CNS Depressant Activity of Ethanol Extract of *Luffa acutangula* Var. *amara* C. B. Clarke. Fruits in Mice

A. V. MISAR, A. S. UPADHYE AND A. M. MUJUMDAR*
Plant Science Division, Agharkar Research Institute, G. G. Agarkar Road, Pune-411004.

Accepted 16 June 2004
Revised 15 March 2004
Received 15 January 2003

The fruits of *Luffa acutangula* Var. *amara* C. B. Clarke were collected in winter season from Western ghat area. These fruits were dried, powdered, defatted and extracted with ethanol. HPTLC pattern of ethanol extract was recorded after removal of solvent and was studied for effect on behavioral changes, exploratory activity, barbiturate sleeping time, using appropriate standards in mice. The extract exhibited dose-dependent CNS depressant activity.

Luffa acutangula Roxb. var. *amara* C. B. Clarke (Cucurbitaceae) a fairly large climber found in western, central and southern India and regarded as a wild form of cultivated species. All parts of this plant are exceedingly bitter[1]. The plant is considered to be laxative, diuretic, antiseptic, antitubercular and used in asthma, skin diseases as well as in enlargement of spleen. The fruits and seeds are employed in dysentery and powder form is used as a snuff for the treatment of jaundice. In *Santhal* tribes it is used for adenitis and sores[2]. A crystalline bitter principle identical with cucurbitacin B has been isolated from seeds[3]. Amarinin was also isolated from seeds, elucidated as 2-deoxy cucurbitacin B[4]. Allied species, *Luffa echinata* Roxb. has been reported to possess CNS depressant activity[5] and an alkaloid isolated from it showed significant local anesthetic and antispasmodic action[6]. In the present investigation, CNS depressant activity of *Luffa acutangula* var. *amara* fruit extract has been explored.

The fruits of *Luffa acutangula* Var. *amara* were collected from Western Ghat area (Kadav, Raigad district, Maharashtra), in September/October 2000. The plant specimen was authenticated by matching with voucher specimen AHMA-18274 available with Agharkar Herbarium of Maharashtra Association at Agharkar Research Institute, Pune (ARI). These fruits were shade dried, powdered and defatted with pet. ether (60-80°) and then extracted with ethanol to yield 2.31% extractive.

The extract was concentrated under reduced temperature and pressure and subjected to HPTLC studies on instrument comprising of Linomat IV for spotting, using Densitometer - TLC Scanner III with CATS software (Camag, Switzerland). These studies were carried out on pre-coated aluminum non-fluorescent plates (E. Merck) with petroleum ether:ethyl acetate:acetone:methanol (60:20:20:2) system at 254 nm and 366 nm , which showed three major spots with Rf values of 0.54, 0.64 and 0.96 at 254 nm. While at 366 nm two spots were observed with Rf values of 0.39 and 0.95.

The experiments were carried out on Swiss mice, which were originally obtained from the National Institute of Virology, Pune. They have been inbred at ARI for several generations at the animal house facility for the last 17 y. These animals were reared and housed in the polypropylene cages at 25±2° and 10:14 h light and dark cycle. They were maintained on commercially available Amrut brand animal feed and water *ad libitum*. For pharmacological studies in all experimental set, 6 mice were used for each treatment. The animal experiments were performed after obtaining the permission of the Institutional Animal Ethics Committee of ARI.

The statistical analysis of results was carried out to calculate mean±SEM. Further analysis was carried out by student's t-test to calculate significance of results. P values > 0.05 were considered as non significant.

There was no mortality upto 50 mg/kg (p.o.) by extract treatment in mice. The effect of extract (10 and 50 mg/kg p.o.) on general behavioral changes were observed initially upto 4 h and then at 24 h. There was reduction in overall activity, respiratory depression (+) at lower dose,

***For correspondence**
E-mail: ammpune @ hotmail.com

while, at higher dose there was marked depression, respiratory difficulty, analgesia, secretions from nose and mouth. These effects were found to be maximal at 30 min after administration of extract and the animals were normal by 4 h.

Based on these observations further studies were carried out after administration at 5 and 10 mg/kg dose by oral route 30 min prior to testing the following CNS activities; exploratory activity was evaluated using the hole-board method with the help of a board (40 cm x 40 cm) with four equidistant holes (1 cm diameter x 2 cm depth). The mouse was placed at one corner of the board and the animal moved about and dipped its head into the holes indicating exploratory behavior. The numbers of dips in 7.5 min were recorded (File and Wardrill, 1975)[7]. The test was carried out 30 min after treatment with extract to various groups of mice, using chlorpromazine HCl, 1 mg/kg as a standard. There was significant dose-dependant reduction due to pre-treatment of *Luffa acutangula* extract *as* compared to control mice, however, it was less as compared to chlorpromazine HCl as shown in Table 1.

TABLE 1: EFFECT OF *LUFFA ACUTANGULA* ETHANOL EXTRACT ON EXPLORATORY ACTIVITY

Pre-treatment	No. of head dips mean±SEM (in 7.5 min)
Control	20.5±1.31
Extract 5 mg/kg.	14.7±1.17*
Extract 10 mg/Kg.	10.8±0.89*
Chlorpomazine HCl 1 mg/kg.	7.17±0.28*

All values for head dips are expressed as mean±SEM, n=6, *Significant as compared to control P< 0.05.

Pentobarbitone sodium (35 mg/kg) and barbital sodium (200 mg/kg) were administered i.p.30 min after pretreatment of extract or chlorpromazine HCl (1 mg/kg i.m.) to various groups of mice to evaluate the effect of the extract on barbibiturate sleeping time . The sleeping time was measured as a time interval between loss and regain of righting reflex. Similar experiment was also performed in mice treated chronically for 5 d with pentobarbitone sodium (35 mg/kg i. p.) only to stimulate the liver microsomal enzyme system and then on next day pentobarbitone sleeping time was studied as described. The extract or chlorpromazine 1 mg/kg pretreatment enhanced the pentobarbitone sleeping time in single as well as in chronically treated mice significantly. This pre-treatment also potentiated barbitone sodium sleeping time as shown in Table 2.

The ethanol extract of *Luffa acutangula* showed significant reduction in exploratory activity of mouse in a dose-dependent manner, which is in agreement with observations in general behavioral studies[8]. However, this reduction is less compared to that observed with chlorpromazine pre-treatment. Further, it enhanced pentobarbitone sodium-induced hypnosis in single dose treated as well as in chronically treated groups of mice. It also enhanced barbitone sodium sleeping time. Usually pentobarbitone sleeping time is potentiated due to inherent depressant activity or effect on degradation by inhibition of liver microsomal enzymatic system of the drug under consideration. The barbitone sodium is known to have central depressant activity, moreover, it is not degraded by liver microsomal enzymatic system. It is also a well-known fact that repeated administration of pentobarbitone produces induction of liver microsomal enzyme system leading to rapid degradation of pentobarbitone thereby leading to a reduction in the sleeping time[9]. Thus, in light of the above observations it can be concluded that the ethanol extract of *Luffa acutangula* exhibited central nervous system depressant activity in mice.

TABLE 2: EFFECT OF *LUFFA ACUTANGULA* ETHANOL EXTRACT ON BARBITURATE SLEEPING TIME

Pre-treatment	Pentobarbitone sodium (in min)		Barbitone sodium (in min)
	Normal	Chronic	
Control	63±1.9	20±2.6	124±3.4
Extract 5 mg/kg.	97±4.4*	41±3.1*	164±1.3*
Extract 10 mg/Kg.	117±1.4*	57±1.3*	186±2.6*
Chlorpomazine HCl 1 mg/kg.	122±4.0*	58±2.8*	194±1.3*

All values of sleeping time are expressed as mean±SEM, n=6, *Significant as compared to control P< 0.05.

REFERENCES

1. Anonymous, In; Wealth of India, (Raw materials), Vol. 6, Publications Information Division, CSIR Publications, New Delhi, 1962, 177.
2. Satyavati, G.V., Gupta, A.K. and Tondon, N., In ; Medicinal Plants of India, Vol. 2., ICMR Publication, New Delhi, 1987 ,178.
3. Nigam, S.K. and Sharma, V.N., **J. Sci. Ind. Res. (India),** 1959,18, 535.
4. Rastogi, R. and Mehrotra, B.N., In; Compendium of Indian Medicinal Plants. Vol. 4, Central Drug Research Institute, Lucknow, Publication and Information Directorate, New Delhi, 1995, 442.
5. Lauria, P., Sharma, V.N., Vanjani, S. and Sangal, B.C., **Indian. J. Pharmacol.,** 1972, 4 , 152.
6. Gajaria, S., Agarawal, S. and Shinde, S., **Indian. J. Pharmacol.,** 1978, 10, 89.
7. File, S.E. and Wardrill, A.G., **Psychopharmacol.**,1975, 44, 53.
8. Turner, R.A. In; Screening Methods In Pharmacology. Academic Press, New York , 1965, 110.
9. Mujumdar, A.M., Naik, D.G., Waghole, R. J., Kulkarni, D.K. and Kumbhojkar, M.S., **Pharmaceutical Biology,** 2000, 38, 13.

Pharmacological Screening of Some Novel Isatin Derivatives

P. SELVAM*, A. RAJASEKARAN, N. MURUGESH[1], M. CHANDRAMOHAN[2], AND E. DE CLERCQ[3]

A. K. College of Pharmacy, Anandnagar, Krishnankoil-626190.

[1]Institute of Pharmacology, Madurai Medical College, Madurai-625020.

[2]Bharat Ratna Kamarajar Liver Hospital and Research Centre, Madurai-625001.

[3]Rega Institute for Medical Research, Katholieke Universiteit-Leuven.

Minder broederstraat 10, Leuven B-3000, Belgium

Accepted 28 June 2004

Revised 4 March 2004

Received 6 October 2003

4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-N(4,6-dimethyl-2-pyrimidiny)-benzene Sulphonamide and its derivatives were evaluated for antibacterial activity, antifungal activity, antiviral activity against ten pathogenic viruses in E_6SM, Vero and HeLa cells and anticancer activity against CNS, breast and lung cancer. The N-acetyl and 5-methyl derivatives showed a minimum inhibitory concentration (MIC) of 48 μg/ml against *herpes simplex virus* type-2 and *respiratory syncytial virus*, without toxicity at a concentration upto 400 μg/ml. The test compounds showed comparable antibacterial activity to that of the parent sulphadimidine, except for the 5-bromo and N-acetyl derivatives.

Isatin (2,3-dioxoindole), a versatile lead molecule for potential bioactive agents, and its derivatives were reported to possess anticancer[1], antibacterial, antifungal and antiHIV activities[2-11]. Methisazone (N-methylisatin-β-thiosemicarbazone) was one of the first clinically used synthetic antiviral agents[12]. Isatin derivatives were reported for antiviral activities against a variety of pathogenic viruses[13] and N,N-disubstituted thiosemicarbazone derivatives of isatin were tested for inhibition of HIV-1 replication[14]. Previously we synthesized some novel isatin derivatives and evaluated for their activities against HIV-1 and HIV-2 in MT-4 cells[15], significant activity was noted with these compounds against HIV-1 replication[16].

In view of the broad spectrum biological activities of isatin derivatives, we aimed at evaluating the antiviral activity of some novel 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene) amino]-N(4,6-dimethyl-2-pyrimidiny)-benzene sulphonamide and its derivatives (fig. 1) against pathogenic viruses in E_6SM, Vero and HeLa cells cultures and compared their activity with that of the standard antiviral agents brivudin (BVDU) and ribavirin. Anticancer activity test were also performed against breast, lung and CNS cancer cell in culture technique (NCI, NIH, USA). The compounds were also tested for antibacterial and antifungal activities in compari-

***For correspondence**

E-mail: periyasamyselvam2001@yahoo.co.in