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Hypocholesterolemic and Antihypercholesterolemic Activity of Extracts of *Trichila Connaroids* on Rats

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Hypocholesterolemic activity was studied on normal rats for hexane, chloroform and methanol extracts of *Trichilia connaroides* (W. & A.) Bentilezen. The parameters studied include free cholesterol, triglycerides and high density lipoprotein levels in blood. Only hexane and methanol extracts produced a rise in high density lipoprotein level ($p<0.05$ at 90 mg/kg) and did not show any fall in cholesterol and triglyceride levels when compared to control group. On Triton-induced hypercholesterolemia, only cholesterol and triglyceride levels were studied for all the three extracts. Chloroform and methanol extracts produced a significant fall in cholesterol level ($p<0.05$) within 24 h of induction when compared to control group.

Trichilia connaroides (W. & A.) Bentilezen (Meliaceae) is known as *Karai* or *Karaivilangu* in Tamil. It is found in moist forests throughout the greater part of India. The bark and leaves possess bitter and tonic properties[1]. The isolation of tri and tetranortriterpenoids from chloroform extract of the leaves has been reported[2-4]. Preliminary pharmacological screening of the chloroform extract of the leaves of *T. connaroides* revealed significant hypotensive activity in rats (unpublished data). In the present work, an attempt has been made to investigate the possible effect of the extracts of *T. connaroides* on normal and Triton-induced cholesterol levels in rats. The parameters recorded were free cholesterol, triglycerides and high density lipoprotein (HDL) levels in mg/dl.

The leaves of *T. connaroides* (2 kg) were collected from Salem, Tamil Nadu, shade dried and coarsely powdered. The powder was first defatted with n-hexane at room temperature. The powder was then extracted with chloroform, distilled and last traces of solvent were removed by vacuum. The marc was then extracted with methanol twice. Chloroform and methanol used were of AR grade. AR grade Triton

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was purchased from S. D. Fine Chem. Ltd. Mumbai and dimethyl sulphoxide (DMSO) from Loba Chemie Pvt. Ltd. Mumbai.

For studying hypocholesterolemic activity in normocholesterolemic rats the method reported by Buchanan *et al.* was used[5]. The animal experiment protocols were approved by the IAEC and CPCSEA registration number of the institution is 626/02/a/CPCSEA.

Groups of six male Sprague-Dawley rats weighing 180-200 g were used. Body weight of each animal was registered before and after the experiment. Food was withdrawn 24 h prior to the experiment but water was given *at libitum*. Before administering the extract, blood samples were withdrawn by retroorbital puncture under light ether anesthesia. Then, the animals were given once daily, in the morning over a period of eight days, 90 mg/kg body weight of hexane, chloroform and methanol extracts of *T. connaroides* via stomach tube. Control group was given 5 ml/kg of 50% DMSO of AR grade. During the whole period, the animals had free access to food and water. Food was again withdrawn 20 h prior to the end of the experiment and blood samples of each group were pooled and centrifuged for 2 min at 1500 rpm. The total cholesterol, triglycerides and HDL concentrations were determined for each blood sample using autoanalyser (RA-50, Bayer-India).

Cholesterol and triglycerides were estimated by employing an enzymatic method[6-10] and for HDL estimation, phosphotungstate method was used[6,11-13]. For the estimation of cholesterol, triglycerides and HDL, serum separated from the blood within 30 min of collection was used. Reagents used for the estimation of cholesterol were a mixture of reagent 1 and reagent 1A. Reagent 1 contains cholesterol esterase (200 U/l), cholesterol oxidase (250 U/l), peroxidase (1000 U/l) and 4-amino antipyrine (0.5mM). Reagent 1A constitutes Pipes buffer; pH 6.90 (50 mM), phenol (24 mM) and sodium cholate (0.5 mM). Equal volumes of reagent 1 and reagent 1A were mixed, kept aside for 5 min before using. The samples and the reconstituted reagents were brought to room temperature prior to use and incubated for 5 min at 37°, mixed and read using autoanalyser.

Reagents used for the estimation of triglycerides contains buffer, pH 7.2 (50 mM), lipase (2000 IU/l), glycerol kinase (300 IU/l), glycerol phosphate oxidase (1000 IU/l), peroxidase (500 IU/l), ATP (1mM) and chromogens (2 mM). The assay mixture was incubated for 10 min at 37° and read using autoanalyser. Chylomicrons, VLDL and LDL fractions in serum were separated for the estimation of HDL by using precipitating reagent which contains phosphotungstic acid (2.4 mM) and magnesium chloride (39 mM), it was then centrifuged at 3500-4000 rpm for 10 min. The cholesterol in the HDL fraction, which remains in the supernatant, was assayed by enzymatic method[6-10].

The method reported by Tamasi *et al.* was used[14] for the estimation of antihypercholesterolemic activity. Male Sprague-Dawley rats (190-230 g) fasted for 16 h received a single intraperitoneal injection of Triton WR-1339 (isooctylpolyoxyethylene phenol) at a dose of 100 mg/kg body weight. The hexane, chloroform and methanol extracts of *T. connaroides* at 90 mg/kg dose level and 50% DMSO were administered orally to test groups and control groups respectively, simultaneously with Triton injection. Cholesterol in serum was estimated at 6, 24 and 48 h after Triton injection. Mean values were calculated for each group at different time intervals and compared statistically by co-variance method with control group ($p<0.05$).

The effect of hexane, chloroform and methanol extracts of *T. connaroides* on cholesterol levels of normal rats was studied. These extracts showed no fall in cholesterol and triglyceride levels, but HDL levels were found to be increased by hexane and methanol extracts ($p<0.05$) at 90 mg/kg dose level when compared to normal animals. The results are shown in Table 1. As the hexane and methanol extracts produced elevated HDL levels, an attempt has been made to find their effects on Triton WR-1339-induced hypercholesterolemia in rats. These extracts produced significant hypocholesterolemia ($p<0.05$) within 24 h of induction of hypercholesterolemia as shown in Table 2.

The mechanism of Triton-induced hypercholesterolemia in phase I is thought to be due to increased hepatic synthesis of cholesterol through the ability of Triton to interfere with the uptake of plasma lipids by the tissues. Drugs interfering with cholesterol biosynthesis were shown to be active in Phase I, while drugs interfering with cholesterol excretion and metabolism were active in Phase II[15]. Since chloroform and methanol extracts of *T. connaroides* produced a significant fall in cholesterol level ($p<0.05$) within 24 h of induction, they are thought to interfere with cholesterol biosynthesis (Phase I) rather than excretion and metabolism (Phase II).

Hyperlipoproteinemia with increased concentrations of cholesterol and triglycerides carrying lipoproteins is considered to be the cause of arteriosclerosis with its duel sequel

TABLE 1: HYPOCHOLESTEROLEMIC ACTIVITY OF EXTRACTS OF *T. CONNAROIDES* IN RATS.

Treatment	Dose (mg/kg)	Free cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	% increase of HDL (mg/dl)
Control	-	38.0±10.00	52.0±1.00	21.0±1.00	—
Hexane extract	90	64.0±3.99	74.5±4.49	39.5± 0.50*	46.8±3.20
Chloroform extract	90	67.0±1.00	61.0±1.00	33.5±3.50	37.3±9.64
Methanol extract	90	54.0±4.00	84.5±4.50	42.0±2.00*	50.0±4.77

*Values are mean±SE of five animals in each group (*p<0.05)

TABLE 2: ANTIHYPERCHOLESTEROLEMIC ACTIVITY OF EXTRACTS OF *T. CONNAROIDES* IN RATS ON TRITON-INDUCED HYPERCHOLESTEROLEMIA.

Treatment	Dose	Plasma cholesterol (mg/dl)			Plasma triglycerides (mg/dl)		
	(mg/kg)	6 h	24 h	48 h	6 h	24 h	48 h
Control	-	93.0±8.99	67.0±17.0	71.5±1.50	43.0±3.00	80.5±0.50	70.5±0.50
Hexane extract	90	73.0±1.00	72.5±2.50	99.0±1.00	62.0±2.00	84.5±4.50	105±5.50
Chloroform extract	90	50.0±10.0*	81.5±1.50	62.5±2.50	29.0±1.00*	51.5±0.50	41.5±1.50
Methanol extract	90	67.0±1.00*	56.5±2.50	50.5±0.50	29.0±1.00*	61.5±1.50*	53.5±33.5

*Values are mean ± SE of five animals in each group (*p< 0.05).

of thrombosis and infarction. HDL promotes the removal of cholesterol from peripheral cells and facilitates its delivery back to the liver. Therefore, increased levels of HDL are desirable. On the contrary, high levels of VLDL and LDL promote atherosclerosis. Therefore, arteriosclerotic drugs should reduce VLDL and LDL and/or elevate HDL[16]. Hence the present study clearly demonstrates that the hexane and methanol extracts of *T. connaroides* produced elevated HDL levels in normal rats, where as chloroform and methanol extracts showed antihypercholesterolemic activity.

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