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CNS activity of *Vitex negundo* Linn. in mice

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Methanolic extract (ME) of the leaves of *V. negundo* potentiated significantly the sleeping time induced by pentobarbitone sodium, diazepam and chlorpromazine in mice. ME possesses analgesic properties and potentiated analgesia induced by morphine and pethidine. ME also showed significant protection against strychnine and leptazole induced convulsions. The results suggest that ME exhibits CNS depressant activity in a dose dependent manner.

Since time immemorial man has been using herbs or plant products as medicine for developing immunity or resistance against various diseases like cold, coryza, joint pains, fever etc[1].The traditional systems of medicine are based on the experience in the use of plant products in amelioration of common diseases and a number of herbal remedies have stood the test of time.The present study has been undertaken to determine the pharmacological action of the plant, *Vitex negundo* (Nishinda in Bengali, family: Verbenaceae) a well known medicinal plant found in Bengal, Southern India and Burma[2-4].Leaves of the plant are used as an indigenous drug in different diseases like rheumatic disease, headache, catarrhal fever, cervical spondylitis etc[5]. The preliminary analysis indicates presence of alkaloids, flavones and benzoic acid (total yield of methanolic extract is 2.1%). Present study deals with CNS activities of the leaf of *V. negundo*.

Materials and Methods

Leaves of the plant were collected from United Chemical and Allied Products, Calcutta, sundried, powdered and finally extracted with methanol in a soxhlet apparatus. After distillation and evaporation the resultant deep green coloured gummy mass thus obtained was dissolved in propylene glycol (E. Merck). This is referred to as methanolic extract (ME; LD_{50}1.25 g/kg in mice)[6, 7] hereafter.

Albino Swiss mice, weighing between 16-20 g were used.Animals were fed pellets (Hindustan Lever Ltd, Calcutta) and given tap water *ad libitum* . The experiments were performed in a quiet room with an ambient temperature of 22° ± 2°C.

Behavioural profile in mice —CNS depressant action of ME at 0.15, 0.20 and 0.30 g/kg body wt. on righting reflex, grip strength, awareness, pinna reflex, touch response on mice was measured by conventional methods.[8]

Effect on sleeping time in mice—Pentobarbitone sodium (40 mg/kg; Nembutal; M&B, India) diazepam (3 mg/kg; Calmpose; Ranbaxy Lab, India), chlorpromazine(10mg/kg; Largactil; M&B, India) were individually injected ip, 15 min after administration of either the vehicle, propylene glycol (PG; 5 ml/kg)or ME at 0.15, 0.20 and 0.30 g/kg body wt. Time interval between loss and regaining of righting reflex was considered as the sleeping time[9].

Effect on morphine and pethidine induced analgesia—The analgesic action was studied by hot plate method[10] .The reaction times were recorded at 15, 30, 45, 60, 90, 120, 150 and 180 min after ip administration of the extract at 0.15, 0.20 and 0.30 g/kg body wt doses. The reaction time was considered at the instant the animals licked their hind paw from the hot surface. The temperature of the bath was maintained at 55°± 0.5°C. A cut off reaction time of 30 sec (when a mouse made no response) was used in order to avoid tissue injury. Eight groups were tested against each standard either morphine or pethidine (n=6/group).

Anticonvulsive property of ME—The anticonvulsive property of ME at 0.15, 0.20, 0.30 g/kg body wt was obtained against two standard drugs, strychnine hydrochloride (2 mg/kg; Sigma) and leptazole (80 mg/kg; Sigma). The average survival time (min) and the percentage mortality of the albino Swiss mice were observed after 24 hr.

Statistical analysis—The unpaired Student's *t* test was applied to evaluate the statistical significance of the data[11].

Results and Discussion

ME at 0.15, 0.20 and 0.30 g/kg body wt showed significant CNS depressant action in a dose dependent manner (Table 1). ME decreases touch response, pain response, righting reflex and grip strength of mice in comparison with respective control groups (vehicle PG) probably due to a pronounced depressant action[12]. Reduction of awareness and depressant action may be due to the action of ME on CNS[13]. The reduction of pinna reflex may be due to blocking synapses of the afferent

pathway [14]. ME does not have any hypnotic action but potentiates the sedative hypnotic action of other reference standard drugs, such as pentobarbitone, diazepam and chlorpromazine. ME at a dose level of 0.15, 0.20 and 0.30 g/kg body wt prolonged sleeping time induced by pentobarbitone, diazepam and chlorpromazine respectively as compared with saline and vehicle control animals (Table 2). Out of these three drugs maximum potentiation of sleeping time takes place with sodium pentobarbitone due to CNS depressant action[15]. ME itself has no analgesic effect but it potentiates significantly ($P<0.001$) the morphine and pethidine induced analgesia in mice in a dose dependant manner (Tables 3 and 4). ME not only increased the average survival time but also decreased the percentage mortality in a dose dependent manner in strychnine / leptazole treated mice (Tables 5 and 6). GABA is known to

Table 1—Effect of methanolic extract of *V.negundo* on behavioural profile in mice (n=6)

Behaviour	Saline control (5 ml/kg)	Vehicle(PG) control (5 ml/kg)	ME dissolved in PG			Pentobarbitone (0.30 g/kg)
			0.15 g/kg	0.20 g/kg	0.30 g/kg	
Awareness	0	0	+	2+	4+	4+
Touch response	0	0	3+	4+	4+	4+
Pain response	0	0	3+	4+	4+	4+
Righting reflex	0	0	+	2+	4+	4+
Pinna reflex	0	+	3+	3+	4+	4+
Grip strength	0	+	2+	3+	4+	4+
Mortality within 24 hr	nil	nil	nil	nil	nil	nil

[0= no effect(normal) , + = slight depression , 2+ = moderate depression , 3+ = strong depression 4+ = very strong depression]

Table 2—Effect of methanolic extract of *V.negundo* on sleeping time in mice
[Values are mean ± SE from 6 animals in each group]

Standard sedatives	Dose (mg/kg)	Normal saline (5 ml/kg)	Propylene glycol (5 ml/kg)	Extract dissolved in PG		
				0.15 g/kg	0.20 g/kg	0.30 g/kg
Pentobarbitone	40	36.0±1.03	57.0±0.82*	74.8±1.12*	105.4±1.32*	137±2.35*
Diazepam	3	68.0±1.10	90.0±0.73*	108.0±0.46*	156.0±1.12*	195±1.16*
Chlorpromazine	10	91.0±4.00	101.3±0.95*	111.0±1.50*	134.0±2.50*	170±1.84*

*$P < 0.001$; results were compared with PG + Ref.standard control and PG+Ref. standard control were compared with saline + Ref. standards.

Table 3—Effect of methanolic extract of *V. negundo* on morphine induced analgesia in mice
[Values are mean ±SE from 6 animals in each group]

Drugs	Average reaction time before treatment (sec)	Average maximum reaction time with treatment of ME (sec)							
		15	30	45	60	90	120	150	180
Morphine (5mg/kg)	2.3 ± 0.04	20.3±0.06	18.7±0.09	14.2±0.06	13.8±0.04	10.3±0.04	8.0±0.05	5.7±0.04	2.0±0.05
PG (5ml/kg) +Morphine	4.3 ± 0.04	23.7±0.06*	22.5±0.07*	17.7±0.07*	13.7±0.01	11.2±0.08	10.3±0.05*	4.7±0.06	3.0±0.05
ME (0.15g/kg)	4.2±0.06	23.5±0.07	22.5±0.06	17.8±0.05	16.3±0.06*	11.8±0.04	10.2±0.04	4.5±0.06	1.3±0.02
ME (0.20g/kg)	4.3±0.09	27.4±0.04*	25.5±0.04*	22.3±0.01*	18.8±0.04*	14.5±0.08*	11.2±0.03	8.7±0.03*	2.7±0.06
ME (0.30g/kg)	3.5±0.07	>30*	>30*	>30*	>30*	>30*	>30*	28.3±0.06*	26.3±0.08*
ME (0.15g/kg)+ Morphine	4.5±0.07	27.5±0.07*	24.2±0.04*	19.8±0.04*	17.2±0.06*	13.8±0.06*	12.5±0.06*	5.5±0.07	3.5±0.06
ME (0.20g/kg)+ Morphine	4.0±0.05	>30*	>30*	23.0±0.06*	21.7±0.04*	18.0±0.05*	12.7±0.03*	10.0±0.03*	4.0±0.05
ME (0.30g/kg)+ Morphine	6.2±0.06	>30*	>30*	>30*	>30*	>30*	>30*	>30*	>30*

*$P<0.001$(compared with vehicle + morphine)

Table 4—Effect of methanolic extract of *V. negundo* on pethidine induced analgesia in mice
[Values are mean ±SE from 6 animals in each group]

Drugs	Average reaction time before treatment (sec)	Average maximum reaction time with treatment of ME (sec)							
		15	30	45	60	90	120	150	180
Pethidine (10mg/kg)	4.0±0.05	24.5±0.07	22.5±0.07	18.5±0.04	17.2±0.03	15.0±0.03	7.3±0.03	5.8±0.04	1.7±0.03
PG 5ml/kg +Pethidine	2.7±0.04	25.2±0.04	23.8±0.03	16.2±0.04	12.3±0.04	9.8±0.04	10.7±0.04*	6.5±0.04	2.2±0.03
ME (0.15g/kg)	3.5±0.07	25.8±0.03	24.0±0.05	16.2±0.06	11.8±0.06	8.7±0.06	7.0±0.05	4.5±0.04	2.3±0.04
ME (0.20g/kg)	4.5±0.07	27.8±0.03	24.3±0.06	22.3±0.03*	18.3±0.06*	15.0±0.05*	10.8±0.04	6.0±0.08	5.7±0.08
ME (0.30g/kg)	6.2±0.06	>30*	>30*	>30*	>30*	>30*	>30*	>30*	>30*
ME (0.15g/kg)+ Pethidine	2.3±0.04	27.5±0.07	24.3±0.04	18.0±0.03	15.8±0.03*	12.7±0.07*	7.0±0.08	5.3±0.07	2.7±0.04
ME (0.20g/kg)+ Pethidine	4.3±0.09	>30*	26.2±0.04*	24.3±0.03*	19.7±0.04*	16.3±0.06*	12.2±0.04	8.5±0.04	3.7±0.04
ME (0.30g/kg)+ Pethidine	4.2±0.06	>30*	>30*	>30*	>30*	>30*	>30*	>30*	>30*

*P<0.001(compared with vehicle + pethidine)

Table 5—Effect of methanolic extract of *V. negundo* on average survival time after strychnine and leptazole induced convulsion
[Values are mean ± SE from 6 animals in each group]

Convulsive drugs	Dose in mg or ml/kg body wt.	Survival time in minutes			
		Saline (5 ml/kg)	PG (5 ml/kg)	ME (0.15 g/kg)	ME (0.20 g/kg)
Strychnine	2	2.7±0.03	4.2±0.06*	33.8±0.06*	85.0±0.18*
Leptazole	80	8.7±0.01	11.7±0.08*	61.7±0.09*	111.5±0.06*

*P (treated vs vehicle control)< 0.001

Table 6—Effect of methanolic extract of *V.negundo* on percentage mortality induced by convulsive drugs on mice (n=10)

Convulsive drugs	Dose in mg or ml/kg body wt	Percentage mortality after 24 hr					
		Saline 5 ml/kg	PG 5 ml/kg	ME 0.15 g/kg	ME 0.2 g/kg	ME 0.25 g/kg	ME 0.3 g/kg
Strychnine	2	100	100	100	80	20	0
Leptazole	80	100	100	100	50	10	0

protect the mice against strychnine and leptazole induced convulsions[16]. ME increases the brain GABA level (unpublished data) and thereby it acts as an anticonvulsive agent.

From these results it can be suggested that methanolic extract of the leaf of *V. negundo* exhibits CNS depressant action in a dose dependent manner.

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