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Effect of Tilliacorine on Haematological and Biochemical Parameters

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Abstract – *Tiliacora racemosa* Colebr. belonging to the family Menispermaceae, is the biggest storehouse of diphenyl bisbenzylisoquinoline (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, MS). Haematological study with tiliacorine proved that there was no abnormal haematological results in comparison with the normal values. Chronic toxicity study with tiliacorine revealed that the alkaloid is devoid of any hepatotoxic and nephrotoxic action.

Key words – *Tiliacora racemosa*, tiliacorine, haematological, biochemical parameters

Introduction

Tiliacora racemosa Colebr., a plant belonging to the family Menispermaceae, is known to be the biggest storehouse of diphenylbisbenzylisoquinoline (DBBI) alkaloids which are 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 *viz.* tiliacorine, tiliacorinine, nor-tiliacorinine A, tiliamosine, *N*-methyltiliamosine, tiliaresine, tiliarine and *N*-methyltiliarine. Tiliacorine potentiated the sleeping time induced by standard hypnotics *viz.* chlorpromazine (**CPZ**), pentobarbitone (**PB**) and diazepam (**DZ**) in a dose dependent manner. Tiliacorine also potentiated the analgesic action of standard analgesic agents *viz.*, morphine and meperidine. Furthermore, tiliacorine was found to possess anti-convulsive activity in the strychnine induced convulsion model (Khasnobis *et al.*, 1999). It possessed neuromuscular blocking activity and produced hypotensive effect in rats and cats and this effect was blocked by atropine. The present study deals with the effect of tiliacorine (**1**) on haematological and biochemical parameters.

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, Institute de Chimie des Substances Naturelles, Gif-Sur-Yvette, France carried out at 70 eV using direct inlet system.

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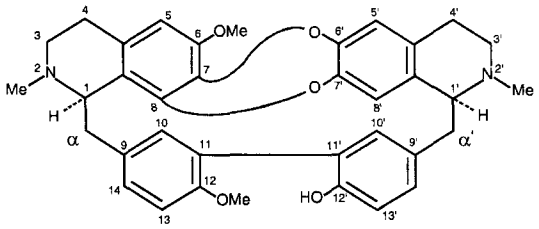
Column chromatography was performed over Silica gel (60~120 mesh), using mixture of chloroform and methanol of increasing polarity.

Animals – Haematological parameters and other toxic effects of tiliacorine were studied on in-bred Swiss albino male mice weighing between 20 to 30 g. They were fed standard pellet food and water *ad libitum*. All mice were kept at room temperature at 25±1°C. The animals were not provided with food for last 17 hrs before the commencement of experiment.

Drugs – Drug used for haematological and biochemical studies was tiliacorine.

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 (Khasnobis *et al.*, 1999).



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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 upto 12.5 mg/kg for tiliacorine and 0.22 mg/kg for d-TC and the animals were observed for 24 hrs.

Haematological studies – Effect of tiliacorine on haematological parameters were studied on mice. The animals were divided into four groups, each group containing 20 mice. The first group received normal saline (10 ml/kg b.w.) once weekly for four weeks. Tiliacorine was injected once weekly for four weeks at the dose level of 1.33, 1.77 and 2.65 mg/kg b.w. to the 2nd, 3rd and 4th groups respectively. Blood was collected from tail vein of each mouse for haematological studies like the total count of red blood cell (RBC), white blood cell (WBC), differ- ential counts of leucocytes as well as the haemo- globin (Hb) content and clotting time using standard methods (D'Amour *et al.*, 1965; Dacie, 1958). Animals were sacrificed 24 hrs after the last dose.

Biochemical studies – All mice were anaesthetised with anasethetic ether, blood was collected by intracardiac puncture. The blood was kept for 30 mins. without disturbing. The clot was dispersed with glass rod and then centrifuged for 15~20 mins. at 2000 rpm to separate serum. Heparinised whole blood was collected for the estimation of blood glucose, blood urea, non-protein nitrogen, cholesterol and total plasma protein.

Total bilirubin content of the serum was estimated by the method of Varely (1980). Serum SGOT and SGPT were determined by the method of Bergmeyer (1968) and alkaline phosphatase by the method of Malany (1964). Whole blood cholesterol and non-protein nitrogen were estimated by the Oser's (1954) method. Total plasma protein and blood urea were estimated by Wooton method (1964). Blood glucose was estimated by the method of Nelson (1944) and Somogy (1945).

Results

Safety evaluation – The LD_{50} of tiliacorine and d-TC are 10.60 mg/kg i.v. and 0.189 mg/kg i.v. respectively as reported earlier (Khasnobis *et al.*, 1999).

Haematological studies – Haematological studies did not show any significant changes in drug treated animals [Table 1 (i and ii)].

Biochemical estimations were carried out to evaluate the effect of tiliacorine on metabolism and on liver and kidney functions. Tiliacorine did not produce any significant change in blood glucose, blood cholesterol and plasma protein as compared to control animals which received normal saline (Table 2).

The levels of serum bilirubin, SGOT, SGPT and

Table 1. Effect of tiliacorine on haematological parameters. Values are mean±SEM of 10 animals

(i)

Group	Drugs mg/kg	Erythrocyte count (million/cmm)	Hemoglobin conc. (g/100 ml blood)	Total leukocytes (thousand/cmm)	Clotting time (sec)
I	Normal saline (0.9%, w/v; 10.00 ml/kg)	9.23±0.27	14.50±0.25	8.20±0.21	11.00±0.17
II	Tiliacorine (1.33)	9.60±0.16	14.20±0.20	8.00±0.15	10.80±0.18
III	Tiliacorine (1.77)	8.90±0.07	14.00±0.17	8.00±0.21	11.00±0.20
IV	Tiliacorine (2.65)	9.00±0.08	14.60±0.11	8.00±0.16	11.10±0.27

(ii)

Group	Drugs (mg/kg)	White Blood Cells				
		Neutrophil (%)	Basophil (%)	Monocyte (%)	Eosinophil (%)	Lymphocyte (%)
I	Normal saline (0.9%, w/v; 10.00 ml/kg)	25.50	0.50	0.40	2.00	68.00
II	Tiliacorine (1.33)	24.40	0.50	0.28	2.00	65.00
III	Tiliacorine (1.77)	24.00	0.48	0.35	2.00	66.00
IV	Tiliacorine (2.65)	25.00	0.52	0.38	2.00	70.00

Table 2. Effect of tiliacorine on blood glucose, plasma cholesterol and plasma protein in mice. Values are mean±SEM of 10 animals

Group	Drugs (mg/kg)	Glucose level (mg/100 ml blood)	Plasma cholesterol (mg/100 ml of blood)	Plasma protein (g/100 ml of blood)
I	Normal saline (0.9% w/v, 10 ml/kg)	85.00±5.25	111.00±2.90	7.50±0.21
II	Tiliacorine (1.33)	90.00±2.24	109.00±9.12	7.61±0.22
III	Tiliacorine (1.77)	90.80±4.10	114.00±8.72	7.80±0.37
IV	Tiliacorine (2.65)	91.20±3.20	125.00±8.64	8.21±0.41

Table 3. Biochemical evaluation of liver function test after treatment with tiliacorine in mice. Values are mean±SEM of 10 animals

Group	Drugs (mg/kg)	Total serum bilirubin (mg/100 ml serum)	Serum SGOT (units/ml serum)	Serum SGPT (units/ml serum)	Alkaline phosphatase (units/ml serum)
I	Normal saline (0.9% w/v, 10 ml/kg)	1.61±0.20	55.10±2.01	35.40±0.61	645.40±70.00
II	Tiliacorine (1.33)	1.58±0.18	55.50±2.02	36.20±0.58	650.80±68.00
III	Tiliacorine (1.77)	1.60±0.21	54.20±1.98	36.40±0.49	654.20±58.00
IV	Tiliacorine (2.65)	1.62±0.18	55.10±2.20	35.80±0.64	645.00±66.00

Table 4. Biochemical evaluation of kidney function test after treatment with tiliacorine in mice. Values are mean±SEM of 10 animals

Group	Drugs (mg/kg)	Blood urea (mg/100 ml blood)	Non-protein nitrogen (mg/100 ml blood)
I	Normal saline (0.9% w/v, 10 mg/ml)	24.00±0.67	27.15±1.62
II	Tiliacorine (1.33)	23.90±0.64	28.63±1.58
III	Tiliacorine (1.77)	24.40±0.53	28.91±2.03
IV	Tiliacorine (2.65)	23.70±0.54	27.58±0.92

alkaline phosphatase levels, the four parameters for studying drug effect on liver function, were not significantly changed in tiliacorine treated mice as compared to control mice (Table 3).

No significant change in the levels of blood urea and non-protein nitrogen, the two parameters used for kidney function study, was observed in tiliacorine treated mice as compared to control mice (Table 4).

Discussion

Administration of alkaloids belonging to Menispermaceae family in mice has been shown to produce various haematological changes. The alkaloid fangchinoline exhibited alteration in erythrocyte membrane and osmotic balance of RBC (Sato *et al.*, 1980). In the present study tiliacorine was administered at different doses over a period of four weeks to mice and there was no significant haematological changes which reveals that it did not have any toxic effect on haemopoetic system.

The results on the effect of tiliacorine on carbohydrate, fat and protein metabolism reveal that tiliacorine does not have any toxic effect on metabolism in mice.

The key hepatic biochemical parameters, total serum bilirubin, SGOT, SGPT and serum alkaline phosphatase, did not exhibit any significant change after treatment with different doses of tiliacorine over a period of four weeks which reveals that this alkaloid does not have any hepatotoxic effect.

Blood urea and blood non-protein nitrogen, two parameters for studying nephrotoxicity remained unaltered after treatment with tiliacorine. This clearly demonstrates that tiliacorine does not have any nephrotoxic effect.

From the above discussion it can be concluded that tiliacorine is devoid of any toxic action on the haemopoetic system, kidney, liver and on general metabolism.

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