

EVALUATION OF PROTECTIVE EFFECT OF CLEOME VISCOSA EXTRACT ON DIET INDUCED ATHEROSCLEROSIS IN DIABETIC RATS

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ABSTRACT

The atherosclerotic effect of Methanolic extract of *Cleome Viscosa* was studied in Streptozotocin induced Diabetic Rats fed with Atherogenic Diet [1.5ml olive oil containing 8mg of vitaminD₃ and 40mg cholesterol] for 5 consecutive days. Oral administration of extract to diabetic rats [250mg/kg and 500mg/kg for 30 days] produced significant (p<0.01) fall in fasting blood glucose levels in a dose dependent manner, when compared to standard drug that is Glibinclamide (5mg/kg). Treatment with extract 250mg/kg and 500mg/kg showed significant (P<0.01) improvement in body weight and serum lipids levels that is total cholesterol, triglycerides(TG), low density lipoprotein(LDL) and very low density lipoprotein(VLDL), when compared to diabetic control. Histopathological studies also confirmed biochemical findings. Thus our study shows that Methanolic extract of *Cleome Viscosa* (250mg/kg and 500mg/kg) significantly improves the homeostasis of glucose and fat and possess anti atherosclerotic activity.

Key words: *Cleome Viscosa*, Methanolic extract, Glibinclamide, Streptozotocin, Vitamin D₃

INTRODUCTION

Elucidation of the role of cholesterol in the pathogenesis of atherosclerosis is often referred to as one of the greatest discoveries of the 20th century. Some of the milestones on the road to acceptance of the 'lipid hypothesis' which proposed that hypercholesterolemia was a causative factor in human atherosclerosis. Atherosclerosis may be defined as degenerative changes in the intima of medium and large arteries. The degeneration includes accumulation of lipids, complex carbohydrates, blood and blood products, and cellular waste products, and is accompanied by the formation of fibrous tissues and calcium deposition in the intima of blood vessels.

Accelerated atherosclerosis occurs in animal models with engineered deficiencies in [apo (a)] or LDL receptors. Other genetic or acquired disorders (e.g., diabetes mellitus, hypothyroidism) that cause hypercholesterolemia lead to premature atherosclerosis. Lowering serum cholesterol by diet or drugs slows the rate of progression of atherosclerosis, causes regression of some plaques, and reduces the risk of cardiovascular events. (Jun Kusunoki, 2000)

MATERIALS AND METHODS

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 *Cleome Viscosa* was collected from Mahabubnagar District; A.P. 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. 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 (Reg No: 769/2010/CPCSEA) (Reenu Singh Tanwar, 2011).

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 plant were powdered and passed through the sieve (coarse 10/44). This powder was used for the preparation of Methanolic extract.

Methanolic extract: Methanolic extract was prepared by Heat Soxhlet extractor. The dried coarse powdered of plant (250 gm) were transferred to a round bottom flask, 99% of Methanol 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 (24.5% yield). The extract was assigned a code name MECV. (G. C. Jain, 2006)

Induction of diabetes: A freshly prepared solution of streptozotocin (45mg/kg in 0.1M buffer citrate solution) is injected to overnight fasted rats. After 48 hrs the rat's blood glucose levels of 250mg/dl or above were considered for the further study. (Suman Bala Sharma, 2010)

Induction of atherosclerosis: Although the cause and pathogenesis of atherosclerosis still remains largely unresolved, it is generally agreed that correlation exists between high blood cholesterol and cardiovascular diseases. Antischkow for the first time succeeded in inducing atherosclerosis in rabbits by feeding cholesterol containing diet. Development of atherosclerosis in rabbits usually takes at least 60 days of feeding atherogenic diet. Rat is said to be resistant to such dietary manipulations for the development of atherosclerosis, but with supplementation of very high doses of vitamin-D₃ along with atherogenic diet, success has been achieved in developing atherosclerosis in rats in a short period of 5 days. The atherogenic diet (AD) consisting of 2 g of cholesterol and 8 g of saturated fat and 100 mg calcium were added to 90 g of powdered standard commercial pellet diet and thoroughly mixed. The rats were fed with high fat diet along with weekly challenge of oral vitamin-D₃ for one month through oral route. (Martha Srinivas, 2008)

Experimental protocol: In order to induce atherosclerosis, the method reported by Bopanna *et al.* was followed. The animals were divided into five groups of six rats each and they received the following diets with or without treatment for 30 days orally:

Group I: Normal diet

Group II: Atherogenic diet + STZ (45mg/kg)

Group III: Atherogenic diet+ STZ (45mg/kg) + Glibinclamide (5mg/kg/day)

Group IV: Atherogenic diet+ STZ (45mg/kg) + MECV (250 mg/kg/day).

Group V: Atherogenic diet+ STZ (45mg/kg) + MECV (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

Measurement of various Parameters:

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

Blood glucose levels: The glucose levels were determined by using commercial glucometer kit on initial and final day of the experiment by collecting blood from rat's tail.

Biochemical Estimations: Lipid parameters were determined in blood serum, at the initial day and on final day of 30 days, animals were fasted overnight and blood was collected from retro orbital plexus under light ether anaesthesia, 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.

$$\text{VLDL-C} = \text{Triglycerides}/5$$

$$\text{LDL-C} = \text{Total cholesterol} - (\text{HDL-C} + \text{VLDL-C}).$$

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. (Reenu Singh Tanwar, 2011)

Statistical Analysis: The results are expressed as mean \pm 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

Atherogenic Diet (AD) Induced Atherosclerosis in Diabetic Rats:

Table.1.Effect of 99.9% MECV extract on Body weight on Atherosclerosis in STZ induced diabetic rats

Groups	Dose (mg/ kg)	Body Weight (Mean± S.E.M)	
		0 th day	30 th day
G I	Normal control	168.3±1.520	232.0 ± 3.109
G II	control(Vehicle)	190.67±3.72	246.5 ± 1.544
G III	10 mg/ kg	173.7±1.820	229.2 ± 3.516
G IV	100 mg/ kg	178.0±2.309	251.5 ± 5.500
G V	200 mg/ kg	177.2±2.713	243.2 ±3.280

Table.2.Effect of 99.9% MECV extract on Fasting Blood Glucose levels on in STZ induced diabetic rat

Group	Normal	Control	Standard	MECV T1	MECV T2
Initial HDL	39.67 ± 1.054	23.00 ± 0.8563	22.83 ± 0.9804	22.67 ± 0.7149	23.17 ± 0.6009
Final HDL	45.67 ± 1.229	13.83 ± 0.6009	32.17 ± 0.9458	30.33 ± 0.8819	35.67 ± 0.6667
Initial LDL	18.80 ± 1.157	61.67 ± 2.086	66.73 ± 1.199	65.33 ± 1.404	67.33 ± 1.303
Final LDL	17.07 ± 1.409	102.3 ± 2.754	35.50 ± 1.670	42.80 ± 2.282	42.82 ± 2.282
Initial VLDL	14.87 ± 0.2231	22.67 ± 0.3887	22.10 ± 0.2864	22.33 ± 0.3040	22.17 ± 0.3667
Final VLDL	15.27 ± 0.1606	31.40 ± 0.6967	16.17 ± 0.1406	18.70 ± 0.3531	15.60 ± 0.3141
Initial TC	73.50 ± 1.335	108.2 ± 2.182	111.7 ± 1.282	110.3 ± 1.542	112.2 ± 1.905
Final TC	78.00 ± 1.238	147.5 ± 2.432	84.67 ± 1.726	91.83 ± 2.104	77.33 ± 1.453
Initial triglycerides	74.33 ± 1.116	113.3 ± 1.944	110.5 ± 1.432	111.7 ± 1.520	110.8 ± 1.833
Final triglycerides	76.33 ± 0.8028	157.0 ± 3.483	80.50 ± 0.7638	93.50 ± 1.765	78.00 ± 1.571

Table.3.Effect of 99.9% MECV extract on Serum Lipids levels in STZ induced diabetic rats

Group	Normal	Control	Standard	MECV T1	MECV T2
0 th day	82.67±1.054	313 ± 1.478	277.2±0.9458	297.0±0.5774	283.3±2.333
6 th day	80.83±1.470	307.3±1.994	309.5±0.7638	311.7 ± 1.054	314.8±1.579
15 th day	82.67±1.542	326.7±2.654	190.3 ± 1.892	223.3 ± 1.542	192.2±1.922
30 th day	83.00±1.265	330.8±2.227	141.3 ± 2.319	169.8 ± 1.302	133.2±1.778

ns= not significant, one way analysis of variance (ANOVA) followed by dunnett multiple comparison.

*** P<0.001 ** P<0.01 and *P<0.005 as compared to control group, ^aP<0.001 ^bP<0.01 and ^cP<0.05 as compared to normal group.

Effect of administration of Methanolic extract of Cleome Viscosa (250 and 500mg/kg P.O once daily for 30days)/ Glibinclamide (5mg/kg/P.O once daily for 30 day) on Histopathological changes in aorta of rats fed with AD for 30 days.

Group 1: The thoracic aorta of animal from group 1 showed normal histological features of the tunica layers. The tunica intima was composed of a continuous layer of endothelial cells. The tunica media appeared to have normal & healthy numerous distinct elastic laminae, which were wavy & arranged concentrically, with smooth muscle cells seen in the interspaces between the concentric lamellae. There was no significant increase in the media thickness. The tunica adventitia was recognized by the normal-looking fibrous tissue elements.

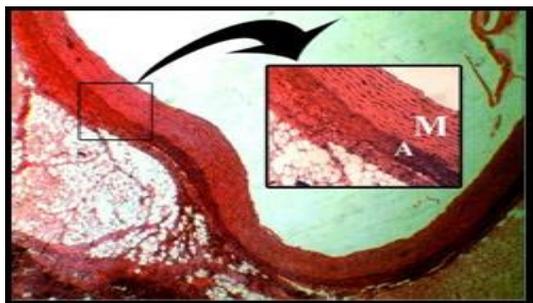
Group 2: In group 2 there was vascular wall thickening resulting from an increase in the thickness of tunica media observed as smooth muscle hyperplasia among regular concentric elastic laminae, on the expense of a relatively thin tunica adventitia.

Group 3: In group 3 the increase in the tunica media was less pronounced but the hyperplasia was irregular & was associated with disruption of the concentric pattern of the elastic laminae regular luminal layers of endothelial cells were observed & the internal elastic laminae showed areas of continuity.

Group 4: In group 4, few fibrocytes were spotted in the subendothelial regions. Fibrous elements of the tunica adventitia were also thickened in both groups as compared to the controls.

Group 5: Transverse section through the thoracic aorta of rats treated with MECV (500mg/kg) showing subendothelial fibrocytes (short white arrows) & intimal continuity (long black arrow). X600

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) in diabetic patients and normal patients is being observed in the developing countries since past few decades. 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 glucose and 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 *Cleome Viscosa*.



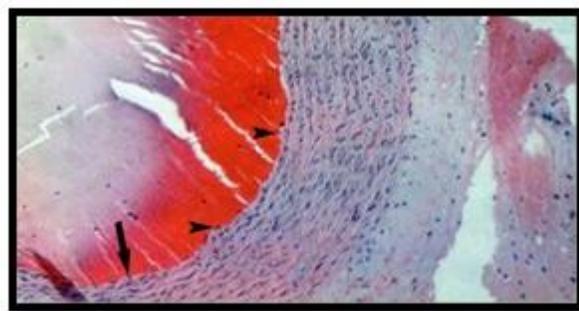
Group.1



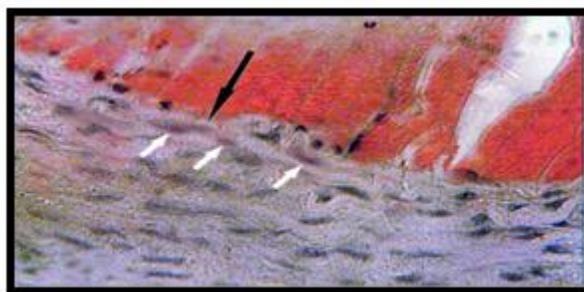
Group.2



Group.3



Group.4



Group.5

CONCLUSION

Treatment with extract of *Cleome Viscosa* produced a significant decrease in the serum level of lipids and glucose in atherogenic diet induced atherosclerosis in Diabetic rats. Hence by considering the effects observed in this model, the possible mechanism of *Cleome Viscosa* 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. 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 *Cleome Viscosa* has anti-atherosclerotic activities in diet induced atherosclerosis in a STZ induced diabetic rats.

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