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Effects of Tiliacorine on Voluntary Muscle and Blood Pressure

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Abstract – *Tiliacora racemosa* Colebr. belongs to the family Menispermaceae, the biggest storehouse of diphenylbisbenzylisoquinoline (DBBI) alkaloids. Exhaustive chemical processing of the root of *T. racemosa* by the application of modern separation techniques yielded a DBBI alkaloid which was identified as tiliacorine using sophisticated spectroscopic methods (UV, IR, ^{1}H-NMR, Mass). Tiliacorine possesses neuromuscular blocking activity. It produces a dose dependent hypotensive effect in rats and cats, and this effect is blocked by atropine.
Key words – *Tiliacora racemosa*; tiliacorine; muscle relaxant; hypotensive.

Introduction

Tiliacora racemosa Colebr. belongs to the family Menispermaceae which is known to be the biggest storehouse of diphenylbisbenzylisoquinoline (DBBI) alkaloids, well known for their pharmacological activities such as antitumor (Kupchan *et al.*, 1973), antimicrobial (Wu, *et al.*, 1976) and hypotensive (Joshi *et al.*, 1974; Wu *et al.*, 1976; 1977) effects. The root of this plant having folkloric medicinal application, e.g., antidote to snake bite and scorpion sting (Kirtikar *et al.*, 1984), was chemically processed for the isolation and identification of alkaloids including tiliacorine, tiliacorinine, nor-tiliacorinine A, tiliamosine, *N*-methyltiliamosine, tiliaresine, tiliarine, *N*-methyltiliarine. d-Tubocurarine, a DBBI alkaloid isolated from *Chondodendron tomentosum* is characterized by quantitative interference with the transmission of nerve impulses to the effectors in voluntary muscle. It has been found that many of the DBBI alkaloids and alkaloidal fractions like thalidazine, thalrugosamine, thalrugosine, *N*-desmethylthalidasine, *N*-desmethylthalistyline, tetrandrine, *O*-methylthalicberine, dimethyltrilobine iodide, etc. have profound effects on blood pressure (Guha *et al.*, 1976; Palit *et al.*, 1969; Wei *et al.*, 1987; Ying, 1987). Tiliacorine potentiated the sleeping time induced by standard hypnotics such as chlorpromazine (**CPZ**), pentobarbitone (**PB**) and diazepam (**DZ**) in a dose-dependant manner. Tiliacorine potentiated the analgesic action of standard analgesic agents like morphine and meperidine. It was also found to possess anti-convulsive activity in the strychnine induced convulsion model (Khasnobis *et al.*, 1999). In continuation of our previous study we report here the effect of tiliacorine (**1**) on voluntary muscle and blood pressure.

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Experimental

Plant materials – Fresh roots of *Tiliacora racemosa* (Menispermaceae) were collected from Indian Botanic Garden, Howrah, and were identified by Mr. Aloke Bhattacharya, Botanist, Botanical Survey of India, Calcutta. A voucher specimen (No. BM/UCM/005) has been preserved in our laboratory.

Instrumentation – The UV spectrum of tiliacorine was recorded in Hitachi U 2000 spectrophotometer in aldehyde free alcohol. IR spectrum was taken in Perkin Elmer 782 spectrophotometer in KBr pellets. ^{1}H-NMR spectrum was recorded in $CDCl_3$ solution on a Bruker AM 300 L spectrometer with TMS as internal standard. Mass spectrum of the compound was kindly supplied by Dr. B.C. Das, Institut

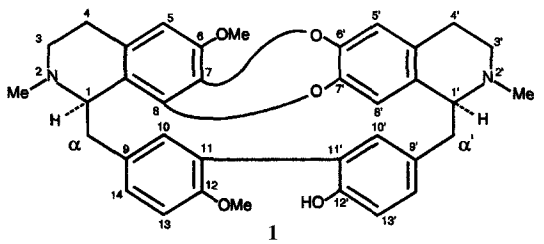
de Chimie des Substances Naturelles, Gif-Sur-Yvette, France, carrying out at 70 eV using direct inlet system. Column chromatography was performed over Silica gel (60-120 mesh), using mixture of chloroform and methanol of increasing polarity.

Animals – Muscle relaxant activity of tiliacorine was carried out using male healthy rabbits (1.5 to 2.0 kg). The rabbits were fed with adequate diet of complete foods *viz.* leaves of cabbage, carrots, grass, etc. and water *ad libitum.* They were kept in the animal house at a temperature of 26±1°C.

Six healthy male cats (2-2.5 kg) and ten albino male rats of Sprague Dawley strain (180-250 g) were used for blood pressure experiments. The animals were maintained at identical laboratory condition for three days.

Drugs – The drugs used were tiliacorine, d-tubocurarine chloride (d-TC), normal saline, acetylcholine chloride, adrenaline bitartrate, atropine sulfate, cyproheptadine hydrochloride, dehydroergotamine tartrate, isoprenaline hydrochloride, histamine acid phosphate, noradrenaline bitartrate, propranolol hydrochloride (Sigma, U.S.A), pentobarbital sodium (Abbott), urethane (E. Merck, Germany), heparin sodium (Biological Evans, India), promethazine hydrochloride (Phenergan, Rhone-Poulence, India), sodium chloride and sodium citrate (BDH, India)

Isolation and identification of tiliacorine – 100 mg (0.01%) tiliacorine (**1**) was isolated from the air-dried roots of *Tiliacora racemosa* and was identified from spectroscopic analysis and by comparing with authentic sample (Guha *et al.*, 1976; Guinaudeau *et al.* 1985 ; Ray *et al.*, 1989 & 1990).



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Preparation of drug – Tiliacorine (75 mg) was dissolved in minimum volume of acetone and excess methyliodide was added to it, filtered and the residue (200 mg) was dried. Methiodide salt of tiliacorine thus prepared was freely soluble in water and was taken as drug for pharmacological investigations.

Pharmacological Studies

Safety evaluation – LD_{50} was determined by the standard method of Litchifield and Wilcoxon (Litchifield *et al.*, 1949). d-Tubocurarine chloride (d-TC) was taken as the reference drug for comparison with tiliacorine. The drugs were administered i.v. to groups of 10 albino mice at doses up to 12.5 mg/kg for tiliacorine and 0.22 mg/kg for dTC and the animals were observed 24 h for mortality.

Muscle relaxant activity – Muscle relaxant activity of tiliacorine was studied following the method described by Varney *et al.* (1949) taking d-TC as standard drug as it is a well-known potent muscle relaxant drug (Crossland, 1971). The rabbits were divided into seven groups, each group containing six rabbits. For all the groups, the time of hind limb paralysis and the onset of recovery were noted. The i.v. doses of d-TC and tiliacorine required to produce onset of head-drop were determined and the recovery times with reference to the vehicle control were noted both for d-TC and tiliacorine. When both the drugs were injected i.v at an intermediate rate into a normal rabbit, a gradual developing relaxation of the muscles of the back and neck resulted and the end point was sharply defined and easily determined. The doses of tiliacorine and d-TC for rabbit were evaluated approximately from the conversion table of different animal species (Ghosh,1984).

The groups and treatments were designed as follows:

Group I: Vehicle control as normal saline (0.9% w/v; 10 ml/kg)

Group II: Tiliacorine at a dose of 0.5 mg/kg, i.v.

Group III: Tiliacorine at a dose of 1.0 mg/kg, i.v.

Group IV: Tiliacorine at a dose of 2.0 mg/kg, i.v.

Group V: d-TC at a dose of 8.75 µg/kg, i.v.

Group VI: d-TC at a dose of 17.5 µg/kg, i.v.

Group VII: d-TC at a dose of 35.0 µg/kg, i.v.

Blood pressure – Arterial blood pressure was recorded via indwelling arterial cannula from common carotid artery of pentobarbitone sodium (40 mg/kg, i.v.) anaesthetized male cats (1.5-2.0 kg) and of urethane (1.4 g/kg, i.p.) anaesthetized male rats (180-250 g) by a mercury manometer on a rotating smoked drum. The drugs were infused through femoral vein.

Statistical analysis: Values are expressed as Mean± SEM and the significance of difference of data

obtained was evaluated statistically using the students t-test.

Results

Safety evaluation – Acute toxicity tests in mice established the LD_{50} of tiliacorine and d-TC to be 10.60 mg/kg i.v. and 0.189 mg/kg i.v. respectively.

Muscle relaxant activity – The intravenous administration of tiliacorine at dose of 2.0 mg/kg i.v induced on set of head-drop within 3.33±1.17 min., whereas the lower doses of 0.5 and 1.0 mg/kg had no effect. The recovery time after head-drop was recorded 19.66±1.28 min. after administration of tiliacorine (Table 1). Similarly three doses of d-TC (8.75, 17.5 and 35.0 μg/kg respectively) were injected intravenously to the marginal ear veins of rabbits. It has been observed that d-TC at doses of 8.75 and 17.5 μg/kg i.v. did not exhibit any head-drop sign. But d-TC at a dose of 35.0 μg/kg produced onset of head-drop within 3.00 min. No respiratory paralysis was observed during the experiment.

Table 1. Effect of tiliacorine and d-TC on Rabbit's Head-drop

Group	Drugs	Head-drop after injection (minutes) Mean±SEM	Duration of head-drop (minutes) Mean±SEM
I	Normal saline (0.9% w/v) (10 ml/kg)	–	–
II	Tiliacorine (0.5 mg/kg)	–	–
III	Tiliacorine (1.0 mg/kg)	–	–
IV	Tiliacorine (2.0 mg/kg)	3.33±0.24	19.66±1.28
V	d-TC (8.75 μg/kg)	–	–
VI	d-TC (17.5 μg/kg)	–	–
VII	d-TC (35.0 μg/kg)	3.00±0.18	30.33±1.17

Number of animals used (n = 6)

Blood pressure – Tiliacorine showed hypotensive action in a dose dependent manner with short duration of action (1-4 min). Tiliacorine produced a biphasic decrease of blood pressure in both rat and cat (Table 2 and Table 3). Hypotensive action of tiliacorine remained unaffected in the presence of β-blocker (Table 4) and histamine blocker (Table 5). However, atropine almost completely blocked the hypotensive action of tiliacorine (Table 5).

Discussion

Though the alkaloid tiliacorine belongs to the same chemical group as d-TC, an established neuromuscular blocking agent, its neuromuscular blocking activity was not reported earlier.

The head drop determination in rabbits has been employed for the biological evaluation of the potency of tiliacorine as neuromuscular blocking agent in comparison to d-TC. It is reported that dau-

Table 2. Effect of tiliacorine on blood pressure in cats (Values are mean±SEM of 6 experiments in each case)

Treatment	Blood pressure (mm Hg)	Decrease of blood pressure (mm Hg)	Duration of fall (Sec)
Normal saline (0.9% w/v, 0.5 ml)	110±2.88	–	–
Tiliacorine (0.8 mg/kg)	80±1.38*	30±2.81	42±2.06
Tiliacorine (1.6 mg/kg)	60±3.6*	50±1.29	60±2.88

*P<0.001

Table 3. Effect of tiliacorine on blood pressure in rats (Values are mean ± SEM of 6 experiments in each case)

Treatment	Blood pressure (mm Hg)	Decrease of blood pressure (mm Hg)	Duration of fall (Sec)
Normal saline (0.9% w/v, 0.5 ml)	128±2.35	–	–
Tiliacorine (0.8 mg/kg)	95±1.75*	33±1.04	102±2.19
Tiliacorine (1.6 mg/kg)	48±1.68*	80±1.87	182±2.22

*P<0.001

Table 4. Effect of blood pressure in cats in the presence of reference drugs (Values are mean±SEM of 6 experiments in each case)

Treatments	Dose	Blood pressure (mm Hg)	Rise of blood pressure (mm Hg)
Normal saline (0.9% w/v)	0.5 ml	90±2.88	–
Adrenaline	2.0 µg/kg	120±0.77*	30±1.27
Tiliacorine + Adrenaline	0.8 mg/kg + 2.0 µg/kg	114±0.95**	24±1.08
Noradrenaline	2.0 µg/kg	128±0.57*	38±1.19
Tiliacorine + Noradrenaline	0.8 mg/kg + 2.0 µg/kg	118±0.92**	28±1.25

*Significant change from normal saline control ($p<0.01$)
**Significant change as compared to respective reference drug control ($p<0.01$)

Table 5. Effect of tiliacorine on blood pressure in rats in the presence of atropine (Values are mean±SEM of 10 experiments in each case)

Treatments	Dose	Blood pressure (mmHg)
Normal saline (0.9% w/v)	0.2 ml	112±2.35
Acetylcholine	0.25 µg/kg	56±1.26
Tiliacorine	0.8 mg/kg	78±0.95
Atropine + Acetylcholine	1.0 mg/kg + 0.25 µg/kg	110±2.02
Atropine + Tiliacorine	1.0 mg/kg + 0.8 mg/kg	108±0.36

risoline and d-TC have a tendency to induce head-drop (Gong, *et al.*, 1979; Patel, *et al.*, 1987). The potency of the experimental drug and head-drop end point in rabbit indicated neuromuscular blocking effect.

In the present study tiliacorine at a dose of 2 µg/kg showed head-drop reaction and its recovery within a time period. Similar action was also observed when d-TC was injected at a dose of 35.00 mg/kg i.v.. However, the recovery period for tiliacorine and d-TC were not similar. The result indicated that recovery period is much faster in tiliacorine than d-TC treatment.

The effect of tiliacorine on blood pressure in cats and rats revealed that like many other diphenylbis-benzylisoquinoline (DBBI) group of alkaloids, tiliacorine also produced a fall of blood pressure in a dose dependent manner (Hu, *et al.*, 1988; Wu, *et al.*, 1977).

Tliacorine induced fall of blood pressure was not inhibited by β-blocker and histamine blocker. Atropine, the muscarinic blocker (Lefkowitz, *et al.*, 1976) almost completely blocked the hypotensive effect of tiliacorine. Hence it can be postulated that the hypotensive action of tiliacorine may be mediated by muscarinic receptors or by stimulating the parasympathetic nervous system like veratrum alkaloids (Avery, *et al.*, 1969). From the results it appears that the hypotensive effect of tiliacorine does not involve histaminergic or beta-adrenergic receptors. Since tiliacorine antagonizes the effect of adrenaline and noradrenaline to a moderate extent, it may be termed as possessing antihypertensive action.

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