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HYPOLIPIDEMIC ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *CNIDOSCOLUS CHAYAMANSA* IN HYPERLIPIDEMIC MODELS OF WISTAR ALBINO RATS

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ABSTRACT

The tree spinch (*Cnidoscolus chayamansa* Mc Vaughn, Euphorbiaceae), called "Chaya" in south texas , is popular in Mexico and Central America and has been introduced into the United states (Mainly south Texas and Florida) and now presently available in and around southern part of India, It is traditionally used for its various properties and hence in the present study, ethanolic extract of leaves of *Cnidoscolus chayamansa* has been screened for its hypolipidemic activity. Hypolipidemic activity is screened by inducing hyperlipidemia with the help of atherogenic diet in wistar albino rats and serum levels of various biochemical parameters such as total cholesterol, triglycerides, LDL, VLDL and HDL cholesterol were determined. Atherogenic index shows the measure of the atherogenic potential of the drugs. Ethanol extract showed significant ($p < 0.01$) hypolipidemic effect by lowering the serum levels of biochemical parameters such as significant reduction in the level of serum cholesterol, triglyceride, LDL, VLDL and increase in HDL level which was similar to the standard drug Atorvastatin. Ethanol extract exhibited significant atherogenic index and percentage protection against hyperlipidemia. These biochemical observations were in turn confirmed by histopathological examinations of aorta, sections and are comparable with the standard hypolipidemic drug Atorvastatin. The overall experimental results suggests that the biologically active phytoconstituents such as flavonoids, glycosides, alkaloids present in the ethanol extract of *Cnidoscolus chayamansa* may be responsible for the significant hypolipidemic activity and the results justify the use of *Cnidoscolus chayamansa* as a significant Hypolipidemic agent.

Key words: Atherogenic diet, Hypolipidemic activity, Atorvastatin, Biological markers, Ethanolic extracts of Chaya.

INTRODUCTION

The tree spinch (*Cnidoscolus chayamansa* Mc Vaughn, Euphorbiaceae), called "Chaya" in south texas, is popular in Mexico and central America and has been introduced into the United states (Mainly south Texas and Florida) and now presently available in and around southern part of India, for potential uses as a leafy vegetable and/or as a medicinal plant. The plant is an attractive shrub 3 to 5 m. tall[1]. The leafs are broad and may consists of 3 or more lobes with fleshy petioles. The white colored flowers which are usually borne on cyme branched inflorescences, may contain 3-forked arrangement in which the pistillate flowers are located on the basal fork. The staminate flowers are expanded distally from the base of the lobes. Mature seeds and fruits are rare and unknown[2].

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The young shoots and tender leafs of Chaya are cooked and eaten like spinach. They comprise part of the staple diet and are the main dietary source of leafy vegetable for the indigenous people of Yucatan Peninsula of Mexico and Kekchi people of Alte Verapaz in Guatemala[3,4].

The edible parts of Chaya plant which taste like spinach when cooked, provide important nutritional sources for proteins, vitamins (A and C), minerals (calcium, iron, phosphorus), niacin, riboflavin and thiamine among populations that cannot afford expensive foods rich in these nutrients[5]. Chaya traditionally has been recommended for a number of ailments including diabetes, obesity, kidney stones, hemorrhoids, acne and eye problems[6].

Chaya shoots and leafs have been taken as a laxative, diuretic, circulation stimulant, to improve digestion, to stimulate lactation, and to harden the finger nails[7]. The leafs contain hydrocyanic glycosides, a toxic compound easily destroyed by cooking, even though some people tend to eat raw Chaya leaves, it is unwise to do so, while the nutritional value of Chaya has been demonstrated[8].

Many people with diabetes have conditions called “risk factor” that contribute to atherosclerosis and its complications. These include high blood pressure, excess weight and high blood glucose levels. Dyslipidemia further raises risk of atherosclerosis in people with diabetes. Dyslipidemia affects people with type 2 diabetes more often than those with type 1 diabetes. The most common Dyslipidemia in diabetes is the combination of high triglycerides and low HDL levels. People with diabetes may also have elevated LDL cholesterol.

Among the drugs available to treat Dyslipidemia, statins are often the first choice for lowering total and LDL cholesterol levels, other drugs that lowers cholesterol include cholesterol-adsorption blockers, bile acids, sequestrants and nicotinic acids. These may be used in combination, if a single drug is not effective in reaching target levels. Fibrates and extended release niacin may be used to lower triglycerides (or) raise HDL cholesterol levels[9].

Hyperglycemia and Dyslipidemia are significant and independent risk factors for the vascular complications and suggested to cause cardio vascular pathological changes in diabetic states through the following molecular mechanism, formation and accumulation of advanced glycation products, increased oxidative stress, activation of proteinkinase C pathway, increased activity of hexosamine pathway and vascular inflammation and the impairment of insulin action in the vascular tissues[10]. As *Cnidoscolus Chayamansa* plant species have been traditionally claimed for the treatment of diabetes, hence in the present study an attempt has been made to screen the herbal extract that is ethanolic extract of *Cnidoscolus Chayamansa* leaves for the Hypolipidemic activity to prove its claim in folklore practice.

The present investigation is undertaken to study the effect of *C. Chayamansa* (CCM) extract on changes in total cholesterol, triglycerides, HDL, LDL, VLDL, AI, and LDL/HDL.

Materials and methods

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 Ethical Committee. (KKP/Ph.D/PMU/2010).

Materials

- Ethanolic Extracts of Leaf of *Cnidoscolus chayamansa* (EECC).
- Cholesterol extra pure for feeding purpose was obtained from SD fine-chem. Limited, Mumbai, India. Coconut oil was used as a vehicle for cholesterol feeding.
- Atorvastatin was obtained from Micro Labs, Bangalore, India.

EXPERIMENTAL

Procedure

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 400 mg/kg body weight for 30 days.

Group III : Fed cholesterol as in Group II and Atorvastatin 1 mg/kg body weight from days 15 to day 30.[11]

Group IV : Fed cholesterol as in Group II and EECC at a dose of 100 mg/kg body weight from days 15 to day 30.

Group V : Fed cholesterol as in Group II and EECC at a dose of 250 mg/kg body weight from days 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 standard procedure[12].

Statistical analysis

- All the values were expressed as mean ± SEM.
- Data was analyzed by one way analysis of variance (ANOVA) followed by Newman Keul's multiple test.
- P values < 0.05 were considered as statistically significant.

Histopathological studies

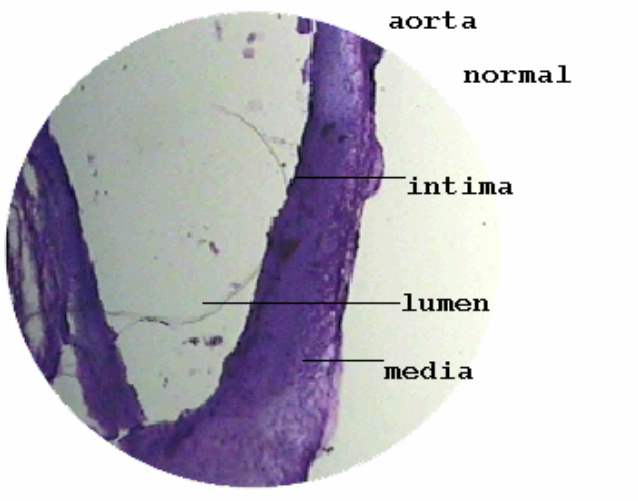

Fig. 1: Normal control

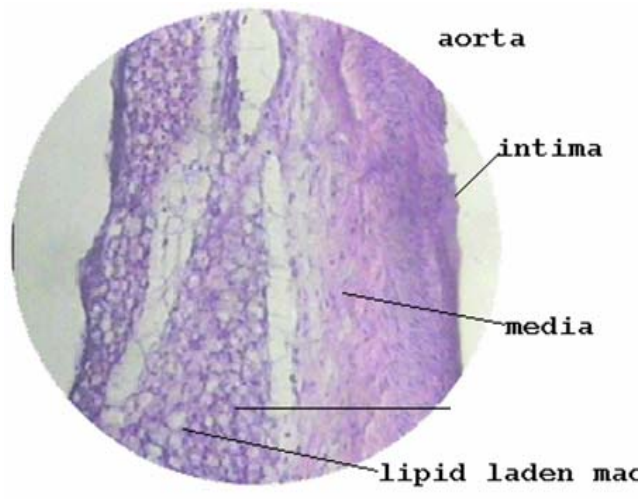

Fig. 2: Cholesterol Control

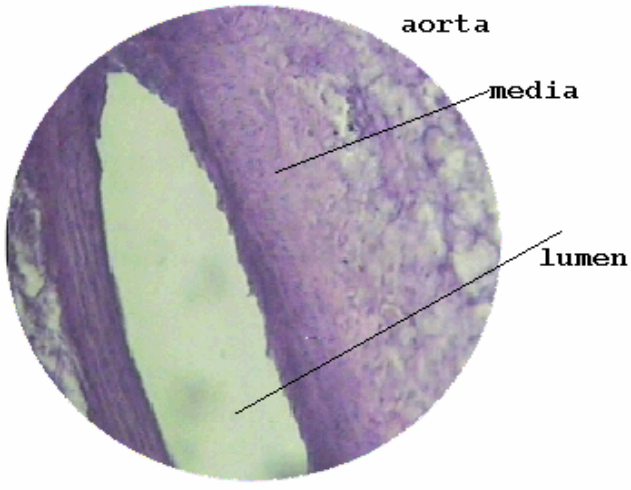

Fig. 3: Standard (Atorvastatin)

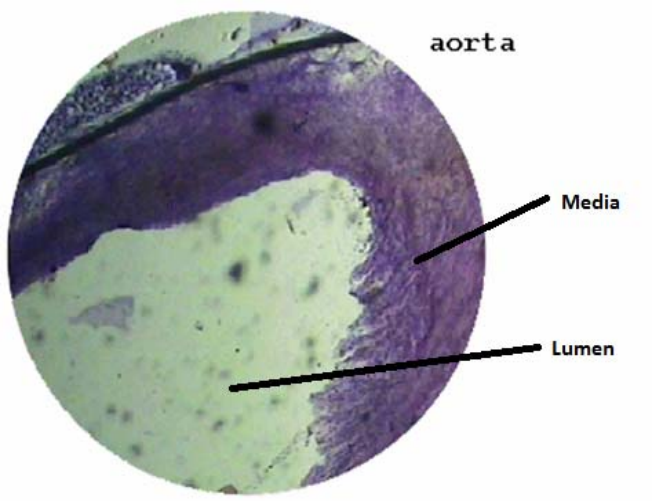

Fig. 4: Ethanolic extract of *Cnidoscolus Chayamansa* 100 mg/kg

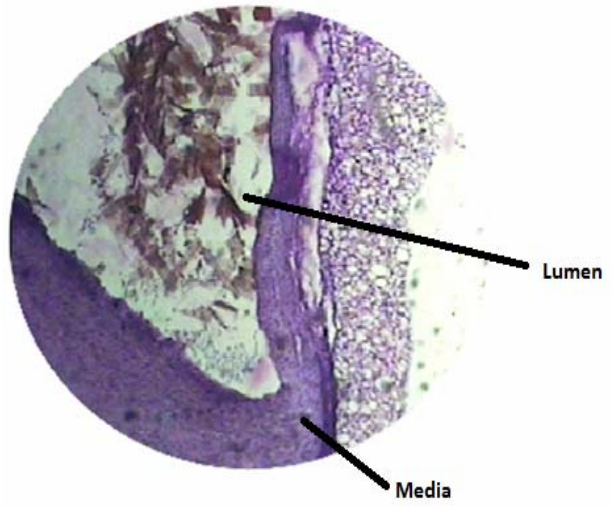

Fig. 5: Ethanolic extract of *Cnidoscolus Chayamansa* 250 mg/kg

RESULTS AND DISCUSSION

Table 1 shows the levels of cholesterol, triglycerides, HDL, LDL and VLDL of control and experiment rats respectively. Serum of hyperlipidemic rats showed significantly increased levels of cholesterol, triglycerides, LDL-C and low HDL-C, when compared with normal rats. In rats treated with both doses of ethanolic extracts of *Cnidoscolus Chayamansa* and Atorvastatin there was significant decrease in the content cholesterol, TGs, LDL-C, and VLDL and increases HDL-C, when compared with cholesterol control rats.

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

Table 1 shows the changes of Atherogenic Index and LDL-C / HDL-C ratio in control and treated rats. It appears clear from these results that the cholesterol induction significantly affects the cardio vascular risk markers.

Indeed, AI was statistically increased in cholesterol control group 90% compared with the values found in their normal control group.

Table 1: Effect on ethanolic extracts of *Cnidoscolus Chayamansa* in lipid profile

Groups	Total cholesterol (mg/d*l*)	Tri-glycerides (mg/d*l*)	HDL (mg/d*l*)	LDL (mg/d*l*)	VLDL (mg/d*l*)	A*I*	LDL/ HDL
Normal control	49.65 ± 1.75	57.06 ± 0.89	26.41 ± 1.16	14.33 ± 0.74	30.88 ± 1.02	0.87 ± 0.50	0.54 ±
Cholesterol control	113.38 ± 1.54**(a)	160.6 ± 1.68**(a)	11.91 ± 0.66**(a)	30.98 ± 1.30**(a)	13.03 ± 0.72**(a)	8.51 ± 1.33**(a)	2.60 **(a)
Standard control	72.61± 1.40**(b)	82.9 ± 1.77**(b)	21.7 ± 0.42**(b)	20.01 ± 0.74**(b)	24.88 ± 0.77**(b)	2.34 ± 2.33**(b)	0.92 **(b)
Treatment control	92.85 ± 1.22**(b)	114.38 ± 1.95**(b)	17.2 ± 0.49**(b)	25.23 ± 0.58**(b)	17.88 ± 0.40**(b)	4.39 ± 1.48**(b)	1.46**(b)
Treatment control	83.83 ± 0.94**(b)	96.4 ± 1.07**(b)	20.26 ± 1.25**(b)	22.08 ± 0.71**(b)	21.26 ± 0.50**(b)	3.13 ± 0.24**(b)	1.08 **(b)

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 were significantly different from normal control at $P < 0.01$.

** (b) values were significantly different from hyperlidemic control at $P < 0.01$.

Besides there were significant further increase of LDL-C / HDL-C ratio in cholesterol control group compared to normal control group.

Promising results in lowering of AI by Ethanolic Extract of *Cnidoscolus Chayamansa* was found in Table 1. The ethanol extract of Cnidoscolus Chayamansa showed an improvement of the cardio vascular risk level by decrease of AI in the treated group by more than 73% and 63% ($p < 0.01$), when compared to the cholesterol control group.

The ratio of LDL-C to HDL-C is also a protective indicator of cardio vascular disease incidence. The cholesterol induction produced a significant increase of this marker. In contrast, elevated ratio in treated group and Atorvastatin group returned to basal value when the data were compared in the same period to the data found for cholesterol rats. (Table 1)

The reduction of plasma total cholesterol was associated with a decrease in its LDL fraction which is a major, potentially modifiable risk factor of cardio vascular disease and the target of drug. Many suggest that the cholesterol lowering activity of this product appears to be due to the enhancement of LDL-C catabolism through hepatic receptors[13]. In addition ethanolic extracts of *Cnidoscolus Chayamansa* showed protective action which is reported to have a preventive function against atherogenesis since an independent inverse relationship between blood HDL-C levels and cardio vascular risk incidence is reported[14]. The lipoprotein called "good cholesterol" facilitates the mobilization of triglycerides and cholesterol from plasma to liver where it is catabolised and eliminated in the form of bile acids. The possible mechanism of this activity may result from the enhancement of lecithin cholesteryl acyl transferase (LCAT) and inhibition of Hepatic Triglyceride Lipase (HTL) on HDL which may lead to a rapid catabolism of blood lipids through enterohepatic tissues[15].

It is also recently reported that triglycerides plays a key role in the regulation of lipoprotein interaction to maintain normal lipid metabolism. Indeed, the elevated plasma TG levels were associated with an increased incidence of coronary artery disease. Moreover these higher plasma TG levels have been

attributed mainly to increase population of small, dense LDL deposits which are very atherogenic and enhanced cholesteryl ester mass transfer from apolipoprotein B containing lipoproteins (VLDL and LDL)[16]. TG has also been proposed to be major determinant of cholesteryl esterification, its transfer and HDL remodeling in human plasma[17].

Ethanolic extracts of *Cnidoscolus Chayamansa* significantly suppress the elevated blood concentration of TGs. This result suggests that the product is able to restore, at least partially, the catabolism of TG. The underlying the mechanism of this activity is not elucidated by the present study. However, as hypothesized by many works with other plants, the restoration of catabolic mechanism of TGs would be due to an increased stimulation of the lipolytic activity of Plasma Lipoprotein Lipase (LPL)[18].

Administration of Ethanolic extracts of *Cnidoscolus Chayamansa* provides a beneficial action on rat lipid metabolism with regard to the reduction of AI. Infact, the AI was decreased in all treated groups. Similar results were reported by others when studying the hypolipidemic effects of natural products[19]. This ameliorative action was due to the plasma lipid lowering activity of different constituents of the plant.

It is also desirable to have higher plasma HDL and lower LDL-C to prevent atherogenesis, since there is a positive correlation between an increased LDL-C/HDL-C ratio and the development of atherosclerosis. Again, the administration of Ethanolic extract of *Cnidoscolus Chayamansa* significantly suppress the higher values of LDL-C/HDL-C ratio showing the beneficial effect of this plant in preventing atherosclerosis incidence.

This result is considered as an important for the treatment of hyperlipidemia induced atherosclerosis and apparently validates the folk medicinal use of hyperlipidemic patients in India.

Histopathological report

Another interesting feature is that the Histopathological abnormalities seen in the aorta of cholesterol control rats are also reversed showing almost normal appearance.

On the basis of above results, it could be concluded that Ethanolic extracts of *Cnidoscolus Chayamansa* is a significant antihyperlipidemic effect.

CONCLUSION

Hyperlipidemia is considered to be major risk factor for the premature atherosclerosis and essentially the cholesterol in atherosclerotic plaque is derived from that of circulatory cholesterol. The antihyperlipidemic effect on Ethanolic extracts of *Cnidoscolus Chayamansa* in particular could be considered as a possible therapeutic value.

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