INH and PTZ in mice. These results suggested that the observed beneficial effect in epilepsy may be by enhancing the GABAergic system. The test drug also inhibited the apomorphine induced climbing and stereotyped behavior and showed significantly reduced levels of brain dopamine, noradrenaline and serotonin which may be due to blocking of central dopaminergic, noradrenergic and serotonergic pathways or by enhancing the GABAergic system. The results obtained in present study suggest that the title plant possesses antiepileptic and antipsychotic activities in rodents.

Keywords: Anticonvulsant, dopamine, GABA, *Merremia emarginata*, sinapic acid

1. Introduction

Ipomoea reniformis (IR) also called as *merremia emarginata* (Burm. f.) is a procumbent herb belonging to the family convolvulaceae. In India, it is commonly known as Undirkana and Mushakparni. The plant is widely distributed in India, Sri Lanka, Philippines, Malaysia, Tropical Africa and mainly grows in rainy and winter season. In India, it is found in Southern part mainly counting Chennai, and some places of Andhra Pradesh [1]. Traditionally, IR has been used to treat diverse clinical conditions ranging from pain; fever to neurological disorders [2]. IR has been claimed to be useful for inflammation, headache, fever, cough, neuralgia, rheumatism and also in liver and kidney diseases [3]. The powder of leaves is used as a snuff during epileptic seizures. Juice acts as purgative and the root is having diuretic, laxative actions and applied in the disease of the eyes and gums [4].

The plant contains various neuroprotective chemical constituents such as caffeic, p-coumaric, ferulic and sinapic acid esters. Petroleum ether extract contains fats and fixed oil while aqueous extract contains amino acids, tannins (condensed and pseudo tannins) and starch [5]. IR has been reported to possess various pharmacological actions, mainly antidiabetic [6], anti-inflammatory [7], nephroprotective [8], antibacterial [9], antioxidant and antimicrobial activity [10]. Further, the principle constituents of IR such as sinapic and ferulic acids have exhibited behavioural and pharmacological

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characteristics including anxiolytic, neuroprotective and antioxidative effects [11]. Because of all these facts, the present study was undertaken to evaluate the effect of IR in different rodent experimental models of epilepsy and psychosis, as IR has not been scientifically studied and reported for its antiepileptic and antipsychotic activities, despite its use in the treatment of nervous disorders in traditional medicines.

2. Materials and Methods

2.1 Drugs and Chemicals

Pentylenetetrazole and apomorphine HCl (Sigma-Aldrich Corporation, St. Louis, USA), isoniazid, pilocarpine, noradrenaline and O-pthaldialdehyde (Himedia, Bombay, India), diazepam injection (Calmpose® Ranbaxy Ltd, New Delhi, India), phenytoin injection (Eptoin®, Abbot, New Delhi, India), haloperidol injection (Mindol®, Micro labs, Bangalore, India), dopamine (United Biotech, New Delhi), serotonin (Merck Co., Germany) were purchased, and all other chemicals used were of analytical grade. Apomorphine HCl and isoniazid were dissolved in distilled water and administered subcutaneously (s.c.) and intra-peritoneally (i.p.), respectively, to induce psychotic behavior (climbing and stereotyped) and seizures. Pentylenetetrazole was dissolved in normal saline and injected i.p. to induce seizures.

2.2 Extraction of Ipomoea *reniformis*

The plant material was collected from Sri Venkateshwara University Campus, Tirupati, Andhra Pradesh, India. The plant specimen was authenticated by Dr. K Madhava Chetty, Assistant Professor, Department of Botany of the same university. The herbarium (SSCP11PC0016) was prepared and kept in the Department of Pharmacology, Sree Siddaganga College of Pharmacy for future reference. The whole plant was washed using water and shade dried for two weeks. About a kilogram of the dried plant material was powdered with mechanical grinder and sieved to get uniform particle size. The powder was packed in soxhlet apparatus for defatting with n-hexane for 3 days. The plant powder (marc) was air dried after defatting. It was once again packed in soxhlet apparatus for methanol extraction to get a clear solution in syphon tube. Then the extract was concentrated under controlled temperature and pressure. Weighed quantity of the extract was then used to prepare the doses.

2.3 Preparation of IR Suspension and Dose

Dried Methanolic Extract of *Ipomoea reniformis* (MEIR) was suspended in distilled water using sodium CMC (1% w/v). Two doses (200 and 400 mg/kg b.w/d, oral route) were selected for the studies. Sodium CMC (1% w/v) was served as vehicle and administered by oral route to vehicle control animals.

2.4 Experimental Animals and Research Protocol Approval

Young Albino Wistar male rats (180–220 g) and Swiss Albino mice of either sex (25–30 g) were obtained from the animal house of our institute and maintained under controlled conditions of temperature (25 ± 2°C) and humidity (45–65%). In addition, the animals were on a 12 h light: 12 h dark cycle and had free access to food and water *ad libitum*. All the animals were acclimatized for a week before the study and randomized into different groups, then housed in sanitized polypropylene cages containing sterile paddy husk as bedding. MEIR and standard prototype drugs (diazepam, phenytoin, haloperidol) were administered once daily (0900) in the morning for a period of 15 days. Food but not water was withdrawn 3 h before the experiment. The protocol was approved by the Institutional Animal Ethics Committee (SSCPT/IAEC.CLEAR/114/2011-12) and conducted according to CPCSEA guidelines, Govt. of India. The different stages of convulsions and climbing as well as stereotyped behaviors were evaluated by an independent observer who was blinded to the treatment.

2.5 Assessment of Antiepileptic Activity

2.5.1 Maximal Electro Shock (MES) Induced Seizures

Albino Wistar rats were divided into five groups of six each and treated as Group 1: vehicle control and received 1% sodium CMC (1 ml/100 g); Group 2: MES control, received 1% sodium CMC (1ml/100 g); Groups 3 and 4 received MEIR 200 and 400 mg/kg, respectively; Group 5: received standard drug phenytoin (90 mg/kg, i.p.). On 15th day, an hour after oral administration of vehicle or test drug and 30 min after phenytoin, all groups received

electric shock (150 mA, 50 Hz for 0.2 s) to pinna of ear by using electroconvulsiometer (INCO, Ambala Pvt. Ltd., India), except the vehicle control. Total duration of Hind Limb Tonic Extension (HLTE), onset of stupor and time taken for recovery were recorded [12].

2.5.2 Isoniazid (INH) Induced Seizures

Swiss Albino female mice were divided into five groups (n = 6). The following treatment was given once daily for 15 days. Groups 1, 3 and 4 received the pretreatments as described under MES model. Group 2 was served as INH control and received 1% sodium CMC (1 ml/100 g); Group 5 received standard drug diazepam (5 mg/kg, i.p.). On the last day of pretreatment, mice of all groups, except vehicle control, were injected with INH (300 mg/kg). INH was given an hour after the vehicle or extract and 30 min after diazepam. Mice were then placed in the isolated perspex chamber for 120 min observation. The occurrence of clonic and tonic seizures as well as death was recorded during the observation. The protection against mortality was expressed in percentage [13].

2.5.3 Pentylenetetrazole (PTZ) Induced Seizures

Swiss Albino mice were divided into five groups of six mice each and pretreated once daily for 15 days. Groups 1, 3, 4, and 5 were received the treatments as described in above models. Group 2 was severed as PTZ control and administered with 1% sodium CMC (1 ml/100 g). On the concluding day of pretreatment, PTZ (80 mg/kg) was administered to groups 2, 4 and 5 after one hour of oral feeding and 30 min after i.p. treatment to group 3. The mice were then placed in the isolated perspex chamber for 60 min observation and the parameters were assessed as per INH model [13].

2.6 Assessment of Antipsychotic Activity

2.6.1 Apomorphine Induced Climbing Behavior in Mice

Swiss Albino male mice were divided into five groups (n = 6) and treated as follows, Groups 1 and 2 vehicle and apomorphine control, respectively were treated with 1% sodium CMC (1 ml/100 g); Groups 3 and 4 were orally treated with a suspension of MEIR in 1% sodium CMC at the doses of 200 and 400 mg/kg, respectively; Group 5 standard control, received haloperidol (0.1 mg/kg, i.p.). On the final day of pretreatment, an hour after oral administration and 30 min after haloperidol, apomorphine was given (3 mg/kg) to all the groups except vehicle control. The mice were then placed individually in vertical wire-mesh stick cages (diameter 12 cm, height 14 cm) for observation of climbing behavior every 10 min for a period of 30 min. Before administration of apomorphine the mice were acclimatized to the new environment for a period of 30 min. The method of scoring was: 0 - four paws on the floor, 1 - forefeet holding the bars and 2 - four feet holding the bars [14].

2.6.2 Apomorphine Induced Stereotyped Behavior in Rats

Albino Wistar rats were divided into five groups of six rats each. The treatments and duration for groups 1, 2, 3 and 4 were similar as described in the above model. Group 5 was received haloperidol (1 mg/kg, i.p.). On behavioral parameter assessment day, after respective treatments, all groups of rats except the vehicle control were received apomorphine (1.5 mg/kg). The rats were individually placed in individual cages and observed for every 10 min for a total period of 90 min. The intensity of stereotyped activity was assessed according to scoring system, 0 - asleep or still, 1 - active, 2 - predominantly active but with bursts of stereotyped sniffing and rearing, 3 - constant stereotyped activity such as sniffing, rearing, or head bobbing, but with locomotor activity still present, 4 - constant stereotyped activity maintained at one location, 5 - constant stereotyped activity but with bursts of licking or gnawing and biting, 6 - continual licking of cage grids and, 7 - continual biting of cage grids [14].

2.7 Biochemical Estimations

2.7.1 Estimation of Brain GABA Neurotransmitter

The brain GABA content was estimated in the convulsion tested models according to the published procedure [15]. On 15th day, after observing the seizures, animals were sacrificed by decapitation and brain was dissected out rapidly. It was blotted, weighed and placed in 5 ml of ice-cold Trichloroacetic acid (10% w/v), then homogenized and centrifuged at 10,000 rpm for 10 min at 0°C. A sample (0.1 ml) of tissue extract was added 0.2 ml of 0.14 M ninhydrin solution in 0.5 M

carbonate-bicarbonate buffer (pH 9.9), kept in a water bath at 60°C for 30 min, cooled and treated with 5 ml of copper tartrate reagent (0.16% w/v disodium carbonate, 0.03% w/v copper sulphate and 0.0329% w/v tartaric acid). After 10 min, fluorescence was recorded at 377/455 nm using spectofluorimeter (Shimadzu, Japan). Rodent brain GABA content was calculated using a standard calibration curve method and expressed in ng/mg of wet brain tissue [16].

2.7.2 Estimation of Brain Dopamine, Noradrenaline and Serotonin Neurotransmitters

2.7.2.1 Preparation of Brain Extract

After the behavioral assessments, rodents of all the groups were sacrificed; brain was dissected out for biochemical (dopamine, noradrenaline and serotonin) estimations. Brain tissue was homogenized in 4 ml hydrochloric acid - butanol, (0.85 ml of 37% v/v HCl in one liter n- butanol) for 1 min. The sample was then centrifuged for 10 min at 5000 rpm. 4 ml of supernatant was removed and added to a tube containing 4 ml of heptane and 0.5 ml of 0.1 M HCl. After 5 min of vigorous shaking, the tube was centrifuged under the same environment as above in order to separate the two phases. Upper organic phase was discarded and the aqueous phase was used for the estimation of dopamine, noradrenaline and serotonin. All the processes were carried out at 0°C [17].

2.7.2.2 Dopamine and Noradrenaline Assay

The assay represents a miniaturization of the trihydroxyindole method. To 1 ml of brain extract, 0.25 ml of 0.4 M HCl and 0.5 ml of EDTA/sodium acetate buffer (pH 6.9) were added, followed by 0.5 ml of iodine solution (0.1 M in ethanol) for oxidation. The reaction was stopped after two min by adding 0.5 ml of 2.5% (w/v) Na_2SO_3 in 5 M NaOH. After 90 s, 0.5 ml of 10 M acetic acid was added and heated to 100°C for 6 min. The samples were cooled to RT and excitation/emission spectra were read at 330/375 nm for dopamine and 395/485 nm for noradrenaline using spectrofluorimeter (Shimadzu, Japan). Concentration of dopamine and noradrenaline in brain samples were calculated using a standard calibration curve and expressed in pg/mg of wet brain tissue [17].

2.7.2.3 Serotonin Assay

Brain serotonin levels were determined by O-Pthaldialdehyde (OPT) method with slight modifications. Briefly, 1.8 ml of OPT reagent (20 mg % in conc. HCl) was added to 1.5 ml of the brain extract. The fluorophore was developed by heating at 100°C for 10 min. The samples were cooled to RT, excitation/ estimation intensity readings at 360/470 nm were taken using spectrofluorimeter (Shimadzu, Japan) was taken and serotonin contents were expressed in pg/mg of wet brain tissue. Concentration of brain serotonin in samples was estimated using a standard calibration curve and expressed in pg/mg of wet brain tissue [17].

2.8 Statistical Evaluation

The data were expressed as Mean ± S.E.M. Statistical comparisons were performed by one-way ANOVA followed by Tukey's post-test using Graph Pad Prism version 5.0, USA. $P < 0.05$ was considered significant.

3. Results

3.1 Effect of MEIR on Different Epilepsy Models

3.1.1 Effect of MEIR on MES Induced Seizures in Rats

Vehicle control group did not exhibit convulsions; whereas electric shock induced HLTE and stupor in MES control rats with recovery time of 5.00 ± 0.71 min. Pretreatment of rats with higher dose of MEIR showed a significant ($P < 0.01$) reduction (36.00%) in the duration of HLTE when compared to MES control rats. MEIR (200 and 400 mg/kg) also significantly ($P < 0.05$ and $P < 0.001$, respectively) reduced (29.41 and 43.09%) the onset of stupor. Phenytoin exhibited complete blockade of HLTE and significant ($P < 0.001$) reduction (60.54%) in stupor phase of convulsion. MEIR (both the doses) and phenytoin, respectively, showed 18.60, 22.40 and 78.00 % reduction in recovery time (Table 1).

3.1.2 Effect of MEIR on INH Induced Seizures in Mice

Convulsions were not seen in vehicle control group, but in INH control group, injection of INH induced fast

onset of clonic and tonic convulsions and 100% death of all mice. MEIR pretreatment, dose dependently (200 and 400 mg/kg) and significantly ($P < 0.05$ and $P < 0.001$, respectively) delayed the onset of clonic convulsion (57.94 and 120.08%, respectively) in comparison to INH control group. Further, MEIR at 400 mg/kg also significantly ($P < 0.01$) deferred (46.80%) the onset of tonic convulsions. Diazepam showed significant ($P < 0.001$) delay (154.77 and 101.36%, respectively) in both the onset of clonic and tonic convulsions. MEIR administration (200 and 400 mg/kg) delayed the time to death and exhibited 33.33 and 83.33% protection against death, whereas diazepam showed 100% protection (Table 2).

3.1.3 Effect of MEIR on PTZ Induced Seizures in Mice

Absence of convulsions was seen in mice pretreated with vehicle alone and diazepam. Administration of PTZ (80 mg/kg) alone induced instant onset of clonic and tonic convulsions and 100% death in PTZ control group. Pretreatment of mice with MEIR (200 and 400 mg/kg) exhibited significant ($P < 0.05$ and $P < 0.01$, respectively) and dose dependent delay in onset of clonic (139.57 and 212.50%, respectively) and tonic (104.35 and 136.96%, respectively) convulsions when compared to PTZ control group. No deaths were observed in higher dose of MEIR and diazepam groups indicating 100% protection, however, lower dose of MEIR showed 83.33% protection (Table 3).

3.1.4 Estimation of Brain GABA Content

Administration of chemical inducers (INH and PTZ), but not electric shock, exhibited significant ($P < 0.01$; $P < 0.001$) reduction in brain GABA content when compared to vehicle control. In MES model, MEIR, both doses, (4.53 ± 0.34 and 5.08 ± 0.53 ng/mg, respectively) and phenytoin (5.87 ± 0.34 ng/mg) for 15 days failed to show significant increase in brain GABA content when compared to MES control (4.05 ± 0.81 ng/mg) group. INH and PTZ control mice showed 15.60 and 19.37% reduction, respectively, in brain GABA content when compared with vehicle control. MEIR at 200 mg/kg produced significant ($P < 0.05$) increase (13.73%) in brain GABA content in PTZ model. However, MEIR at higher dose exhibited a significant ($P < 0.01$) increase in brain GABA content (16.57 and 16.19%, respectively) in both INH and PTZ models, whereas diazepam showed 20.89 and 20.80% increase, respectively (Table 3).

3.2 Effect of MEIR on Different Behavior Models

3.2.1 Effect of MEIR on Apomorphine Induced Climbing Behavior in Mice

Vehicle alone pretreated mice exhibited normal behavior. Mice injected with apomorphine alone showed significant increase in the climbing behavior characterized by forefeet holding the vertical bars and four feet holding the bars in wire-mesh stick cages. Pretreatment with MEIR, 200 mg/kg, significantly

Table 1: Effect of MEIR on MES induced seizures in rats

Group	Treatment	Various phases of convulsions		
		Extension (s)	Stupor (s)	Recovery (min)
1	Vehicle control (1 ml/100 g)	Nil	Nil	Nil
2	MES control (1 ml/100 g) + ES	25.00 ± 1.16	96.33 ± 7.09	5.00 ± 0.71
3	MEIR (200 mg/kg) + ES	31.17 ± 2.21 (24.69)	68.00 ± 4.94[a] (29.41)	4.07 ± 0.63 (18.60)
4	MEIR (400 mg/kg) + ES	16.00 ± 0.63[c] (36.00)	54.83 ± 5.90[c] (43.09)	3.88 ± 0.44 (22.40)
5	Phenytoin (90 mg/kg, i.p.) + ES	Nil	38.00 ± 8.11[c] (60.54)	1.10 ± 0.41[a] (78.00)

Results are expressed in Mean ± S.E.M., (n = 6), [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; compared with MES control. Values in parentheses indicate percentage change in time taken for different phases of convulsions. ES = Electric Shock.

Table 2: Effect of MEIR on INH and PTZ induced seizures in mice

Group	Treatment		Onset of clonic action (s)		Onset of tonic action (s)		No. of animals recovered/ used		Time to death in min (No. of deaths)		Protection against mortality (%)	
	INH	PTZ	INH	PTZ	INH	PTZ	INH	PTZ	INH	PTZ	INH	PTZ
1	Vehicle control (1 ml/100 g)	Vehicle control (1 ml/100 g)	Nil	Nil	Nil	Nil	6/6	6/6	Nil	Nil	Nil	100
2	INH Control (1 ml/100 g) + INH	PTZ control (1 ml/100 g) + PTZ	1260 ± 34.64	160.0 ± 20.66	1840 ± 71.69	230.00 ± 42.19	0/6	0/6	52.76 ± 1.17	5.84 ± 0.72	0.00	0
3	MEIR (200 mg/kg) + INH	MEIR (200 mg/kg) + PTZ	1990 ± 63.19[a] (57.94)	383.3 ± 65[b] (139.57)	2150 ± 175.60 (16.85)	470 ± 64.65[a] (104.35)	3/6	2/6	98.00 ± 6.92 (3)	8.00 ± 1.78 (4)	50.00	33.33
4	MEIR (400 mg/kg) + INH	MEIR (400 mg/kg) + PTZ	2773 ± 46.09[c] (120.08)	500.00 ± 34.16[c] (212.50)	2701 ± 56.12[b] (46.80)	545.00 ± 66.12[c] (136.96)	5/6	6/6	125.0 (1)	Nil	83.33	100
5	Diazepam (5mg/kg, i.p.) + INH	Diazepam (5mg/kg, i.p.) + PTZ	3210 ± 287.10[c] (154.77)	Nil	3705 ± 158.30[c] (101.36)	Nil	6/6	6/6	Nil	Nil	100.00	100

Results are expressed in Mean ± S.E.M., (n = 6), [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; compared with their respective INH and PTZ control group. Values in parentheses indicate percentage change in time taken for different phases of convulsions.

($P < 0.05$ and $P < 0.001$) reduced (69.88 and 79.38%) the intensity at 20 and 30 min intervals, respectively. Higher dose of MEIR exhibited significant ($P < 0.01$ and 0.001) reductions (80.13 and 90.00%) at 20 and 30 min intervals, respectively. Haloperidol did not exhibit any climbing behavior indicating 100% protection and higher activity (Table 4).

3.2.2 Effect of MEIR on Apomorphine Induced Stereotyped Behavior in Rats

Administration of apomorphine exhibited a stereotyped behavior characterized by intermittent or constant sniffing, rearing, licking, gnawing or biting in a limited area of the cage. Pretreatment of rats with MEIR (200 mg/kg) produced significant reduction (8.50 to 58.50%, respectively) in stereotyped score at 70 ($P < 0.001$) to 90 ($P < 0.05$) min intervals. Higher dose of MEIR exhibited the gradual increase in reduction (15.48 to 58.50%) at the period of 60 ($P < 0.01$) to 90 ($P < 0.05$) min intervals. Haloperidol showed significant reductions ($P < 0.001$; 56.66 to 100%) at 20 to 90 min intervals (Table 5).

Table 3: Effect of MEIR on brain GABA content in INH and PTZ induced seizures in mice

Group	GABA estimation (ng/mg of wet brain tissue)	
	INH	PTZ
1	11.80 ± 1.15	12.10 ± 1.10
2	9.96 ± 0.18	9.76 ± 0.25
3	10.05 ± 0.35 (0.9)	11.10 ± 0.23[a] (13.73)
4	11.61 ± 0.27[b] (16.57)	11.34 ± 0.28[b] (16.19)
5	12.04 ± 0.14[c] (20.89)	11.79 ± 0.14[c] (20.80)

Results are expressed in Mean ± S.E.M., (n = 6). [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ compared with their respective INH and PTZ control group. Values in parentheses indicate percentage increase in brain GABA content.

Table 4: Effect of MEIR on apomorphine induced climbing behavior in mice

Group	Treatment	climbing behavior scores at min		
		10	20	30
1	Vehicle control (1 ml/100 g)	0 ± 0	0 ± 0	0 ± 0
2	Apomorphine control (1 ml/100 g) + apomorphine	1.50 ± 0.22	1.66 ± 0.21	1.60 ± 0.43
3	MEIR (200 mg/kg) + Apomorphine	0.66 ± 0.42 (56.00)	0.50 ± 0.34[b] (69.88)	0.33 ± 0.21[c] (79.38)
4	MEIR (400 mg/kg) + Apomorphine	0.50 ± 0.71 (66.67)	0.33 ± 0.33[b] (80.13)	0.16 ± 0.16[c] (90.00)
5	Haloperidol (0.1 mg/kg, i.p.) + Apomorphine	0 ± 0[c] (100)	0 ± 0[c] (100)	0 ± 0[c] (100)

Values are expressed in mean ± S.E.M., (n = 6), [b] $P < 0.01$; [c] $P < 0.001$ compared with vehicle control group. Parenthesis indicates the percentage reduction compared to apomorphine control.

Table 5: Effect of MEIR on apomorphine induced stereotyped behavior in rats

Group	Treatment	Stereotyped scores at min								
		10	20	30	40	50	60	70	80	90
1	Vehicle control (1 ml/100 g)	0.33 ± 0.21	0.50 ± 0.37	0.16 ± 0.16	0.50 ± 0.22	0.15 ± 0.15	0.41 ± 0.27	0.66 ± 0.21	0.33 ± 0.47	0.33 ± 0.21
2	Apomorphine control (1 ml/100 g) + Apomorphine	3.66 ± 0.65	3.83 ± 0.16	4.00 ± 0.25	4.33 ± 0.21	4.16 ± 0.16	4.50 ± 0.73	4.66 ± 0.21	4.83 ± 0.16	4.00 ± 0.81
3	MEIR (200 mg/kg) + Apomorphine	3.16 ± 0.16 (13.67)	3.28 ± 0.21 (14.37)	3.66 ± 0.33 (8.50)	3.92 ± 0.16 (9.47)	3.66 ± 0.21 (12.02)	3.66 ± 0.74 (18.67)	2.66 ± 0.21[c] (42.92)	2.33 ± 0.21[c] (51.76)	1.66 ± 0.33[a] (58.50)
4	MEIR (400 mg/kg) + Apomorphine	3.50 ± 0.33 (4.38)	3.66 ± 0.42 (4.44)	3.16 ± 0.40 (21.00)	3.66 ± 0.49 (15.48)	3.17 ± 0.16 (23.80)	2.83 ± 0.40[b] (37.12)	2.50 ± 0.22[c] (46.36)	2.17 ± 0.33[c] (55.08)	1.66 ± 0.49[a] (58.50)
5	Haloperidol (1 mg/kg, i.p.) + Apomorphine	2.80 ± 0.50 (23.50)	1.66 ± 0.21[c] (56.66)	1.00 ± 0.37[c] (70.00)	0.16 ± 0.16[c] (96.31)	0 ± 0[c] (100)	0 ± 0[c] (100)	0 ± 0[c] (100)	0 ± 0[c] (100)	0 ± 0[c] (100)

Values are expressed in mean ± S.E.M., (n = 6), [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ when compared with apomorphine control group. Parenthesis indicates the percentage reduction compared to apomorphine control.

3.2.3 Estimation of Brain Dopamine, Noradrenaline and Serotonin Content

Apomorphine alone induced a significant increase in brain dopamine ($P < 0.01$; 27.23 and 15.87%), noradrenaline ($P < 0.01$; 22.50 and 23.33%) and serotonin ($P < 0.05$ and $P < 0.001$; 30.60 and 24.76%, respectively) levels in mice and rats, respectively when compared to vehicle control. MEIR (200 and 400 mg/kg) and haloperidol pretreatment in mice exhibited significant reduction in brain dopamine, noradrenaline ($P < 0.01$ and $P < 0.001$, respectively) and serotonin ($P < 0.05$ and $P < 0.01$, respectively) levels when compared to apomorphine control mice. Further, in rat model, MEIR at both the doses showed statistically significant decrease in brain dopamine, noradrenaline ($P < 0.05$ and $P < 0.01$, respectively) and serotonin ($P < 0.01$ and $P < 0.001$, respectively) content and also by haloperidol when compared to apomorphine control rats (Table 6).

4. Discussion

In the present study, antiepileptic effect of *Ipomoea reniformis* was evaluated by using MES, INH and PTZ induced seizure models. MES is a novel standard procedure that evaluates the antiepileptic activity of testing materials by its ability to protect against HLTE. The seizure pattern in MES for all laboratory animals and man are similar except for time scale [18]. MEIR exhibited a significant antiepileptic action in MES induced seizures, dose dependently and showed maximum protection at 400 mg/kg. Phenytoin suppresses HLTE by limiting the repetitive firing of action potentials and this effect is mediated by a slowing of the voltage activated Na^+ ion channels. Protection against HLTE in MES predicts antiepileptic activity of drugs that prevent the spread of the epileptic seizure from an epileptic focus during seizure activity [19]. Since, MEIR showed antiepileptic activity in the MES, it may act through any of the aforementioned mechanisms.

The convulsant action of INH and PTZ involves disruption of GABAergic neurotransmission in the CNS [13]. Decreased levels of GABA are believed to lead to seizures. INH inhibits glutamic acid decarboxylase, an enzyme that catalyzes the synthesis of GABA from glutamic acid [20]. PTZ, the most popular chemoconvulsant used for evaluation of AEDs, is a selective blocker of the Cl^- channel coupled to the GABA receptor complex [21]. Several AEDs in current clinical use facilitate GABA neurotransmission by different mechanisms: benzodiazepines such as diazepam modulate the action of GABA by enhancing Cl^- currents in channels linked to different receptor sites [22]. MEIR exhibited antiepileptic activity against INH and PTZ induced seizure in mice. Highest antiepileptic activity was observed at higher dose (400 mg/kg) in both the models with a significant increase in mean time of

Table 6: Effect of MEIR on brain dopamine, noradrenaline and serotonin content in apomorphine induced climbing and stereotyped behavior in mice and rats, respectively

Group	Neurotransmitter estimation (pg/mg of wet brain tissue)					
	Apomorphine induced climbing behavior in mice			Apomorphine induced stereotyped behavior in mice		
	Dopamine	Noradrenaline	Serotonin	Dopamine	Noradrenaline	Serotonin
1	2652.00 ± 41.30	710.80 ± 31.25	646.20 ± 89.54	2364.00 ± 48.34	587.70 ± 28.44	687.00 ± 33.25
2	3374.00 ± 75.57	870.70 ± 22.9	843.90 ± 17.6	2739.00 ± 75.22	724.80 ± 29.43	857.10 ± 60.64
3	2666.00 ± 158.30[b] (20.99)	727.60 ± 06.50[b] (16.44)	459.81 ± 104.10[a] (45.52)	2106.00 ± 186.30[a] (23.12)	589.70 ± 28.86[a] (18.64)	620.30 ± 37.93[b] (27.63)
4	2478.00 ± 120.70[c] (26.56)	700.40 ± 29.47[b] (19.56)	435.90 ± 86.81[b] (43.38)	2015.00 ± 170.10[b] (26.44)	568.60 ± 23.62[b] (21.56)	612.10 ± 34.18[c] (28.59)
5	2482.00 ± 107.90[c] (26.44)	678.20 ± 32.18[c] (22.18)	432.90 ± 64.01[b] (48.72)	2033.00 ± 146.40[b] (25.78)	574.40 ± 30.54[b] (20.76)	597.60 ± 40.14[c] (30.28)

Results are expressed in Mean ± S.E.M., (n = 6). [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ compared with their respective apomorphine control. Parenthesis indicates the percentage reduction in brain neurotransmitter compared to their respective apomorphine control.

latency in onset of clonic action and tonic action which are comparable to diazepam.

To further support the antiepileptic activity of MEIR, brain GABA estimation was done. An increase in brain GABA content was observed in mice pretreated with MEIR when compared with the INH and PTZ controls, thus suggesting the protective effect against epilepsy probably through elevation of brain GABA content. Further, only higher dose of MEIR showed a significant elevation in INH model. However, in PTZ model MEIR showed dose dependent elevation in brain GABA content. MEIR failed to show significant increase in brain GABA content in MES model. This may be due to the fact that GABAergic system is not disturbed in MES model unlike other tested models [23]. It is also well known that anxiolytic drugs inhibit clonic and tonic seizures elicited in mice [14]. Sinapic acid, a major chemical constituent of IR is reported to possess anxiolytic like effects, thus justifying the observed antiepileptic effect in the present study [23]. IR also contains some derivatives of phenylpropanoids, such as p-coumaric acid, caffeic acid, and ferulic acid, have also been reported to have good antioxidative properties [24]. Sinapic acid, a cinnamic acid derivative, exhibited neurobehavioral protective characteristics in Alzheimer's disease including attenuation of kainic acid-induced hippocampal neuronal damage in mice [25–26]. Hence, it is reasonable to assume that the antiepileptic action of IR may also be because of above neurodefensive parameters. The other major active constituents of IR such as p-coumaric acid, caffeic acid, and ferulic acid may be responsible for the tested activity alone or in combination, which needs to be investigated.

The study results also indicated antipsychotic activity of MEIR against apomorphine induced climbing and stereotype behavior. Apomorphine induces stimulation of central mesolimbic and striatal dopaminergic, noradrenergic, serotonergic pathways and inhibition of GABA system [14, 27, 28]. Apomorphine injection to rodents typically results in an increased locomotion and stereotyped behavior (rearing, sniffing, licking, biting and gnawing), respectively which was also observed in the present study [14]. Dopamine D_2, noradrenergic and serotonin receptor blockade as well as GABA mimetic actions are suggested in the management of psychosis [14, 27–29]. The ability of a drug to antagonize apomorphine induced climbing and stereotyped behaviors in the rodents has been correlated with neuroleptic activity and is suggestive of D_2 receptor blockade [14]. The ability of MEIR to antagonize apomorphine induced stereotyped behavior supports the hypothesis of central activity which might be related to anti-dopaminergic, noradrenergic receptor blockade, anti-serotonergic and GABA mimetic actions. MEIR and haloperidol (dopamine D_2 receptor antagonist) significantly minimized apomorphine induced climbing and stereotyped behavior in mice and rats, respectively. The observed blockades were better at higher dose of MEIR.

The antipsychotic activity of MEIR was further supported by brain neurotransmitters estimation. Elevated levels of brain neurotransmitters are indicators of the severity of apomorphine induced climbing and stereotyped behavior [13]. In the present study, a significant increase in the content of brain neurotransmitters was observed after peripheral administration of apomorphine to rodents. Apomorphine acts on central dopamine D_2 and noradrenergic neurotransmitters, involved in the motor activity. Acting on these pathways, apomorphine causes increase in the level of brain dopamine and noradrenaline [30]. It also selectively increases serotonin concentrations in the dorsal raphe and striatum [27]. Some noradrenergic neurone blocking agents like reserpine blocks the granular reuptake of noradrenaline and 5-HT by the vesicular amine transporter and inhibits stereotyped behavior [30]. Furthermore, serotonin ($5\text{-}HT_{2A}$) receptor antagonists such as clozapine, olanzapine and amperozide are used as antipsychotics [14].

MEIR including sinapic acid was reported to possess radical scavenging and neuroprotective activities and traditionally in neurological disorders [3, 11, 25]. MEIR pretreatment significantly reversed the increased brain neurotransmitters level. A correlation in the results was observed with better reduction by the higher dose. The observed biochemical changes supported the antipsychotic effect of MEIR, which could be due to its antidopaminergic, noradrenergic blocking action and serotonin uptake blockade.

It has been also demonstrated that the psychotic behavior in rodents induced by apomorphine can be antagonized by GABA agonists. The GABAergic and dopaminergic systems influence one another to enhance their antagonistic activity [28]. GABA agonists act

by inhibiting feedback activation of the nigrostriatal dopamine neurons by stimulation of GABA receptors, action similar to that of neuroleptics. Since, the anxiolytic and antiepileptic effects of MEIR were suggested to be mediated through effect on the GABA mimetic action [23], similar mechanism of antipsychotic action cannot be ruled out. Further, natural products such as phenylpropanoid derivatives, considered to possess better safety and efficacy report, facilitate the inhibitory activity of the GABAergic system probably through a competitive agonist action in the benzodiazepine site of the GABA receptors [11]. Moreover, decrease in locomotion is due to decrease in dopaminergic transmission and thus increase in GABAergic transmission [28]. Hence, it is rational to presume that the antipsychotic action of MEIR may also be by modifying the GABAergic system.

In conclusion, methanolic extract of *Ipomoea reniformis* antagonized MES, INH and PTZ induced seizures and also increased brain GABA levels decreased by INH and PTZ in mice. MEIR also exhibited antipsychotic activity by inhibiting the apomorphine induced climbing and stereotyped behavior in rodents along with normalization of elevated brain neurotransmitters such as dopamine, noradrenaline and serotonin. Further research is warranted to determine the exact mode of its antiepileptic and antipsychotic activities.

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