Current Research in Pharmaceutical Sciences
Available online at www.crpsonline.com

ISSN 2250 – 2688

Received: 08/11/2013
Revised: 15/12/2013
Accepted: 22/12/2013

Veerendra Sharma, Pooja Sinoriya
Department of Pharmaceutical Sciences, LNAP,Gwalior

S C Mehta
G.R.Medical College, Gwalior, Madhya Pradesh

Correspondence:
Veerendra Sharma
LANP, Gwalior-474011

Anticonvulsant and CNS Depressant Activity of Methanolic Extracts of Whole Plant of *Sida acuta* and *Sida rhombifolia* in Mice

Veerendra Sharma, Pooja Sinoriya and S C Mehta

ABSTRACT

To investigate the anticonvulsant and CNS depressant activity of methanolic extracts of whole plant of *Sida acuta* and *Sida rhombifolia* in mice. The methanolic extract of whole plant of *Sida acuta* (MESA) and *Sida rhombifolia* (MESR) (100, 200 and 400 mg/kg, p.o.) was studied for its anticonvulsant effect on maximal electroshock (MES) induced seizures and Pentylenetetrazole (PTZ) induced seizures and CNS depressant activity at the same dose level using traction test, rota rod and locomoter activity in mice. MESA and MESR at dose 100, 200 and 400 mg/kg significantly reduced the duration of the seizures as compared to control group. In CNS depressant activity and the mean time duration on rotating rod was significantly decreased (P<0.0001) and decline in motor co-ordination

.**Keywords:** *Sida acuta*, *Sida rhombifolia*, MES, Pentylenetetrazole, CNS activity

1. INTRODUCTION

Epilepsy is a collective term for a group of chronic seizure disorder having in common sudden and transient episodes (seizure) of loss or disturbance of consciousness, usually but not always with a characteristic body movements (convulsions) and sometimes with autonomic hyperactivity.[1,2] The risk of having epilepsy at some point in the average life span of any individual varies between 2% and 5%. In some countries, particularly, phytotherapy in traditional medicine still plays an important role in the management of diseases, mainly among populations with very low income.[3]

The genus *Sida* is used as 'Bala' in Ayurveda. *Sida* (Bala) is of great importance in the Indian traditional system of medicine and this is perhaps the most widely used raw drug in the production of diffeent Ayurvedic formulations.[4] *S. acuta* is a profusely, branching annual, herbaceous weed, cosmopolitan in distribution. The bark is smooth, greenish, the root is thin, long, cylindrical and very rough; leaves are lanceolate, nearly glabrous, peduncles equal to the petioles, the flowers are yellow, solitary or in pairs; seeds are smooth and black.[5,6] Medicinally, it is used as stomachic, diaphoretic antipyretic, cooling, astringent, tonic, useful in treating nervous and urinary diseases and also disorders of the blood, bile and liver.[7,8] It is also reported to possess antibacterial activity due to its flavonoid content[9,10,11,12], antispasmodial activity[13], larvicidal and repellent activities.[14] *S. rhombifolia* is a weed of waste places, in all plain districts. It grows in over 70 countries throughout the tropical, subtropical and warm temperate regions. It is a perennial, woody, fibrous stemmed shrub, deeply rooted grows upto 2m high. Pounded leaves of the plant are applied as a paste to reduce swelling and rid of boils and headaches. Root decoction is taken as tea to treat diarrhea.[15] It is used in malaria, chestpain, fever, abdominal pain and as a tonic.[16]It is reported to show antioxidant,[17] cytotoxicity and antibacterial activity,[18] anti-inflammatory and hepatoprotective activities.[19]

2. MATERIALS AND METHODS

2.1 Animals

Swiss albino mice of either gender, weighing 25-30 g were used for the study. Animals were procured from Defense Research and Development Establishment, Gwalior and maintained at Central Animal Facility of the Department. They were maintained at standard environmental condition (R.H. – 55-65%, room temperature 25±2°C and 12 h light/ dark cycle) and were fed standard pellet diet and water *ad libitum*. Each experimental group consisted of five animals, housed in separate cages. All experimental protocols were approved by Institutional Animal Ethical committee of the Institute (approved by 1039/AC/07/CPCSEA dated 19-08-2009).

2.2 Drugs and Chemicals

All standard drugs and chemicals used in this study were of analytical grade. The drugs Diazepam was obtained from Ranbaxy Ahmedabad, India; Phenytoin from Sain medicaments, Hyderabad; Pentylenetrazole from Sigma Aldrich, USA. The extracts were dissolved in Tween 80 or Sod. CMC.

2.3 Plant Material

The plant materials and the parts used were represented below. The selected plant were collected from Jorasi and Malanpur village Dist. Gwalior in the month of July and were identified and authenticated by Mr. N.K. Pandey, Research officer (Botany) at central research institute (Ay) Gwalior (M.P.).

2.4 Preparation of extract

The collected plants were dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh. The powdered materials (about 150 gm) were extracted with methanol using soxhlet extraction apparatus. This methanol extract was then concentrated and dried under reduced pressure. The methanol free semi-solid mass thus obtained was used for the experiment. [20] The extractive yield was found to be 13.02% and 6.63% in *Sida acuta* and *Sida rhombifolia* respectively. Both extracts were stored at 4ºC until use.

2.5 Toxicity studies

The aim of the toxicity studies was to determine the non observable adverse effect level of the extract and to find out safe dose range of extract for administration.[21,22]

(1) Acute oral toxicity: The limit test for acute toxicity was carried out at 2000 mg/kg oral dose of MESA and MESR in rats as per OECD 423 guidelines (OECD, 2001). The rats were observed continuously for 2h for behavioral, neurological and autonomic profiles and, after a period of 24 and 72 h, for any lethality, moribund state or death.

(2) Acute dermal toxicity: The acute dermal toxicity testing of the methanolic extract was done by applying the ointments containing methanolic extracts of the highest concentrations of 5% (w/w) on the shaved back of the rats. The OECD guidelines no. 402 (OECD Guidelines, 1987) were followed for the study. Five rats of either male or female were taken for the toxicity study. Extract of 2000 mg/kg body weight in a single dose was applied by dermal route and observed for 14 days.

Table 1 Effect of MESA and MESR on MES-induced seizures

Treatment	Duration of HLTE (mean ± SEM)
Control	16.44 ± 0.12
Phenytoin	2.85 ± 0.14*
MESA-100	10.73 ± 0.01
MESA-200	9.62 ± 0.02
MESA-400	5.38 ± 0.04*
MESR-100	11.35 ± 0.02
MESR-200	8.62 ± 0.03
MESR-400	4.84 ± 0.01*

mean ± SEM * P<0.001, #P<0.0001 when compared to vehicle

2.6 Evaluation of Anticonvulsant activity

2.6.1 MES induced seizures

Electroconvulsive shock (50 mA for 0.2 sec) was delivered through corneal-electrode to induce hind limb tonic extensor phase (HLTE) in mice. Prior to delivery, the current output was checked by using Millimeter.[23] Mice were divided into different groups (n=5). The current was delivered to mice 1h after administration of MESA or MESR (100, 200, 400 mg/kg) or Phenytoin sodium (25 mg/kg) or in normal saline (10 ml/kg). The control group received 0.9 % saline or vehicle of the extract. After the electric stimulation, occurrence and duration of hind limb tonic extensor (HLTE) and incidence of mortality was noted. The animals that did not exhibit

HLTE and death were considered protected.

2.6.2 PTZ induced seizures

PTZ (80 mg/kg) was injected i.p. to induce general clonic convulsions in mice. After PTZ injection, the mice were observed for onset and duration of general clonuses and mortality. PTZ was administered to mice 1h after administration of MESA or MESR (100, 200, 400 mg/kg) or diazepam (4 mg/kg) or in normal saline (10 ml/kg). If no general clonuses occurred the animal were considered protected.

Table 2. Effect of MESA and MESR in Pentylenetetrazole-induced convulsion in mice

Treatment	Onset of Seizure (Sec)	Duration of clonic seizure (Sec)	Seizure Protection (%)	Mortality Protection (%)
Control	68.20 ± 0.20	40.55 ± 0.12	0	0
Diazepam	532.8 ± 2.23	5.53 ± 0.10	100	100
MESA-100	163.2 ± 1.44	8.30 ± 0.06	12.5	62.5
MESA-400	484.5± 1.44**	3.57 ± 0.02**	50	37.5
MESA-200	293.7 ± 1.49	4.24 ± 0.02	37.5	62.5
MESR-100	135.8 ± 1.34	8.00 ± 0.97	25	87.5
MESR-200	317.2 ± 2.56	4.84 ± 0.01##	37.5	75
MESR-400	480 ± 3.23	3.10 ± 0.02##	50	50

Values are the mean ± SEM for 8 mice. *P<0.05, #P<0.001 Compared to control, Dunnett's test.

2.7 Evaluation of CNS depressant activity

2.7.1 Doses and treatments

Mice were divided in different groups (n=5). MESA and MESR were administered orally 1h prior to the assessment of traction test, rota rod test and motor activity. The control group received 0.9% saline or vehicle of the extract. In another set of experiments mice were pre-treated with Diazepam (5 mg/kg) and after 30 min MESA (100, 200 and 400 mg/kg) or MESR (100, 200 and 400 mg/kg) were administered.

Table 3. Effect of MESA and MESR on CNS depressant activity

Treatment/dose (mg/kg)	Traction test		Rota rod test		Locomotor activity count/ 5 min
	Time of Holding (Sec.)	% Failure to put hind limb	Fall off time (Sec.)	% decrease in time	
Control	5.39 ± 0.056	0%	310.6 ± 3.42	0%	541.94 ± 7.49
Diazepam (5 mg/kg)	1.38± 0.055	100 %	18.61 ± 0.11	94%	152.86 ± 0.95
MESA -100	4.51 ± 0.024	25%	192.1 ± 2.22	38.15 %	317.67 ± 3.23
MESA – 200	3.15 ± 0.020	50%	146 ± 1.16 #	52.9%	294.44 ± 1.10
MESA – 400	2.41±0.022#	75%	94.88 ± 1.58 *	69.7%	272.15 ± 2.34
MESR – 100	4.54 ± 0.013	37.5 %	210.1 ± 3.27	32.3%	360.62 ± 3.15
MESR – 200	3.45 ± 0.029	62.5 %	156.7 ± 2.14	49.5%	304 ± 3.73
MESR – 400	2.17±0.018*	87.5 %	116.2 ± 2.14 *	62.5%	291.06 ± 3.29

2.7.2 Traction test

Traction test was used to assess the CNS depressant action and screening of centrally acting muscle relaxants. Forepaws of a mouse were placed on a 15 cm long twisted wire rigidly supported and 20 cm above the table top. Normal mice grasped the wire with

forepaws and when allowed to hang free, placed at least one hind foot on the wire within 5 s. Inability to put up at least one hind foot considered failure to the traction.[24] The test was conducted on different groups of previously screened mice, 1h after the administration of 0.5% Sod. CMC in normal saline (10ml/kg), MESA or MESR (100, 200, 400 mg/kg), and diazepam (4 mg/kg).

2.7.3 Assessment of motor activity

Locomotor activity was studied by using Actophotometer (MAC India Pvt. Ltd., New Delhi) in five groups of five animals each. Group I received control, Group II received diazepam (5mg/kg), Group III, IV and V received methanolic extracts 100, 200 and 400 mg/kg. The locomotor activity for each animal was recorded for 5 minutes at an interval of 2hrs.[25]

2.7.4 Rota rod test

The Rota-rod test was used to determine the effect of MESA or MESR on motor incordination. Mice were placed on horizontal metal-coated rod (2.5cm diameter) rotating at speed of 22 rpm. The time each mouse was able to maintain its balance walking on top of the rod was measured and cut off time was kept 300 s. Before the beginning of all experiments, the riding ability of the animals on Rota-rod was checked. Thus, the mice were initially put on a rotating rod and mice that immediately dropped off (within 60s) were excluded from the experiment.[26] The test was conducted on different groups of previously screened mice (n=5), 30 min after the administration of vehicle (10ml/kg), MESA or MESR (100, 200, 400 mg/kg) and diazepam (4 mg/kg).

2.7.5 Statistical Analysis

The data were analyzed by one way ANOVA followed by Turkey's multiple comparison post hoc test using Graph Pad Prism 5, software (Graph Pad Software Inc, USA). The data from anti-seizure evaluation studies were analyzed by Chi Square test for the assessment of protection of convulsion. The ED50 and TD50values were calculated by Litchfield and Wilcox an method using computer software (PHARM/ACS). The data from traction test were analyzed by Chi Square test while the data from Rota rod test and convulsion parameters were analyzed by one way ANOVA followed multiple comparisons test. The difference of $p<0.05$ was considered significant in all the cases.

3. RESULTS AND DISCUSSION

3.1 Toxicity studies

(1) Acute oral toxicity

Acute oral toxicity studies revealed that the both the extracts MESA and MESR were safe up to a dose level of 2000 mg/kg of body weight (limit test) and LD50is more than 2000mg/kg. No lethality or any toxic reactions or moribund state were observed up to the end of the study period.

(2) Acute dermal toxicity

Acute dermal toxicity studies revealed that the ointments of both the extracts, MESA and MESR were safe up to a dose level of 2000 mg/kg of body weight (limit test) and LD50 is more than 2000mg/kg. No lethality or any toxic reactions or moribund state were observed up to the end of the study period.

3.2 Assessment of Anticonvulsant activity

3.2.1 MES induced seizures

Administration of methanolic extracts at 100, 200 and 400 mg/kg resulted in HLTE for 10.73 ± 0.01, 9.62 ± 0.02 and 5.38 ± 0.04 seconds in MESA and 11.35 ± 0.02, 8.62 ± 0.03 and 4.84 ± 0.01 seconds in MESR respectively. It significantly ($P<0.001$ and $P<0.0001$) reduced the duration of seizures as compared to control groups. Phenytoin completely reduces the seizures in all the animals.

3.2.2 PTZ induced seizures

A significant increase ($P<0.05$ and $P<0.001$) in the latency to clonic convulsions was observed with the MESA and MESR treated groups compared with control. Treatment with MESA and MESR (100 and 200 mg/kg) showed a significant increase ($P<0.05$ and $P<0.001$) in latency to tonic phase, whereas MESA and MESR (400 mg/kg) exhibited higher latency period, but statistically insignificant. A significant increase in latency to death was observed with all tested doses of MESA and MESR, however the effect was not dose-dependent in the diazepam treated group none of the animals showed any phases of convulsions or death.

3.3 Assessment of CNS depressant activity

One-way ANOVA showed significant ($p<0.0001$) influence of MESA and MESR on traction test, rota rod and locomotor activity compared to vehicle treated control. Dunnett's test showed that MESA and MESR extracts both caused significant effect on traction test, rota rod test and motor activity. The dose of 100 mg/kg did not produce significant effect on CNS depressant activity.

The present study was designed to evaluate anticonvulsant and CNS depressant activities of methanolic extract of whole plant of *S.acuta* and *S.rhombifolia* in mice. The maximal electroshock test

is the most widely used animal model in evaluation of antiepileptic drugs. The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic clonic seizures.[27] In addition to identifying drug activity against generalized tonic clonic seizures, it has often been proposed that the maximal electroshock test predicts anticonvulsant drug effects against partial seizures.

MESA and MESR at dose 100, 200 and 400 mg/kg significantly reduced the duration of the seizures as compared to control group. Phenytoin completely inhibited the MES induced tonic seizures in all the animals. PTZ induced seizures in all the mice used. Pentylenetetrazole may elicit seizures by inhibiting gabaergic mechanisms.[28] Standard antiepileptic drugs, diazepam and phenobarbitone, are believed to produce their effects by enhancing GABA-mediated inhibition in the brain.[29] In PTZ model, MESA and MESR increased the latency of seizures, dose dependently.

The present study also revealed that MESA and MESR produced motor impairment and decreased spontaneous locomotor activity at anticonvulsant dose. There are some evidences about anticonvulsant effect of this fatty acids,[30,31] triterpenes and some flavonoids.[32,33] Therefore, it seems that anticonvulsant and CNS depressant activity of *S.acuta* and *S.rhombifolia* may be due to part of linoleic acid, triterpenes (amyrin) and flavonoids[34] present in the extracts.

Thus, it can be concluded that the MESA and MESR having significant anticonvulsant activity with CNS depressant activity. But the exact mechanism by which MESA and MESR exerts its anticonvulsant activity is not determined yet and needs further investigation to elucidate the other active compounds and underlying mechanism(s).

REFERENCES

1. Tripathi KD. Essentials of Medical Pharmacology. New Delhi: Jaypee Publishers; 2008.p.401-5.
2. Arzimanoglou A, Hirish E, Nehlig, Castelnau AP, *et al.* Epilepsy and neuroprotection- An illustrated review, Epileptic Disord 2002;3:173-83.
3. Geoffrey C and Kirby M. Medicinal plants and the control of protozoa disease with particular reference to malaria. Transaction of the royal society of Tropical medicine and hygiene. London: Great Britain. ISSN 0035-9203.
4. Ajithabai MD, Sunitha Rani SP,Balachandran S, Jayakumar G. The use of *Sida* plant in the preparation of Nayopayam Kashayam. International journal of Research and reviews in Pharmacy and applied science 2012;3(1):99-104.
5. Gamble JS. Flora of the Presidency of Madras. London: Adlard & Sons;1935:1(A).
6. Sreedevi CD, Latha PG, Ancy P, Suja SR, *et al.* Hepatoprotective studies on *Sida acuta* Burm. F. J Ethanopharmcol 2009;124:171-75.
7. Kirtikar KR and Basu BD. Indian Medicinal Plants. In: Bishen Singh, Mahendrapal Singh, editor. 2nd ed. Dehradun: International Publisher; 1975.p.205-6.
8. Khare M, Srivastava SK, Singh AK. Chemistry and pharmacology of genus *Sida* (Malvaceae) – a review. Journal of Medicinal and Aromatic Plant Science 2002;24:430-40.
9. Jindal Alka, Kumar Padma and Singh Geeta. Extraction and pharmacological evaluation of flavonoids of *Sida acuta* Burm. f. International J Green Pharm 2012;6(3):208-11.
10. Oboh IE, Akerele JO, Obasuyi O. Antimicrobial activity of the ethanol extract of the aerial parts of *Sida acuta* burm.f. (malvaceae). Trop J Pharma Res 2007;6:809-13.
11. Ekpo MA, Etim PC. Antimicrobial activity of ethanolic and aqueous extracts of *Sida acuta* on micro-organisms from skin infections. J Med Plant Res 2009;3:621-4.
12. Saganuwa AS, Gulimbe ML. Evaluation of *Sida acuta* subspecies leaf/flower extract for antimicrobial activity and phytochemical constituents. Afr J Exp Microbiol 2006;7:83-8.
13. Banzouzi JT, Prado R, Menan H *et al.* Studies on medicinal plants of Ivory Coast: Investigation of *Sida acuta* for in vitro antispasmodial activities and identification of an active constituent 2004;11(4):338-41.
14. Marimuthu Govindarajan. Larvicidal and repellent activities of *Sida acuta* Burm. f. (family- Malvaceae) against three important vector mosquitoes. Asian Pacific Journal of Tropical Medicine 2010;3(9):691-95.
15. Kaushik P, Kaushik D, Khokra S, Chaudhary B. Abutilon indicum (Atibala): Ethnobotany, Phytochemistry and Pharmacology- A review. International Journal of Pharmaceutical and Clinical research 2009;1(1):4-9.
16. Wilcox ML and Bodekar G. Traditional herbal medicines for malaria.

BMJ 2004;329(7475):1156-59.

17. Dhahval K, Deshpande YS and Purohit AP. Evaluation of In-vitro anti-oxidant activity of *Sida rhombifolia* (L.) Ssp. Retusa (L.). Journal of Medicinal food 2007;10(4):683-88.
18. Islam ME, Haque ME, Mosaddik MA. Cytotoxicity and antibacterial activity of *Sida rhombifolia* (Malvaceae) grown in Bangladesh. Phytotherapy research 2003;17(8):973-75.
19. Kumar RS, Mishra SH. Anti-inflammatory and hepatoprotective activities of *Sida rhombifolia* linn. Indian J Pharmacol 1997;29(2):110-16.
20. Harbone JB. Phytochemical Methods. London: Chapman and Hall Publishers; 1998.p.4-32.
21. OECD guidelines for testing of chemicals. Acute oral toxicity-Acute toxic class method 2001;423:1-14.
22. OECD guidelines for testing of chemicals – Acute dermal toxicity 1987;402:1-7.
23. Kulkarni SK. Handbook of Experimental Pharmacology. In: Jain MK, editor. 3rd ed. Delhi: Vallabh Prakashan;1999.p.122-3, 131-2.
24. Hossein H, Marjan NA. Anticonvulsant, sedative and muscle relaxant effects of carbenoxolone in mice. BMC Pharmacol 2003; 3:3.
25. Achliya GS, Wadodkar SG, Dorle AK. Evaluation of CNS activity of *Brahmi Ghrita*. Indian J Pharmacol 2005;37:33-6.
26. Cartmell S.M, Gelgor L, Mitchell D. A revised- rota rod procedure for measuring the effect of antinociceptive drugs on motor function in the rat. J Pharmacol Methods 1991;26(2):149-59.
27. Loscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations,Epilepsy Res 1988; 2: 145-81.
28. De Sarro A, Cecchetiv, Fravoloni V, Naccari F, *et al.* Effects of novel 6-desfluoroquirolones and classic quinolones on PTZ-induced seizure in mice. Antimicrob Agents Chemother 1999;43:1729-36.
29. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology.5th ed. India: Churchill Livingstone;2005.p.456-73.
30. Yehuda S, Carrasso RL, Mostofsky DI. Essential fatty acid preparation (SR-3) raises threshold in rats. Eur J of Pharmacol 1994;254:193-98.
31. Voskuyl RA, Vreugndenhil M, Kang JX, Leaf A. Anticonvulsant effect of Polyunsaturated fatty acids in rats using cortical stimulation models. Eur J of Pharmacol 1998;341:145-52.
32. Kauvadias D, Saud P, Youdium KA, Qaiser MZ, *et al.* The flavones hispidulin, a benzodiazepine receptor ligand with positive allosteric properties, traverses blood brain barrier and exhibits anticonvulsive effect. Br J Pharmacol 2004;142:811-20.
33. Griebel G, Perrault G, Tan S, Shoemaker H, Sanger D. Pharmacological studies on synthetic flavonoids: comparison with diazepam. Neuropharmacology 1999;38:965-977.
34. Otero R, Nu'nez V, Jime'nez SL *et al.* Snake bites and ethnobotany in the northwest region of Colombia part II: neutralization of lethal and enzymatic effects of Bothrops atrox venom. J Ethanopharmacol 2000;71:505-11.