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RESEARCH ARTICLE

Evaluation of the Anti hyperlipidemic and Anti atherosclerotic Activities of Ethanolic Extract of Cissus Pallida in Atherogenic Diet Fed Rat

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ABSTRACT

Atherosclerosis is one of the risk factors for coronary artery disease. Hyperlipidemia is an abnormally high level of fatty substances called lipids, largely cholesterol and triglycerides, in the blood. The present study highlights the anti hyperlipidemic and anti atherosclerotic activity of Ethanolic extract of Cissus Pallida preparation in atherogenic diet induced atherosclerosis in rats. Atherosclerosis was developed in male Albino Wistar rats, which were randomly divided into five groups of six animals each; by feeding with atherogenic diet for 45 days. Group 1 received normal diet. Group 2 received atherogenic diet (AD) which served as control. Group 3 served as standard, administered with Atorvastatin (50 mg/kg) along with AD and Group 4 and Group 5 were administered with ethanolic extract of Cissus Pallida preparation (250 mg/kg and 500 mg/kg) along with AD. Serum lipid profile, fecal cholesterol execration, and atherogenic index were estimated for all rats on the last day of the experiment. At the end of the experiment one rat from each group was sacrificed and aorta was isolated for histopathology studied. The results were analyzed statistically using analysis of variance (ANOVA). Ethanolic extract of Cissus Pallida preparation reduced the raised serum level of total cholesterol, triglyceride, LDL, VLDL and increased the serum HDL level as compared to the control group(AD).

KEYWORDS

Atherosclerosis, Atherogenic Diet, Hyperlipidemia, Atorvastatin, Cissus pallid.

INTRODUCTION

Hyperlipidemia is the disorders of lipid metabolism have been ranked as one of the greatest risk factors contributing to the prevalence and severity of atherosclerosis, stroke and coronary heart diseases[1,2]. Hyperlipidemia is characterized by elevated serum total cholesterol, low density lipoprotein, very low- density lipoprotein (LDL, VLDL) cholesterol and decreased high-density lipoprotein (HDL) levels.

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Atherosclerosis refers to deposition of fatty substances on the inner lining of the blood vessels. Lipids undergo peroxidative change in the arterial wall and eventually result in tissue injury. It is characterized by vascular areas containing mononuclear and proliferation of smooth muscle cells resulting in hardening and thickening of the arterial walls[3]. The high concentration of cholesterol, particularly LDL-cholesterol is one of the principal risk factors. Coronary heart disease caused by atherosclerosis continues to be a leading cause of mortality in developed and developing nations of the world[4]. Myocardial and cerebral infarctions are also main clinical syndromes

resulting from atherosclerosis and are the leading causes of death in all over the world[5]. Lipid lowering drugs like Fibrates, Statins and bile acid Sequestrants used in the treatment of hyperlipidemia possess toxic side effect[6,7]. Therefore, there is an urgent need to have a lipid lowering drug with fewer side effects. A number of herbal medicines are used for controlling hyperlipidemia and related complications in patients[8].

Cissus pallida is a climbing shrub that is widely used in veterinary folk medicine and ayurvedic medicine. The root of this plant has shown rheumatic activity[9]. The Phytochemical investigation reveals that the plant contains phenolic compound called Pallidol. Pallidol is a dimer of resveratrol[10].

MATERIALS AND METHOD

Materials

All chemicals were of analytical grade and obtained locally. Cholesterol, Triglycerides and HDL-C kit were procured from Robonik diagnostics, Hyderabad, India.

Plant Material

The fresh plant Cissus pallida (Figure 6) were collected from Dakshina Kannada district, Karnataka. Identification of the plant was done by Dr. K. Madhava Chetty assistant professor, department of botany, Sri Venkateswara University, Tirupati, A.P, India.

Animals

Wister albino adult male rats weighing 200-220g were selected and housed in polypropylene cages in a room where the congenial temperature was 27°C ± 1°C and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet and water ad libitum. The composition of atherogenic diet used during the study was as given in Table-1. Each of these treatment groups consisted of six animals/group. The protocol of this study was approved by the Institutional Animal Ethics Committee (IAEC) constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Method

Preparation of the Extract

The whole plant were isolated, chopped into small pieces and dried under shade at room temperature for seven days. The dried roots were powdered and passed through the sieve (coarse 10/44). This powder was used for the preparation of ethanolic extract.

Ethanolic Extract

Ethanolic extract was prepared by Heat Distillation Process. The dried coarse powdered of plant (250 gm) were transferred to a round bottom flask, 75% of ethanol was added to the flask and soaked for 2 hours. This was then boiled for 4 hours. The extract so obtained was decanted in a beaker and then concentrated to 1/6th of the total volume on a water bath. This was preserved by adding a few drops of chloroform and kept in the refrigerator. This extract was administered to the animals by making the concentration required by weighing the water-evaporated extract (14.2% yield). The extract was assigned a code name ECP[11].

Dose Selection and Administration

According to reported activities, ECP at the doses of 250 and 500 mg/kg p.o. /day 45 was selected for the study.

Experimental Induction of Hyperlipidemia

In order to induce hyperlipidemia, the method reported by Bopanna *et al.*[12] was followed. The animals were divided into five groups of six rats each and they received the following diets with or without treatment for 45 days orally:

Group I: Normal diet

Group II: Atherogenic diet

Group III: Atherogenic diet+ Atorvastatin (50mg/kg/day)

Group IV: Atherogenic diet + ECP (250 mg/kg/day).

Group V: Atherogenic diet + ECP (500 mg/kg/day).

At the end of the treatment the rats were fasted overnight, blood was drawn from retro orbital plexus as per CPCSEA guidelines. Serum was separated and stored in refrigerator until assay.

Table 1: Composition of normal and atherogenic diet

S. No.	Ingredients	Normal diet (%)	Atherogenic diet (%)
1	Protein (milk powder)	12	10
2	Carbohydrates (wheat flour)	71	61
3	Sugar	05	05
4	Fat (butter)	05	16
5	Salts	04	04
6	Vitamins	01	01
7	Fibres	02	01
8	Cholesterol	-	01
	Total weight	100g	100g

Measurement of Various Parameters

Physical Parameters [15]

The body weight was recorded on the first day and then last day of the study period in each group.

Biochemical Estimations

Lipid parameters were determined in blood serum. At the end of 45 days, animals were fasted overnight and blood was collected from retro orbital plexus under light ether anesthesia, centrifuged at 2500 rpm for 20 minutes. The serum obtained will be kept at 4°C until used.

The quantitative estimation of lipid profile was carried out using Infinite triglycerides liquid for triglycerides, Infinite cholesterol liquid for total cholesterol and Autozyme for HDL-C, ACCUREX in SICRA laboratory. Estimation of VLDL-C and LDL-C will be done by using the Friedward's formula.

VLDL-C = Triglycerides/5 LDL-C = Total cholesterol – (HDL-C + VLDL-C).

Measurement of Coronary Disease Risk Factor [17]

Atherogenic Index (AI), was calculated using the following formula and the results were tabulated.

Calculation of Atherogenic Index (AI)

$$\text{Atherogenic index} = \frac{\text{Total serum cholesterol}}{\text{Total serum HDL-C}}$$

Calculation of Percentage Protection

$$\text{Protection (\%)} = \frac{\text{AI of Control} - \text{AI of Treated group}}{\text{AI of Control}} \times 100$$

Histopathology of Aorta

For histopathology, the rats were sacrificed by cervical decapitation and their aortas were dissected out. During the procedure, ice was used to keep the aorta samples fresh and avoid any degradation. The aortas were stored in 10% formaline solution and sent to a local pathological laboratory for hematoxyline and eosine staining.

Statistical Analysis

The results are expressed as mean ± standard error of mean (SEM).The data were analyzed using one-way analysis of variance (one-way ANOVA) followed by Dunnett multiple comparison test for comparison between groups. The criterion for statistical significance was $p<0.05$.

RESULTS AND DISCUSSION

Effect of administration of ethanolic extract of cissus pallida (250 and 500mg/kg P.O once daily for 45days) / atorvastatin (50mg/kg/P.O once daily for 45 day) on Histopathological changes in aorta of rats fed with AD for 45 days.

Group: 1

Light microscopy of the aortic sections of normal control group showed normal histology of the tunica intima, media and adventia. The

Table 2: Effect of administration of ethanolic extract of cissus pallida (250 and 500mg/kg P.O once daily for 45days)/ atorvastatin (50mg/kg/P.O once daily for 45 day) on serum lipids levels in rats fed with A.D for 45 days

Groups	**Total cholesterol (mg/dl)**	**HDL (mg/dl)**	**Triglycerides (mg/dl)**	**LDL (mg/dl)**	**VLDL (mg/dl)**
I	65.66±0.45	25.23±0.3	56.71±0.51	29.08±0.6	11.33±0.51
II	115.86±0.41[b]	19.25±0.2[b]	146±0.86[b]	67.41±0.59[a]	29.19±0.66[a]
III	86.35±0.79**	28.57±0.29**	76.18±0.47**	45.85±0.6***	15.23±0.09***
IV	97.84±0.2**	24.64±0.31**	92.16±0.6**	51.13±0.43***	18.52±0.11***
V	88.3±0.51**	25.49±0.22**	81.26±0.6**	46.39±0.46***	16.24±0.11***

Values are expressed as MEAN±SEM for 6 animals.

One way analysis of variance (ANOVA) followed by dunnett multiple comparisons.

*** P<0.001and ** P<0.01 as compared to control group.

[a] P<0.001 and [b]P<0.01 as compared to normal group.

Table: 3 Effect of administration of ethanolic extract of cissus pallida (250 and 500mg/kg P.O once daily for 45days)/ atorvastatin (50mg/kg/P.O once daily for 45 day) on serum lipids levels in rats fed with A.D for 45 days on coronary risk factors

Groups	**Atherogenic index (A.I)**	**Atherogenic protection (%)**
I	1.60±0.036	68.44
II	5.07±0.073[a]	-
III	2.03±0.08***	59.96
IV	2.95±0.02***	41.81
V	2.45±0.02***+	51.67

Values are expressed as MEAN±SEM for 6 animals.

One way analysis of variance (ANOVA) followed by dunnett multiple comparisons.

***P<0.001 as compared to control group.

[a]P<0.001 as compared to normal group

ECP= ethanolic extract of cissus pallida.

intima was composed of a continuous layer of endothelial cells (Fig: 1).

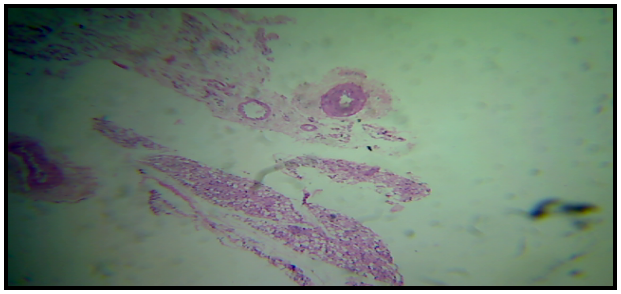

Figure 1: Group 1

Group: 2

Light microscopy of the aortic sections of AD control group showed thickening of vascular wall of aortic musculature with fatty tissue. Also there is a formation of neointima containing vascular smooth muscle cells of tunica media (Fig: 2).

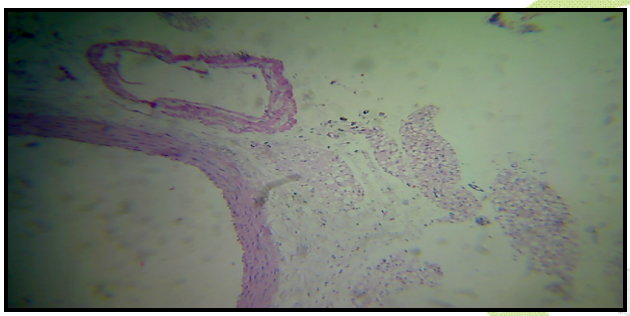

Figure 2: Group 2

Group: 3

Light microscopy of the aortic sections of AD and Atorvastatin (40mg/kg) group showed thickening of vascular wall of aortic musculature with fatty tissue. Moderately decreased the formation of the neointima and the vascular smooth muscle cells of tunica media are less in the neointima. (Fig: 3).

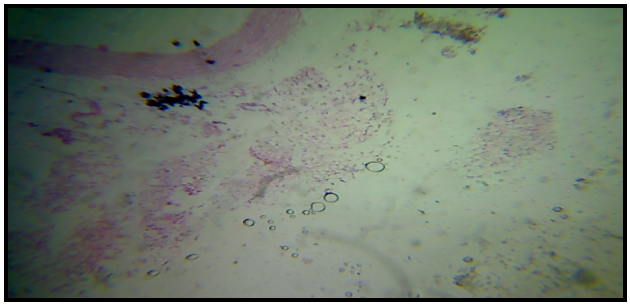

Figure 3: Group 3

Group: 4

Light microscopy of the aortic sections of AD and Cissus pallida (250mg/kg) treated group showed thickening of vascular wall of aortic musculature with fatty tissue. Mild decrease of formation of neointima containing vascular smooth muscle cells of tunica media. (Fig: 4).

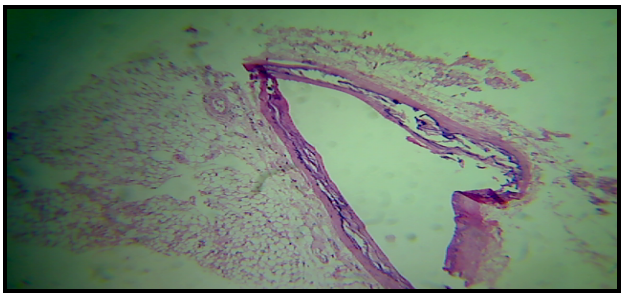

Figure 4: Group 4

Group: 5

Light microscopy of the aortic sections of AD and Cissus pallida (500mg/kg) treated group showed thickening of vascular wall of aortic musculature with fatty tissue. There is mild formation of neointima containing vascular smooth muscle cells of tunica media and almost there is an appearance of normal architecture. (Fig: 5).

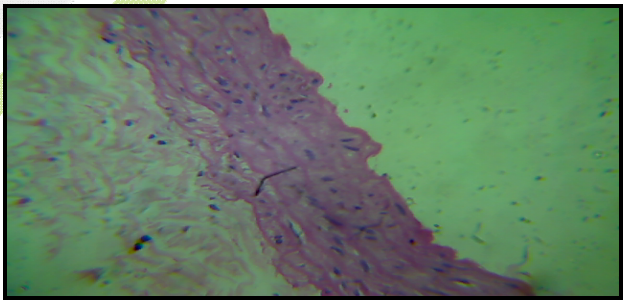

Figure 5: Group 5

High fatty diet is a very common cause of heart disease. Particularly, with an increase in tendency towards fast foods, which are rich in saturated fats, an increase in coronary heart disorder (CHD) is being observed in the developing countries since past few decades[14]. A one percent decrease in HDL-cholesterol is associated with a 3-4% increase in the risk of heart disease. In the present study an increase in plasma HDL-cholesterol with a concomitant

percentage decrease in other lipid parameters were observed (Tables 2 and 3).

It can be concluded from the present data that the levels of total serum cholesterol, triglyceride and total protein which are actually raised in atherogenic diet, can be lowered significantly with extract of Cissus Pallida.

CONCLUSION

Treatment with extract of Cissus Pallida produced a significant decrease in the serum level of lipids in atherogenic diet induced hyperlipidemia in rats. Hence by considering the effects observed in this model, the possible mechanism of Cissus Pallida may involve increase of HDL-cholesterol, which is attributed to the mobilization of cholesterol from peripheral cells to the liver by the action of Lecithin Cholesterol O-acyltransferase (LCAT) enzyme[15]. LCAT enzyme is involved in the transesterification of cholesterol, the maturation of HDL and the flux of cholesterol from cell membranes into HDL. Thus from the above results we can conclude that Cissus Pallida has anti hyperlipidemic and anti atherosclerotic activities.

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