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Research Article

AN EXPERIMENTAL EVALUATION OF THE ANTIHYPERLIPIDEMIC EFFECT OF ETHANOLIC EXTRACT OF TINOSPORA CORDIFOLIA STEMS IN CHOLESTEROL DIET INDUCED HYPERLIPIDEMIA IN RATS

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A B S T R A C T

T. Cordifolia is commonly known as Guduchi in India. Guduchi is one of the most important herbs of Ayurveda used as Rasayana (rejuvenator) having wide ranging of health benefits. The prevalence of obesity is increasing to epidemic proportions globally. Hypercholesterolemia is a major risk factor for the development and progression of atherosclerosis and related cardiovascular diseases. There is an urgent need for an effective understanding of hyperlipidemia and its management. The present study was aimed to evaluate the antihyperlipidemic effect of ethanolic extract of stem of *T.cordifolia* in diet induced hyperlipidemic rats. Hyperlipidemia was induced in rats by feeding cholesterol (400mg/kg b.w) on diet basis in coconut oil as vehicle orally throughout the experimental period and simultaneously, the treatment groups received *T. cordifolia* (100 and 200 mg/kg b.w.) orally for 15 days. Atorvastatin (1 mg/kg b.w.) was used as a standard antihyperlipidemic drug. Serum cholesterol, triglycerides, HDL and LDL were analyzed at the end of the experiment. The aorta part is extracted and subject to histopathological examination. Aqueous extract of *T. cordifolia* at 200 mg/kg b.w. dose significantly decreased the serum cholesterol, triglycerides and LDL level when compared to that of standard drug. Thus the study revealed that the ethanolic extract of *T. cordifolia* was found to be effectively controlling the hyperlipidemic conditions.

INTRODUCTION

Hypercholesterolemia is a major risk factor for the development and progression of atherosclerosis and related cardiovascular diseases [1]. In India, there has been an alarming increase in the prevalence of CVD over the past two decades so much so that accounts for 24% of all deaths among adults aged 25–69 years [2]. Regional disparity exists with the highest rates of hypercholesterolemia observed in Tamilnadu (18.3%), highest rates of hypertriglyceridemia in Chandigarh (38.6%), highest rates of low HDL-C in Jharkhand (76.8%) and highest rates of high LDL-C in Tamilnadu (15.8%) [3]. Ayurvedic medicine as define in the drugs and cosmetic act 1940, includes all medicines intended for internal or external use, for or in the diagnosis, treatment, or prevention of diseases or disorders in human being or animal and manufactured in accordance with the formulae describe in the authoritative books of ayurvedic system of medicine specified in the first schedule of the act [4].

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Tinospora cordifolia (Menispermaceae) is an important medicinal plant of tropical and sub-tropical India. Its medicinal usage has been reported in the Indian herbal pharmacopoeia, the ayurvedic pharmacopoeia and in traditional systems of medicine such as Ayurveda, unani and siddha [5]. *Tinospora cordifolia* (Giloy) plant is found mainly in Malaysia, India and Srilanka. There are about 40 species of Giloy which are found throughout the world, comprising parts of Africa, Southern Eastern Asia, and Australia. Out of 40 species only 4 species have been found in India [6].

This plant species has been over exploited by the pharmaceutical companies and folk people for their traditional remedies [7]. *Tinospora cordifolia* is an important medicinal plant. Flavonoids were isolated from leaves, stem, seeds and in-vitro callus of the species. The isolated compounds were identified through FT-IR spectroscopy [8].

Thin layer and HPLC analysis were carried to compare the methanolic extracts of *T.cordifolia* and *T.sinensis*. TLC perfomed on precoated silica gel $60F_{254}$ TLC plates. HPLC chromatogram shows standard berberine, retention time

8.6min at wavelength 266nm [9]. Hexane extract (2g) was subjected to sillica gel (60-120) column chromatography. Column was packed in n-hexane and eluted by increasing polarity with ethyl acetate. A single compound was obtained at polarity 5% ethyl acetate in hexane. Spectral analysis by using HNMR and Carbon 13 NMR & a melting point of 132.33°C showed that the isolated compound is ß-sitosterol [10].

Tinospora cordifolia extract decreased the activity of glycogen phosphorylase in the liver and widely used in the treatment of diabetes mellitus. The *in silico* study aimed to screen the active compounds of *T. cordifolia* which play a role in its hypoglycemic activity as glycogen phosphorylase inhibitor by molecular docking analysis. The results indicate that magnoflorine, cordiofolioside A, and syringin exhibited good affinity to glycogen phosphorylase, by interacted at catalytic site of enzyme [11].

The extract of stems alone and with honey is useful as a tonic in jaundice, skin diseases and fever, stem starch (satva) is used as a tonic. A combination of root and stem is prescribed as an antidote to snake bite and scorpion sting [12]. A study assess the antioxidant capacity of *Tinospora cordifolia* stem methanol extract in daily oral administration of 500 mg/kg of body weight for 40 days in alloxan induced diabetic rats [13]. A study indicates that the greater activity resides in ethanolic stem extracts of plant since other extracts including chloroform and aqueous did not effectively inhibit the growth of the bacteria. This may due to the chemical constituents responsible for the antibacterial activity are more soluble in ethanol extracts. It can be interpreted that the antibacterial activity against microorganisms is due to any one or more alkaloids of the plants [14].

A study revealed that the aqueous extract of *T. cordifolia* was found to have potential effective in controlling the diabetes associated hyperlipidemic conditions effectively [15]. The confirmation of an immunomodulatory protein in guduchi stem showing lymphoproliferative and macrophage-activating properties reinforces the rationale of the use of guduchi preparations in several ayurvedic medicines for immunomodulation. To our knowledge, this is the first report of an immunomodulatory protein isolated from guduchi [16]. A study suggested that the synthesized silver nano particles showed higher antioxidant and antibacterial activity than the plant extract [17].

EXPERIMENTAL METHODS

Plant collection and authentication

The plant was collected from Uthangudi village, Madurai during January 2019 and the plant was identified and authendified by Dr. Stephen Ph.d., Taxonomist and Assitant professor, Department of botany, American college, Madurai, Tamilnadu.

Extraction

Collected plant material was washed under running tab water to remove foreign earthy adherable matter. The stem parts are cut into pieces and dried under sun for two weeks. The stem parts were crushed and sieved into coarse powder by using mechanical blender and sieve. Then kept in an airtight polythene bags for further use and stored at room temperature. The extraction was carried out by using soxhlet apparatus for 8 hours with ethanol as solvent. Rotary evaporator was used to concentrate the extract (ESETC) and recover the solvent. The obtained extract was subject to phytochemical screening.

Study of Antihyperlipidemic activity of ESETC

Animals

Wister albino rats were obtained from central animal house, K.M.College of pharmacy, Madurai. The animals were given standard rodent diet and water ad libitum throughout the study. The rats used in the present study were maintained in accordance with guidelines of the national institute of nutrition, Indian council for medical research, Hyderabad, India and study approved by Institutional animal ethicalcommittee.(IAEC/S.A.MOHAMEDSHIEKARABATH/ TNMGRMU/PhD/KMCP/2017-18).

MATERIALS

- Ethanolic extract of stem of *Tinospora cordifolia* (ESETC).
- Cholesterol extra pure for feeding purpose was obtained from S D fine-chem. limited, Mumbai, India. Coconut oil was used as a vehicle for cholesterol feeding.
- Atorvastatin was obtained from Acumen Pharma, Puduchery, India.

Experimental design

All the animals were weighed and divided into five groups each of six animals.

Group I : Normal control.
Group II : Cholesterol control. Fed **cholesterol** at a dose of 400mg/kg body weight for 30 days.
Group III: fed cholesterol as in group II and **Atorvastatin** 1mg/kg body weight from day 15 to day 30.

Group IV: fed cholesterol as in group II and ESETC at a dose of 100mg/kg body weight from day 15 to day 30.
Group V : fed cholesterol as in group II and ESETC at a dose of 200mg/kg body weight from day 15 to day 30.

At the end of 30 days all the rats were sacrificed, blood was collected, allowed to clot and serum was obtained by centrifugation. The serum samples were used for various biochemical procedures.

Biochemical analysis

The serum was analyzed for total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), by using standard protocol methods. (Auto Analyzer)

Atherogenic index (AI) and LDL-C/HDL-C ratio

- The AI was calculated by the following formula
- AI = (total cholesterol – HDL-C)/HDL-C
- LDL-C/HDL-C ratio was calculated as the ratio of plasma LDL-C to HDL-C Levels

Histopathological studies

Small portion of aorta was recovered and histopathological studies carried out according to standard procedure.

Statistical analysis

- All the values were expressed as mean ± SEM.

- Data was analyzed by one way analysis of variance (ANOVA) followed by Newmankeuls multiple test.
- P values <0.05 were considered as statistically significant.

RESULTS

Extract Yield

The percentage yield of crude ethanolic extract of stems of *Tinospora cordifolia* – 3.56%w/w.

Table 1 Phytochemical screening

Sl. No	Functional group	Inference
1	Saponin	Presence
2	Terpenoids	Presence
3	Tannins	Presence
4	Steroids	Presence
5	Glycosides	Absence
6	Alkaloids	Presence
7	Flavonoids	Presence
8	Anthraquinones	Absence
9	Phenol	Presence

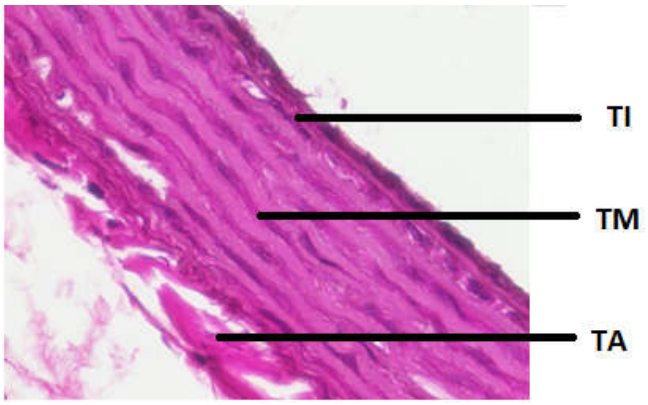

Fig 3 Standard Group

Table 2 Effect of ESETC on lipid Profile

Groups	Total cholesterol	Triglycerides	HDL	LDL	VLDL	CH/HDL (AI Index)	LDL/HDL	% Protection
G -1(NC)	112.10±3.15	122.83±2.85	43.16±2.46	73.25±2.18	28.38±1.57	2.59±1.28	1.69±0.88	NA
G-2(TC)	395.83±8.28*a	194.66±4.65*a	24.52±1.72*a	388.26±7.54*a	58.35±2.78*a	16.14±4.81*a	15.83±1.28*a	NA
G-3(ST)	174.60±5.20*b	138.05±3.39*b	40.83±2.38*b	104.20±3.55*b	34.41±1.85*b	4.27±2.18*b	4.38±1.49*b	73.54
G-4(T I)	232.50±7.86*b	151.35±2.85*b	37.50±2.17*b	138.75±4.16*b	42.36±2.37*b	6.20±3.62*b	3.70±1.91*b	61.58
G-5(T II)	184.45±5.67*b	147.15±3.45*b	41.18±2.58*b	126.20±3.79*b	38.35±1.99*b	4.47±2.19*b	3.06±1.46*b	72.30

- Values are expressed as Mean ± SEM.
- Values were find out by using ONE WAY ANOVA followed by Newman Keul's multiple range tests.
- *a- Values are significantly different from Normal control at P<0.01
- *b- Values are significantly different from Toxic control at P<0.01

Histopathology

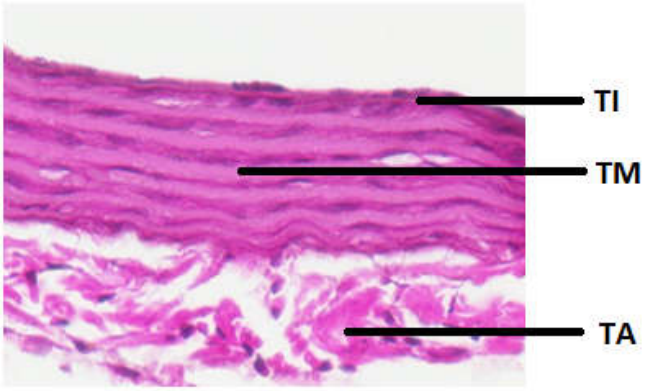

Fig 1 Control Group

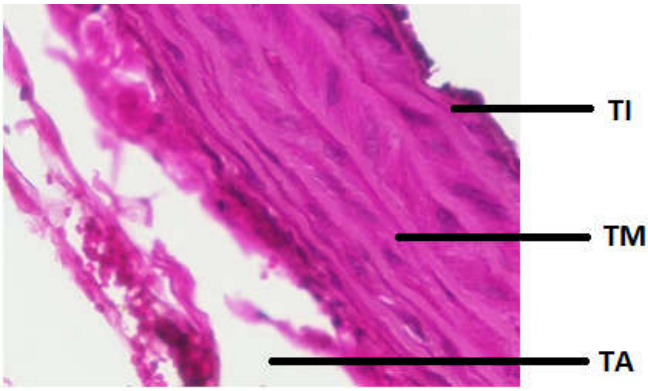

Fig 2 Toxic Group

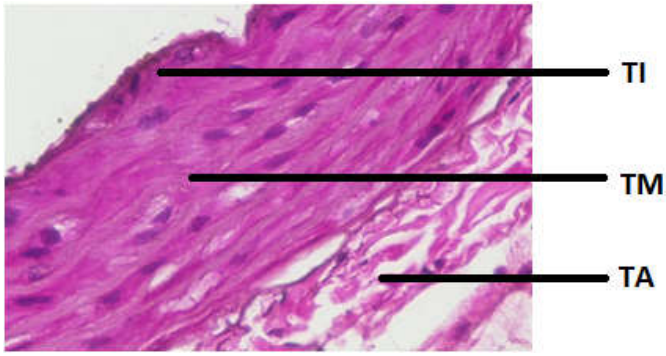

Fig 4 ESETC 100mg/kg

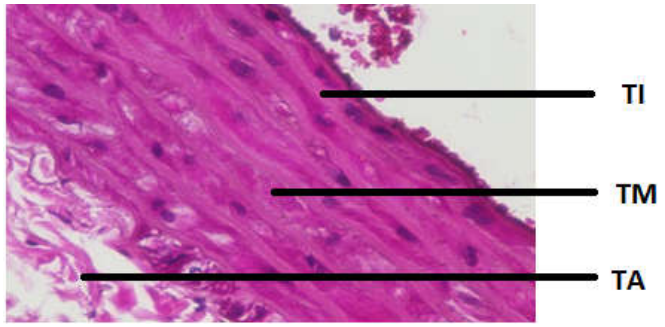

Fig 5 ESETC200mg/kg

DISCUSSION

The soxlet method of extraction by using ethanol as a solvent gave a good extract yield. The crude extract was subject to phytochemical screening which shows the presence of various important phytocompounds like alkaloids, flavonoids, saponins and terepenes etc (Table 1). When comparing the

Percentage change in lipid levels on day 30 the test groups were compared with atorvastatin group and they showed lesser percentage reduction in the serum total cholesterol, triglyceride, LDL and VLDL but test group II showed greater percentage elevation in the serum HDL than atorvastatin group (Table 2). Both the atorvastatin group and the test groups reduced AI significantly (P<0.001) (Table 2). Sections of thoracic aorta from the atherogenic diet-fed, saline treated rats showed significant damage to the tunica media (TM) layer and an increase in the thickness of the tunica adventitia (TA) layer; in addition many mast cells and lipid-containing cells were also seen(Fig. 2). No such alterations were noted in sections of the thoracic aorta from control rats (Fig. 1). The sections of thoracic aorta from atherogenic diet-fed with atorvastatin-treated rats showed a lower degree of damage to the tunica media layer than the sections from atherogenic diet-fed, saline-treated rats(Fig. 3). However, the overall histo architecture of sections of the thoracic aorta from the atherogenic diet-fed with *Tinospora cordifolia* extract-treated rats at both doses appeared to be similar to that seen in thoracic aorta sections from control rats, with almost normal tunica media and with only a small number of lipid-containing cells in the tunica adventitia.(Fig. 4 &5).

CONCLUSION

The observation of the present study indicated that the *T. cordifolia* has a definite hypolipidemic and hence cardioprotective and antiatherosclerotic potential. Hence, the present study helps to support the traditionally claimed antihyperlipidemic activity of *T. cordifolia*. *T. cordifolia* can be considered as an important addition to the therapeutic armamentarium for the treatment of hyperlipidemia. Further studies at the cellular and molecular level, may give clues regarding its mechanism in detail.

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