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Analgesic and anticonvulsant effects of saponin isolated from the leaves of *Clerodendrum infortunatum* Linn. in mice

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Saponin (SN1) isolated from *C. infortunatum* leaves in doses of 30, 50, 75 and 100 mg/kg, ip provided 36.28, 60.47, 90.71, 100% protection respectively from writhing induced by 1.2% v/v acetic acid. In hot plate method, SN1 not only produced analgesia in mice but also potentiated the analgesic action of pentazocine and aspirin. The anticonvulsant activity was tested by leptazol-induced seizures. SN1 decreased the duration of seizures and gave protection in a dose dependent manner against leptazol-induced convulsions. The results suggest that saponin has significant analgesic and anticonvulsant effects.

Keywords: Analgesic and anticonvulsant activity, Aspirin, *Clerodendrum infortunatum*, Pentazocine, Saponin

Clerodendrum infortunatum Linn. (Verbanaceae; Bhant in Hindi, Ghentu in Bengali, Bhania in Oriya) is a terrestrial shrub having square, blackish stem and simple, opposite, decussate, petiolate, exstipulate, coriacious, hairy leaves with a disagreeable odour[1]. The plant is common throughout the plains of India. Various parts of the plant have been used by tribes in colic, scorpion sting and snakebite, tumor and certain skin diseases[2,3]. Fresh juice of leaves has been used as vermifuge and in treatment of malaria[3,4]. *C. infortunatum* leaves on preliminary chemical analysis are found to contain saponin, clerodin (a bitter diterpene)[3,5] and some enzymes. The objective of the present study was to isolate the saponin from the leaves of *C. infortunatum* and investigate its analgesic and anticonvulsant effects in mice.

Materials and Methods

Plant material—The fresh leaves of *C. infortunatum* Linn. were collected locally (Bankura district of West Bengal) in autumn as it contains the maximum active constituents at that time. It was authenticated by Dr H J Chowdhury, Joint Director, Central National Herbarium, Botanical Survey of India, Howrah, West Bengal. A voucher specimen has been preserved in our laboratory for further reference (DKP 02/2005). After collection the leaves were washed properly with water and fungal leaves were picked out.

Isolation of saponin—Fresh leaves (3.2 kg) were grinded with water and stored overnight in a refrigerator for the separation of pigments and other material. The supernatant was taken after centrifugation at 10,000 RPM for 30 min and mixed well with 10 g of activated charcoal. It was filtered repeatedly to get a colorless solution which was taken in a separating flask and 200 ml petroleum ether was added and shaken thoroughly and a jelly like mass was fractionated (16.2 g). This fraction (SN1) was collected, dried in air (yield 3.5 g) and it gave a positive test for saponin (foam test, Liebermenn-Burchard reaction, high hemolytic activity).

Part of the crude saponin mixture (SN1) (2 g) on preparative TLC on 1.0 mm silica gel layers prepared in the laboratory using applicator (solvent system, $CHCl_3$-MeOH, 3:2 and $CHCl_3$-EtOH, 1:1) gave a pure compound SN2 (yield 105 mg) (R_f value: 0.41 and 0.43 respectively, m.p.193°-195°C, λ_{max}215 nm in water) showed characteristic IR (Jasco 530 FTIR, in KBr) peaks at 3371 cm^{-1} (for –OH group), 2934 cm^{-1} (for –CH), 1735 cm^{-1}, 1601 cm^{-1} (for aromatic and

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unsaturation), 1409 cm^{-1}, 1077 cm^{-1}, 599 cm^{-1} suggesting the structural similarities with 2α-hydroxyuvaol[5-7] which was identified and confirmed by comparable physicochemical properties, m.p., λ_{max} value and superimposable I.R. spectra of authentic sample obtained from Central Drug Laboratory, Kyd Street, Kolkata.

Animals—Adult Swiss albino mice of either sex (22 ± 2 g) obtained from B.N. Ghosh & Co., Kolkata were acclimatized to normal laboratory conditions for one week and given pellet diet and water *ad libitum*. All experiments were performed between 0800 hr to 1200 hr to minimize circadian influences. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared before initiating the experiments. The animals were handled as per guidelines of committee for the purpose of control and supervision on animals (CPCSEA, registration no 787), New Delhi.

Toxicity studies—Acute toxicity study was carried out in mice with different doses of SN1 intraperitoneally (ip) into different group of mice, each containing 10 animals, as per the method described by Litchfield and Wilcoxon[8]. The behavioral profiles and mortality was observed for 72 h.

Assessment of analgesic activity

Acetic acid-induced writhing tests—This method involved ip injection of freshly prepared 1.2% v/v acetic acid. The number of abdominal constrictions (writhing) and stretching with a jerk at the hind limbs and bending of trunk were counted between 5 and 15 min after adminstration of acetic acid. For the test, SN1 was administered ip at 30, 50, 75 and 100 mg/kg. Acetylsalicylic acid (ASA, Cipla Ltd, Mumbai, India), paracetamol (PCM, Tablets India Ltd, Chennai, India) and morphine sulphate (M; Modi-Hundi Pharma Ltd, New Delhi, India) were used as reference standards at doses of 40, 68, 100 mg/kg; 40, 68, 100 mg/kg; 0.75, 1.15, 1.50 mg/kg, ip respectively[9-12] to compare with SN1 activity. The decrease in acetic acid induced writhing numbers was calculated. The analgesic activity was expressed in terms of percentage and compared with other reference drugs. The ED_{50} value and therapeutic index of SN1 were also calculated.

Hot plate method—Animals were divided into various groups as shown in the Table 1. Reaction time was recorded at 15, 30, 45, 60, 90, 120, 150 and 180 min after ip injection of SN1 at doses of 50, 75, 100 mg/kg. The temperature of the hot plate was maintained at 55° ± 0.5°C. A cut off reaction time of 10 sec was chosen in order to avoid the physical injury. Pentazocine (Pharma Impex Laboratories Pvt Ltd, Kolkata, India; 40 mg/kg, ip) and aspirin (Cipla Ltd, Mumbai, India; 68 mg/kg, ip) were used as a reference drug. SN1 was given individually and also 15 min prior to the administration of reference drugs to investigate the potentiation of pentazocine and aspirin activity[13].

Assessment of anticonvulsant activity

Leptazol-induced seizures—SN1 was administered ip in varying doses (20-100 mg/kg) 30 min prior to the administration of leptazol (HiMedia Laboratories Pvt Ltd, Mumbai, India) (80 mg/kg, ip) in mice. The time needed for the development of unequivocal sustained clonic seizures activity involving the limbs was carefully noted; isolated myoclonic jerks or other preconvulsive chewing behavior were not counted. Seizure free state for a period of 1 hour was taken as protection[14-16].

Statistical analysis—LD_{50} and ED_{50} values of SN1 were calculated with 19/20 (95%) confidence limit using commercial computer and Therapeutic Index (T.I.) number was calculated by dividing the LD_{50} by the ED_{50} in each case. Results were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test[17]. $P < 0.05$ was considered significant.

Results

In toxicity studies, the SN1 was found to have the LD_{50} value of 375 (349-403) mg/kg, ip. SN1 not only produced analgesia in mice but also potentiated the action of pentazocine and aspirin (Table 1). In acetic acid-induced writhing tests, protection produced by SN1 against acetic acid-induced writhing was dose dependent and its ED_{50} value was found to be 40 (32.50-49.20) mg/kg, ip and therapeutic index was 9.4. The ED_{50} values of morphine, paracetamol and acetyl salicylic acid were found to be 0.82 (0.73-0.92) mg/kg, ip, 55 (46.8-64.1) mg/kg, ip and 57 (48.5-66.5) mg/kg, ip respectively. In hot plate method, the minimum effective dose of SN1 was found 35 (29.4-42.0) mg/kg, ip (Table 2). SN1 significantly inhibited the onset and incidence of convulsion against leptazol-induced seizures. Protection produced against leptazol-induced convulsion was dose dependent and ED_{50} and

Table 1—Effect of SN1 on analgesia induced by pentazocine and aspirin in mice by hot plate method
[Values are mean ± SE from 6 animals in each group]

Treatment (ip)	Base value	Reaction time (sec) at different time intervals (min)							
		15	30	45	60	90	120	150	180
Control (NS)	4.6 ± 0.25	5.4 ± 1.00	5.2 ± 0.60	5.1 ± 0.89	4.9 ± 0.44	4.6 ± 0.40	4.2 ± 0.45	4.1 ± 0.39	4.0 ± 0.57
SN1 (50 mg/kg)	5.2 ± 0.64	225.3 ± 1.49*	22.6 ± 1.61*	21.5 ± 1.49*	18.6 ± 1.10*	17.2 ± 0.99*	15.6 ± 0.99*	13.2 ± 0.95*	6.5 ± 0.96
SN1 (75 mg/kg)	5.6 ± 0.55	>30*	>30*	28.2 ± 0.97*	26.4 ± 1.65*	19.9 ± 1.44*	18.5 ± 1.28*	15.5 ± 0.98*	9.2 ± 0.84*
SN1 (100 mg/kg)	5.8 ± 0.70	>30*	>30*	>30*	29.3 ± 1.98*	24.3 ± 1.39*	20.2 ± 1.45*	19.3 ± 0.91*	13.4 ± 1.00*
Pentazocine (40 mg/kg)	4.9 ± 0.16	27.2 ± 1.0*	>30*	>30*	27.5 ± 1.70*	21.1 ± 1.24*	19.8 ± 0.97*	16.8 ± 0.99*	12.4 ± 0.96*
SN1 (50 mg/kg) + pentazocine (40 mg/kg)	5.7 ± 0.86	>30*	>30*	>30*	>30*	29.2 ± 1.35*	27.3 ± 1.69*	26.8 ± 1.05*	21.1 ± 1.04*
SN1 (75 mg/kg) + pentazocine(40 mg/kg)	5.1 ± 0.75	>30*	>30*	>30*	>30*	>30*	>30*	28.4 ± 1.17*	25.3 ± 1.35*
SN1 (100 mg/kg) + pentazocine(40 mg/kg)	6.7 ± 1.01	>30*	>30*	>30*	>30*	>30*	>30*	>30*	27.6 ± 1.37*
Aspirin (68 mg/kg)	5.0 ± 0.49	26.1 ± 1.0*	24.5 ± 0.79*	23.0 ± 0.71*	20.2 ± 0.60*	18.1 ± 0.55*	17.2 ± 0.58*	14.7 ± 0.45*	10.4 ± 0.85*
SN1 (50 mg/kg) + aspirin (68 mg/kg)	5.5 ± 0.75	>30*	>30*	>30*	>30*	26.5 ± 1.12*	24.2 ± 1.00*	23.8 ± 0.85*	18.0 ± 0.95*
SN1 (75 mg/kg) + aspirin (68 mg/kg)	5.7 ± 0.80	>30*	>30*	>30*	>30*	>30*	>30*	26.3 ± 1.05*	22.4 ± 1.10*
SN1 (100 mg/kg) + aspirin (68 mg/kg)	5.8 ± 0.74	>30*	>30*	>30*	>30*	>30*	>30*	>30*	26.5 ± 1.20*

Statistical analysis done by ANOVA followed by post-hoc test of significance, Dunnett's test, *P <0.05 *vs* resting value (average reaction time before treatment). Results of SN1 + pentazocine and SN1 + aspirin were significant (P <0.05) *vs* SN1. NS: Normal saline, >30: animals fail to react within 30 S (30-s response latency).

therapeutic index of SN1 was determined to be 45 (30.2-65.0) mg/kg, ip and 8.3 respectively (Table 3).

Discussion

In the present study, SN1 elicited analgesic and anticonvulsant activity in mice in a dose dependent manner and therapeutic index of SN1 was found to be large, there by indicating its safety margin.

The analgesic activity of SN1 was studied to see the involvement of central and peripheral components. The SN1 treatment significantly prolonged the reaction time (hot plate method) and also reduced the number of writhing episodes induced in mice by acetic acid administration suggesting its central as well as peripheral analgesic activity, respectively[9,18]. SN1 not only produced analgesia in mice, but also potentiated the analgesic activity of pentazocine (Table 1). The present results suggest that SN1 is also effective against leptazol-induced scizures.

The promising analgesic activity shown by SN1 is probably mediated either by depleting endogenous levels via dopamine-β-hydroxylase inhibition or by blocking its effects at the receptor level and there by inhibition of post-synaptic specific sensitive mechanism[19]. The increase of brain serotonin and GABA level may also be responsible for analgesic and anticonvulsant activities[19]. It was found that SN1 increased the brain serotonin and GABA level in mice (personal communication). Therefore, analgesic and anticonvulsant activities produced by SN1 may be related to the increased brain serotonin and GABA level in mice[16,19,20]. Since various saponins have been reported to possess anti-epileptic activities[21-23], the anticonvulsant effects of SN1 in mice may be due to the presence of such compound.

The present results demonstrate the potential effectiveness of saponin isolated from the leaves of *C. infortunatum*, which supports the claim by traditional medicine practitioners as an analgesic and anticonvulsant remedy.

Table 2—Effect of SN1 (30, 50, 75, 100 mg/kg, ip) on the writhing and stretching induced in mice by ip injection of 1.2% acetic acid (writhing test) [Values are mean ± SE from 6 animals in each group]

Treatment	Dose (mg/kg, ip)	Number of writhing	Protection (%)	ED_{50} (mg/kg, ip)* (95% confidence limit)
Vehicle (NS)	5	80.32 ± 0.95	-	-
SN 1	30	51.18 ± 1.87*	36.28	
	50	31.75 ± 1.68*	60.47	40 (32.50-49.20)
	75	7.46 ± 0.55*	90.71	
	100	A	100	
PCM	40	51.30 ± 1.70*	36.13	
	68	30.98 ± 1.85*	61.43	55 (46.8-64.1)
	100	7.76 ± 0.50*	90.33	
ASA	40	51.9 ± 1.00*	35.38	
	68	32.00 ± 1.79*	60.15	57 (48.5-66.5)
	100	9.27 ± 0.90*	88.46	
M	0.75	43.59 ± 1.86*	45.73	
	1.15	24.00 ± 1.20*	70.12	0.82 (0.73-0.92)
	1.50	6.86 ± 0.85*	91.46	

The activity of SN1 was compared with paracetamol (PCM), acetyl salicylic acid (ASA), morphine sulphate (M).
Therapeutic index (T.I.) of SN1 = LD_{50}/ED_{50} = 375/40 = 9.4.
*P <0.001 compared to vehicle control (ANOVA following by Dunnett's test).
A = Absence of writhing.
*Calculated from the 3 point regression of response on dose.

Table 3—Effect of SN1 against leptazol-induced convulsion in mice [Values are mean ± SE from 10 animals]

Dose (mg/kg, ip)	Latency (min)	Protection (%)	Duration of seizure (Min)	ED_{50} value (mg/kg, ip)# (95% confidence limit)
20	5.32 ± 1.12	0	16.73 ± 1.95	
40	13.80 ± 8.05	25	10.12 ± 2.00	
45	32.05 ± 10.0	50	05.65 ± 2.98	45 (30.2-65.0)
75	52.60 ± 7.20	90	02.40 ± 1.90	
100	Nil	100	Absent	

Therapeutic index of SN1 = LD_{50}/ED_{50} = 375/45 = 8.3.
Calculated from the 3 point regression of response on dose.

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