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Evaluation of anticonvulsant activity of hydroalcoholic extract of *Mussaenda philippica* on animals

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ABSTRACT

Objective: To evaluate the anticonvulsant activity of hydroalcoholic extracts of leaves and sepals of *Mussaenda philippica (M. Philippica)* and its fractions (methanol, dioxin and aqueous) against pentylene tetrazole (PTZ), maximal electroshock (MES), Strychnine (STR), Picrotoxin induced convulsions at different dose levels. **Methods:** The anticonvulsant effect of extracts was studied by the MES, PTZ, STR and Picrotoxin-induced seizure. **Results:** The extract at 100 and 200 mg/kg, produced a significant ($P<0.01$) dose dependent increase in onset of convulsion compared to the control in MES, PTZ, strychnine and picrotoxin-induced seizures. **Conclusions:** The data obtained indicates that Hydroalcoholic extracts of *M. philippica* leaves and sepals may help to control grandmal and petitmal epilepsy.

1. Introduction

Epilepsy, which has been described as a chronic disorder of the central nervous system of various etiologies, characterized by recurrent seizures due to excessive discharge of cerebral neurons is a major medical and social problem[1]. The incidence of the disease in developing countries is higher than that in developed countries and is reported to be 190 per one lakh people. There are many classes of anticonvulsants that are of clinical usefulness with good prognosis for controlling seizures in most patients. The currently available anticonvulsant drugs suffer from drawbacks like neurotoxicity, teratogenic and other dose related side effects[2]. In order to reduce the risk of side effects, overcome resistance and achieve safety and effectiveness, the use of anticonvulsant drugs combination is a fundamental strategy in assessment of epilepsy[3]. In the last years a number of herbal products have been demonstrated to have promising anticonvulsant activity[4]. These herbal products are candidates to be included in combination therapy of epilepsy due to their considerable safety and lower side effects. *Mussaenda philippica* (*M. philippica*) (Aurorae) of family Rubiaceae is distributed in throughout India, South East Asia. The flowers are small, tubelike, expanded into five, ovate lobes, yellowish orange in colour. In traditionally the plant is used as dysentery, jaundice, emollient and snake bites. Thus, it is necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in items of drug related toxicity. The aim of treating an epileptic is not only to abolish the occurrence of seizures. Hence the objective of this study was to investigate the effect of different extracts of *M. philippica* on the anticonvulsant activity.

2. Materials and methods

2.1. Plant material

Fresh leaves and sepals of the *M. philippica* (Aurorae) collected from commercial nurseries of Bhubaneswar,

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Khurda district, Odisha, during the month of July and August 2011 in the early morning. The plant was identified, confirmed and authenticated by taxonomist Prof. Sushil Kumar Mallick in the Department of Botany, S.V.M Autonomous College, Bhubaneswar.

2.2. Animals

Swiss albino mice (20-25 g) and Wistar albino rats (150-180 g) of either sex were used in the study. Animals were procured from SPS, SOAU, BBSR (RegdNo. 1171/C/08/CPCSEA). The animals were acclimatized in the laboratory conditions for 7 d before experimentation. The animals had free access to food and water *ad libitum* and maintained under 12 h day and night cycles. All experiments were carried out during day time from 9.00 am to 14.00 pm.

2.3. Preparation of extracts and fractions

The air dried plant materials was powdered and extracted successively with the different solvents. First the powered was extracted with petroleum ether in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place. The defatted marc of the drug was subjected to hydroalcoholic extraction for a period of 6-7 d and filtered. All the filtrates were subjected to evaporation by using Rotary Evaporator to dryness, till the thick paste remained in the evaporator. However, it was kept in a refrigerator below 4 ℃ till the experimental study. The extract obtained was fractionated by using ethyl-acetate, 1, 4-dioxane, methanol & ethanol in the order of increasing polarity. Extract was subjected to qualitative chemical investigation of phytoconstituents such as Alkaloids, Flavonoids, Ttannins, Carbohydrates, Proteins, Vitamins, Coumarin etc by using different methods[5,6].

2.4. Toxicity tests

Nine groups with each group containing five mice and five rats were used for each of the three extracts. Groups 1-8 were injected intraperitoneally with 25, 50, 100, 200, 400, 800, 1 600, and 2 000 mg/kg, while the ninth group received 10 mL/kg normal saline. Mortality rate was observed and recorded for 24 h period[7].

2.5. Anticonvulsant activity

The anticonvulsant effect of extracts was studied by the Maximal electroshock (MES), Picrotoxin, Pentylenetetrazole (PTZ) and Strychnine-induced seizures.

2.6. Maximal electroshock (MES) induced convulsion

Protection against electroshock induced seizures in mice or rats is used as an indication for compounds which may prove effective in 'grand mal epilepsy'. Electric stimuli evoke tonic hind limb extensions, which are suppressed by anti-epileptic drugs. The animals were given maximal electroshocks of 150 mA for 0.2 s to the cornea by using electroconvulsometer[8,9]. Fifty healthy and convulsion free Swiss albino rats (120-150 g) were randomly divided into 10 groups (n=5). The plant aqueous extract and it's fractions at the dose of 100 and 200 mg/kg, standard drug phenytoin and vehicle control were administered 30 min prior to MES. The vehicle treated animals exhibit the characteristic maximal electroshock convulsions which can be divided into 5 phases- (a) tonic flexion, (b) tonic extensor, (c) clonic convulsions, (d) stupor and (e) recovery or death. The animals are observed for 2 min after the shock. Disappearance of the hind limb extensor tonic convulsions is taken as the criterion of protection[10].

2.7. Pentylenetetrazole (PTZ)-induced seizure

This test is considered as indicative of anticonvulsant activity of drugs against 'petit mal seizures'. PTZ produces generalized asynchronized clonic movements which are superceded by tonic convulsion characterized by flexion of limbs followed by extension. The excitatory effects of PTZ may be due to decrease in neuronal recovery time in the postsynaptic pathway of the spinal cord[11,12]. The plant aqueous extract and it's fractions at the dose of 100 and 200 mg/kg, standard drug phenobarbitone sodium and vehicle control were administered 30 min prior to PTZ (80 mg/kg). The onset and number of death after showing tonic hindlimb extension were also recorded. Mice that did not convulse 30 min after pentylenetetrazole administration were considered protected[13].

2.8. Picrotoxin-induced seizure

Picrotoxin is a GABA-antagonist and it modifies the function of the Cl^- ion channel of the GABA receptor complex. Activation of GABA receptor effects inhibition of the post-synaptic cell by increasing the flow of Cl^- ions into the cells, which tends to hyperpolarize the neurons. The plant aqueous extract (AEMP) and it's fractions at the dose of 100 and 200 mg/kg, standard drug phenobarbitone sodium and vehicle control were administered 30 min prior to Picrotoxin (6 mg/kg). Time for onset of action (clonic and tonic seizures) and death were the two parameters used to evaluate antiepileptic activity of the drugs. Delay in onset of action and death of animals was considered as anticonvulsant property[14,15].

2.9. Strychnine (STR) induced seizure

Strychnine is a powerful convulsant. The convulsant

action of strychnine is due to interference with post-synaptic inhibition that is mediated by glycine. Glycine is an important inhibitory transmitter to motorneurons and interneurons in the spinal cord. The test drug at the doses of 100 and 200 mg/kg, standard drug phenobarbitone sodium and vehicle control were administered 30 min prior to Strychnine (2.5 mg/kg). Sixty healthy and convulsion free Swiss albino mice (20-25 g) were randomly divided into 10 groups (n=6). Then the treatment was started in similar manner as described above. Onset to forelimb clonic and tonic seizures was recorded. Mice that did not convulse 30 min after strychnine administration were considered protected[16,17].

2.10. Statistical analysis

Results are presented as mean±S.E.M. Statistical significance between the groups was analyzed by means of an analysis of variance followed by Dunnett's multiple comparison tests. P values less than 0.05 were considered significant.

3. Results

Many plants have been used for the treatment of various CNS disorders in Indian system of medicine and in other ancient systems of the world, out of these only a few have been evaluated as per modern system of medicine. From many such plants only extracts have been prepared and their usefulness evaluated in experimental animals.

3.1. Acute toxicity study

An acute toxicity study of ALETD was determined in mice, as per OECD guidelines No. 423. The extract was administered orally to different groups of mice at different dose levels and extract produced no mortality up to 2 000 mg/kg. So 1/5th, 1/10th, and 1/20th of LD_{50} doses were selected for the present study.

3.2. MES induced convulsion

The result of MES induced convulsion depicted in Table 1. The duration of extensor phase was recorded in control and drug treated animals before the electroshock. A significant (P<0.001) reduction in the extensor phase and a significant reduction in stupor phase was observed with Phenytoin when compared with control. In the test, the MF, AF with AEMP at the dose of 100 mg/kg significantly (P<0.05) failure in extensor phase, where as the MF, AF with AEMP at the high dose of 200 mg/kg more significantly (P<0.01) failure in extensor phase when compared with the solvent control group and the result is comparable to that produced by phenytoin. In case of stupor phase, similarly the MF, AF with AEMP at the dose of 100 mg/kg 99% significantly (P<0.01) decrease, where as the MF, AF with AEMP at the high dose of 200 mg/kg more significantly (P<0.001) decrease when compared to the solvent control group. There was no death observed for all the fractions and the test drug.

Table 2.

Effect of fractions and extracts of *M. philippica* on Pentylenetetrazole (PTZ)-induced seizure in mice.

Group	Treatment	Dose (mg/kg)	Onset of convulsion in minute	Duration of convulsion in minute
Gr I	NS + Tween	10 mL/kg	1.79±0.05	2.01±0.07
Gr II	Phenobarbitone	40	5.54±0.19[c]	10.43±0.30[c]
Gr III	MF	100	2.10±0.09	2.83±0.24[a]
Gr IV		200	2.68±0.16[a]	3.35±0.18[a]
Gr V	DF	100	2.08±0.10	1.92±0.08
Gr VI		200	2.12±0.25	2.09±0.09
Gr VII	AF	100	2.04±0.12	3.17±0.13[a]
Gr VIII		200	2.89±0.15[a]	3.52±0.17[a]
Gr IX	AEMP	100	1.88±0.21	3.18±0.15[a]
Gr X		200	2.91±0.26[a]	3.47±0.17[a]

Values are expressed in MEAN±S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *P<0.05, **P<0.01) (t-value denotes statistical significance at [a]P<0.05, [b]P<0.01 and [c]P<0.001 respectively, in comparison to group-I).

3.3. PTZ induced convulsion

The result of PTZ induced convulsion depicted in Table 2, the test extract and all the fractions with standard drug

Table 1.

Effect of fractions and extracts of *M. philippica* on MES induced convulsion in rats.

Group	Treatment	Dose (mg/kg)	Time in various phases of convulsion (s)				
			Flexion	Extensor	Clonus	Stupor	R/D
I	NS+Tween	10 mL/kg	4.53±0.26	10.95±0.31	4.55±0.32	181.00±13.75	R
II	Phenytoin	25	3.81±0.20[a]	2.80±0.33[c]	4.81±0.30	79.31±3.88[c]	R
III	MF	100	4.26±0.17	9.80±0.17[a]	4.31±0.16	154.33±5.84[a]	R
IV		200	3.76±0.18[a]	8.47±0.21[b]	3.89±0.22[a]	114.26±5.75[c]	R
V	DF	100	4.07±0.07	10.25±0.21	4.24±0.15	151.83±6.22[a]	R
VI		200	4.33±0.18	10.14±0.21	4.32±0.18	152.66±6.89[a]	R
VII	AF	100	4.22±0.16	9.92±0.16[a]	4.28±0.21	153.65±4.62[a]	R
VIII		200	3.48±0.21[b]	8.24±0.21[b]	3.78±0.27[a]	108.96±3.43[c]	R
IX	AEMP	100	3.89±0.24[a]	9.06±0.12[a]	4.30±0.18	150.16±4.60[a]	R
X		200	3.51±0.18[b]	8.12±0.15[b]	3.91±0.29	111.08±5.18[c]	R

Values are expressed in MEAN±S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *P<0.05, **P<0.01) (t-value denotes statistical significance at [a]P<0.05, [b]P<0.01 and [c]P<0.001 respectively, in comparison to group-I).

delayed the onset of time and increased the duration of convulsion except DF at the dose of 100 mg/kg. The MF, AF with AEMP at the dose of 200 mg/kg significantly (P<0.05) delayed the onset of convulsion and the MF, AF with AEMP at the both dose of 100 and 200 mg/kg 95% significantly (P<0.05) increased the duration of convulsion except DF against PTZ induced convulsion when compared to the solvent control group in (1.79±0.05) (onset of convulsion) and (2.01±0.07) (duration of convulsion), effect also comparable with those result produced by standard drug.

3.4. Picrotoxin-induced seizure

The result of Picrotoxin induced convulsion depicted in Table 3, showed the fractions and the extract delayed the onset of time and the duration of convulsion except DF in comparison to solvent control, the effect also comparable with phenobarbitone. The MF, AF with AEMP at the dose level of 100 mg/kg significantly (P<0.05) delayed the onset of time whereas the high dose of 200 mg/kg more significantly (P<0.01) delayed the onset of time against Picrotoxin induced convulsion respectively, when compared with control group. Similarly in case of the time of death, the MF, AF with AEMP at the dose level of 100 mg/kg significantly (P<0.05) delayed and at high dose of 200 mg/kg more significantly delayed except DF. The fractions and the extract dose dependently and significantly delayed the onset of time and the time of death except DF.

Table 3.

Effect of fractions and extract of *M. philippica* on Picrotoxin-induced seizure in rats.

Group	Treatment	Dose (mg/kg)	Onset of convulsion (sec)	Time of death(sec)
Gr I	NS + Tween	10 mL/kg	130.49±1.77	1 256.50±40.93
Gr II	Phenobarbitone	10	1 794.90±7.78^c	Recovery
Gr III	MF	100	156.62±6.34^a	1 353.03±40.61^a
Gr IV		200	214.98±8.81^b	1 496.28±67.31^b
Gr V	DF	100	134.02±3.09	1 081.59±52.14
Gr VI		200	133.83±3.56	1 147.64±50.16
Gr VII	AF	100	163.69±3.76^a	1 357.79±36.51^a
Gr VIII		200	222.23±12.73^b	1 497.87±48.87^b
Gr IX	AEMP	100	156.97±5.51^a	1 337.18±51.26^a
Gr X		200	208.92±6.12^b	1 602.13±53.34^b

Values are expressed in MEAN±S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *P<0.05, **P<0.01) (t-value denotes statistical significance at $^a P$<0.05, $^b P$<0.01 and $^c P$<0.001 respectively, in comparison to group-I).

3.5. Strychnine induced convulsion

The result of Strychnine induced convulsion depicted in Table 4, showed the fractions and the extract delayed the onset of convulsion and the duration of convulsion except DF. Phenobarbitone in a dose of 40 mg/kg; i.p, 99.99% significantly delayed the onset of convulsion and duration of convulsion respectively, when compared with control group. The MF, AF with AEMP at the dose level of 100 mg/kg significantly (P<0.01) delayed the onset of time and while at the high dose of 200 mg/kg more significantly (P<0.001) delayed the onset of time against strychnine induced convulsion. Similarly in case of the duration of convulsion, the MF, AF with AEMP at the dose level of 100 mg/kg significantly (P<0.05) delayed and at high dose of 200 mg/kg more significantly delayed. The DF at the dose level of 200 mg/kg significantly (P<0.05) delayed the onset of time with duration of convulsion. The fractions and the extract dose ependently and significantly delayed the onset of time and the duration of convulsion except DF.

Table 4.

Effect of fractions and extracts of *M. philippica* on Strychnine induced seizure in mice.

Group	Treatment	Dose (mg/kg)	Onset of convulsion in minute	Duration of convulsion in minute
I	NS + Tween	10 mL/kg	3.28±0.14	1.78±0.13
II	Phenobarbitone	40	9.23±0.16^c	7.90±0.18^c
III	MF	100	5.66±0.18^b	2.95±0.09^a
IV		200	7.06±0.16^c	4.45±0.18^b
V	DF	100	3.13±0.11	2.32±0.14
VI		200	4.01±0.13^a	3.01±0.16^a
VII	AF	100	5.96±0.10^b	3.10±0.08^a
VIII		200	7.21±0.14^c	4.70±0.17^b
IX	AEMP	100	4.40±0.12^b	3.09±0.13^a
X		200	6.96±0.14^c	4.56±0.16^b

Values are expressed in MEAN±S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *P<0.05, **P<0.01) (t-value denotes statistical significance at $^a P$<0.05, $^b P$<0.01 and $^c P$<0.001 respectively, in comparison to group-I).

Preliminary phytochemical analysis performed showed that Carbohydrates were not found to be present in the aqueous extract AEMP as well as in all the fractions, while flavonoids, glycosides, tannins, phenols, reducing sugar, terpenoids and saponins were detected in the aqueous extract and in all the fractions. In addition to DF, AF with AEMP, alkaloids also present in the DF, AF with AEMP while the MF was not contained. In MES induced convulsion, the MF, AF with AEMP at the dose of 100 mg/kg significantly (P<0.05) failure in extensor phase, where as the MF, AF with AEMP at the high dose of 200 mg/kg more significantly (P<0.01) failure in extensor phase when compared with the solvent control group and the result is comparable to that produced by phenytoin. The effectiveness of the extract and the MF, AF of *M. philippica* in the experimental convulsion paradigm used probably suggests that the herb could be used in both the petitmal and grand mal types of epilepsy. In study of Picrotoxin-induced convulsion, the MF, AF and the extract AEMP significantly (P<0.05 to P<0.01) and dose dependent manner increase in onset of convulsion while a significant (P<0.05 to P<0.01) and dose dependant delay in the time of death. Similarly in study of Strychnine induced convulsion, the MF, AF and the extract AEMP significantly (P<0.05 to P<0.01) and dose dependently manner increase in onset of convulsion while a significant (P<0.05 to P<0.01) and dose dependant increase in the time of death and in the PTZ induced convulsion, the MF, AF and the extract AEMP at the dose level of 200 mg/kg significantly (P<0.05) increased the onset of convulsion while a significant (P<0.05) increase in the duration of convulsion. Picrotoxin (PCT), Strychnine (STC) and Pentylenetetrazole (PTZ) are convulsants drugs used to induce convulsions, while ability of an agent to inhibit

convulsion in comparison with the untreated mice. The test fractions (MF and AF) and the extract AEMP appears to be relatively more effective in Picrotoxin- and Strychnine-induced convulsions. Picrotoxin (PCT) and strychnine (STC) produce their convulsions by blocking gamma-aminobutyric acid (GABA) and glycine receptors respectively, while pentyltetrazole (PTZ) destabilizes nervous cell membrane to produce convulsion[18]. GABA is the predominant inhibitory neurotransmitter in the mammalian CNS, and is widely implicated in epilepsy, mediating inhibition of neuronal responsiveness (excitability) and activity by increasing the chloride ion conductance through opening of the chloride-ion channel[19]. The findings of the present study, therefore, tend to suggest that the *M. philippica* extract & fractions might have inhibited and/or attenuated PTZ-induced seizures by enhancing, or in some ways interfering with GABAergic neurotransmission & also protection of mice against Strychnine induced seizure. Strychnine blocked glycine mediated neuronal activities in the spinal cord thereby inducing a tonic type of convulsion in animal. The fact that the MF, AF with the extract AEMP was capable of offering protection against different types of seizure models indicate that the fractions (MF & DF) and the extract AEMP must be acting through different mechanisms to elicit its anticonvulsant effects.

4. Conclusion

It was concluded that the hydroalcoholic extract of *M. philippica* in small to moderate doses (100-200 mg/kg) enhances the anticonvulsant effects in different animal models. This effect may be attributed to different mechanisms including the brain glutamate and increasing brain GABA levels, inhibition of free radical generation, scavengering of reactive oxygen species and reactivation of antioxidant defenses. Results of this study direct the light towards the possible use of conventional anticonvulsant and *M. philippica* exatracts combination in treatment of epilepsy in human.

Conflict of interest statement

The authors report no conflict of interest.

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