

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 [1.5 ml olive oil containing 8 mg of vitamin D-2 and 40 mg cholesterol] for 5 consecutive days. Oral administration of extract to diabetic rats [250 mg/kg and 500 mg/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 (5 mg/kg). Treatment with extract 250 mg/kg and 500 mg/kg showed significant ($P < 0.01$) improvement 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 of aorta also confirmed biochemical findings. Thus, our study shows that Methanolic extract of Cleome viscosa (250 mg/kg and 500 mg/kg) significantly improves the homeostasis of glucose and fat and possess anti atherosclerotic activity.

Keywords: Cleome viscosa, Methanolic extract, Atherosclerosis, diabetic rats, Glibinclamide, Streptozotocin, Vitamin D2.

Introduction

Diabetes mellitus is a group of metabolic disorders with one common manifestation hyperglycemia. Chronic hyperglycemia causes damage to eyes, kidneys, nerves, heart and blood vessels. It is caused by inherited and/or acquired deficiency in production of insulin by

the pancreas, or by the ineffectiveness of the insulin produced. It results either from inadequate secretion of hormone insulin, an inadequate response of target cells to insulin, or a combination of these factors. This disease requires medical diagnosis, treatment and changes in life style. It is projected to become

one of the world is main disablers and killers within the next 25 years [1].

Elucidation of the role of cholesterol in the pathogenesis of atherosclerosis is often referred to as one of the greatest till date discoveries. 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 [2].

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 [3].

The management of diabetes is a global problem until now and successful treatment is not yet discovered. There are many synthetic medicines developed for patients, but it is the fact that it has never been reported that someone had recovered totally from diabetes. The modern oral hypoglycemic agents produce undesirable and side effects. Thus, alternative therapy is required, a need of hour is to shift towards the different indigenous plant and herbal formulations. The traditional medicines demonstrated a bright future in therapy of diabetes and to understand the importance of traditional herbs, in the present study the *Cleome viscosa* has reported to have nutritional, antioxidant and free radical scavenging properties in has been reported to possess anit oxidant, wound healing, analgesic, antidiarrheal, antipyretic, anti-emetic, antimicrobial and diabetic hepato-protective and diabetic neuroprotective [4,5].

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 *Cleome viscosa* was collected from Mahabubnagar, 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 200-220 g were selected and housed in polypropylene cages in a room where the congenial temperature was $27^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and 12 h 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) [6].

Method

Preparation of the Extract

The whole plant was isolated, chopped into small pieces and dried under shade at room temperature for seven days. The dried plant was 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 h. This was then boiled for 4 h. The extract 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.

Induction of Diabetes

A freshly prepared solution of streptozotocin (45 mg/kg in 0.1 M buffer citrate solution) is injected to overnight fasted rats. After 48 hrs the rat's blood glucose levels of 250 mg/dl or above were considered for the further study [7].

Induction of Atherosclerosis

Although the cause and pathogenesis of atherosclerosis remains largely unresolved, it is generally agreed that correlation exists between high blood cholesterol and cardiovascular diseases. Atherogenic Diet 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 D-2 along with Atherogenic diet, success has been achieved in developing atherosclerosis in rats in a short period. The atherogenic diet (AD) consisting of 1.5 ml olive oil containing 8 mg (3,20,000 IU) vitamin D2 and 40 mg cholesterol and was given for 5 consecutive days (Table 1). The rats were fed with high fat diet along with weekly challenge of oral vitamin D-2 for one month through oral route [6,7].

Toxicological Studies

The Methanolic extract of plant is subjected to toxicological studies and the rats were observed for every 4 h till for 24 h for 30 days and behavioural changes and mortality were observed, it was found to be safe. The low dose of 250 mg and high dose of 500 mg/kg body weight of rat is being selected for the study.

Experimental Protocol

In order to induce atherosclerosis, the method reported by Sharma et al. [7] and Tanwar et al. [6] 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 (45 mg/kg)

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

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

Group V: Atherogenic diet + STZ (45 mg/kg) + MECV (500 mg/kg/days).

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 was done by using the **Friedward's formula**
VLDL-C=Triglycerides/5
LDL-C=Total cholesterol-(HDL-C+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 [6].

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

The Methanolic extract of the *Cleome viscosa* has shown significant effect on the serum lipid profile and blood glucose levels. Whereas it hasn't show a significant effect on controlling the body weight. The blood glucose levels were initially high with no significant effect till 6th day of the study but gradually from the 15th day the decrease in the blood glucose levels was observed which on the final day shown the highly significant decrease in the elevated blood glucose levels at high dose of the plant extract and standard drug treatment.

The serum lipid profile was checked at initial and final day of the study, the high dose and standard drug treatment has shown the significant effect of the plant extract.

Effect of administration of Methanolic extract of *Cleome viscosa* (250 and 500 mg/kg P.O once daily for 30days)/ Glibinclamide (5 mg/kg/P.O once daily for 30 days) 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 (Figure 1).

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 (Figure 2).

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 (Figure 3).

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 (Figure 4).

Group 5

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

Discussion

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 (Table 2 and Table 3). 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 [8]. LCAT enzyme is involved in the transesterification of cholesterol, the maturation of HDL and the flux of cholesterol from cell membranes into HDL [3].

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

Conclusion

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. Further study is required for the detailed elucidation of mechanism for the activity shown.

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Table 1: Atherosclerotic diet

Ingredients	Weight in grams
Olive oil	1.5 ml
Cholesterol	40 mg
Standard pellet powder (Q.S)	100

Table 2: Effect of 99.9% MECV on Body weight and Lipid profile on Atherosclerosis in STZ induced diabetic rats

Group	Normal	Control	Standard	MECV T1	MECV T2
Initial body weight	166.7 ± 1.89	168.3 ± 1.52	173.7 ± 1.82	178.0 ± 2.30	177.2 ± 2.71
Final body weight	202.0 ± 3.10	266.5 ± 1.54	219.2 ± 3.51*	231.5 ± 5.50	225.2 ± 3.28*
Initial HDL	39.67 ± 1.05	23.00 ± 0.85	22.83 ± 0.98	22.67 ± 0.71	23.17 ± 0.60
Final HDL	45.67 ± 1.22	13.83 ± 0.60 ^a	32.17 ± 0.94**	30.33 ± 0.88*	35.67 ± 0.66**
Initial LDL	18.80 ± 1.15	61.67 ± 2.08	66.73 ± 1.19	65.33 ± 1.40	67.33 ± 1.30
Final LDL	17.07 ± 1.40	102.3 ± 2.75 ^a	35.50 ± 1.67***	62.80 ± 2.28*	42.82 ± 2.28***
Initial VLDL	14.87 ± 0.22	22.67 ± 0.38 ^c	22.10 ± 0.28	22.33 ± 0.30	22.17 ± 0.36
Final VLDL	15.27 ± 0.16	31.40 ± 0.69 ^c	16.17 ± 0.14**	18.70 ± 0.35*	15.60 ± 0.31**
Initial TC	73.50 ± 1.33	108.2 ± 2.18 ^b	111.7 ± 1.28	110.3 ± 1.54	112.2 ± 1.90
Final TC	78.00 ± 1.23	147.5 ± 2.43 ^a	84.67 ± 1.72**	91.83 ± 2.10*	77.33 ± 1.45**
Initial triglycerides	74.33 ± 1.11	113.3 ± 1.94 ^b	110.5 ± 1.43	111.7 ± 1.52	110.8 ± 1.83
Final triglycerides	76.33 ± 0.80	157.0 ± 3.48 ^a	80.50 ± 0.76**	93.50 ± 1.76*	78.00 ± 1.57**

*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. ^a P<0.001 ^bP<0.05 and ^cP<0.1 as compared to normal group.*

Table 3: Effect of 99.9% MECV extract on Fasting Blood Glucose levels on in STZ induced diabetic rats

Group	0 th day	6 th day	15 th day	30 th day
Normal	82.67 ± 1.05	80.83 ± 1.47	82.67 ± 1.54	83.00 ± 1.26
Control	313 ± 1.47	307.3 ± 1.99	326.7 ± 2.65 ^a	330.8 ± 2.22 ^a
Standard	277.2 ± 0.94	309.5 ± 0.76	190.3 ± 1.89 ^{**}	141.3 ± 2.31 ^{***}
MECV T1	297.0 ± 0.57	311.7 ± 1.05	223.3 ± 1.54 [*]	169.8 ± 1.30 ^{**}
MECV T2	283.3 ± 2.33	314.8 ± 1.57	192.2±1.92 ^{**}	133.2 ± 1.77 ^{***}

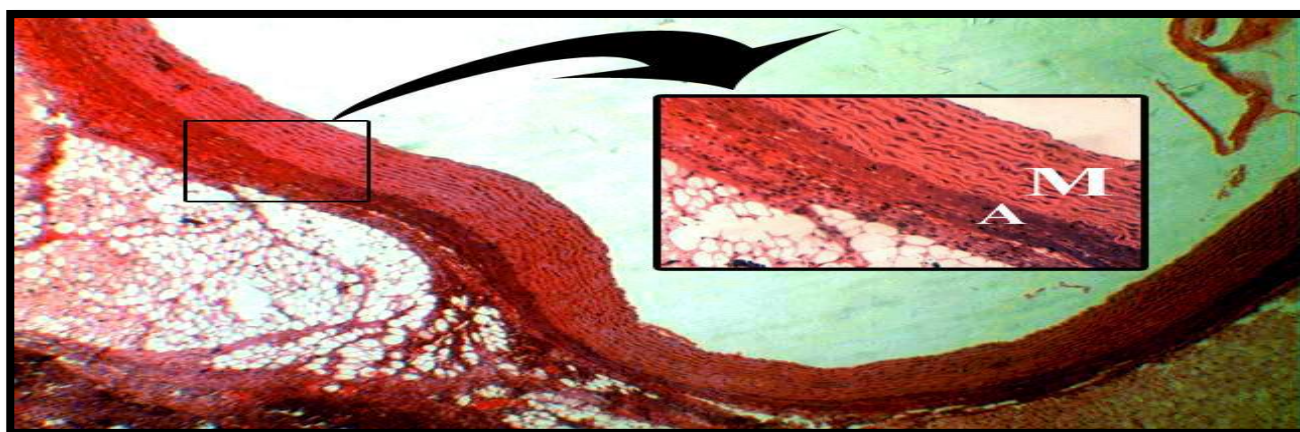


Figure 1: Histopathology of Aorta of group-I

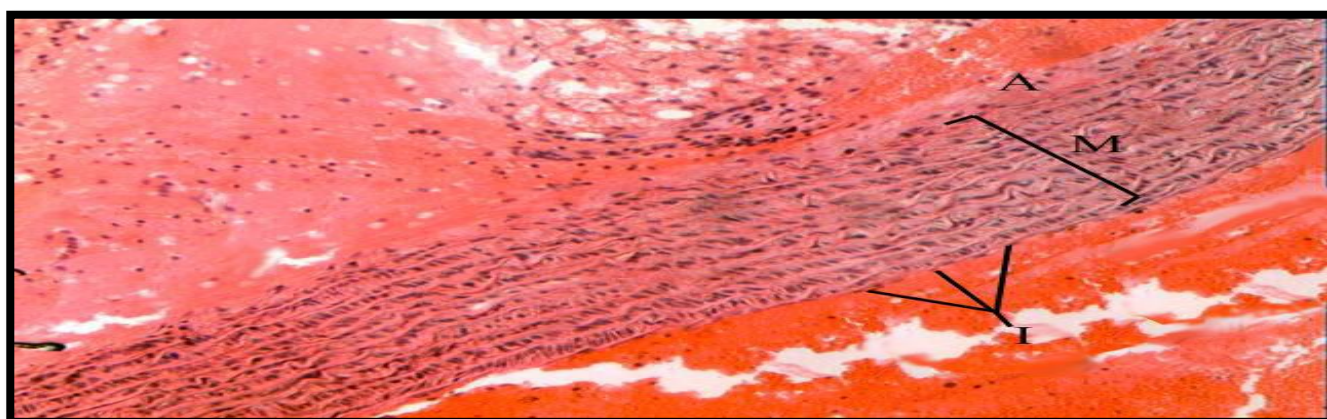


Figure 2: Histopathology of Aorta of group-II

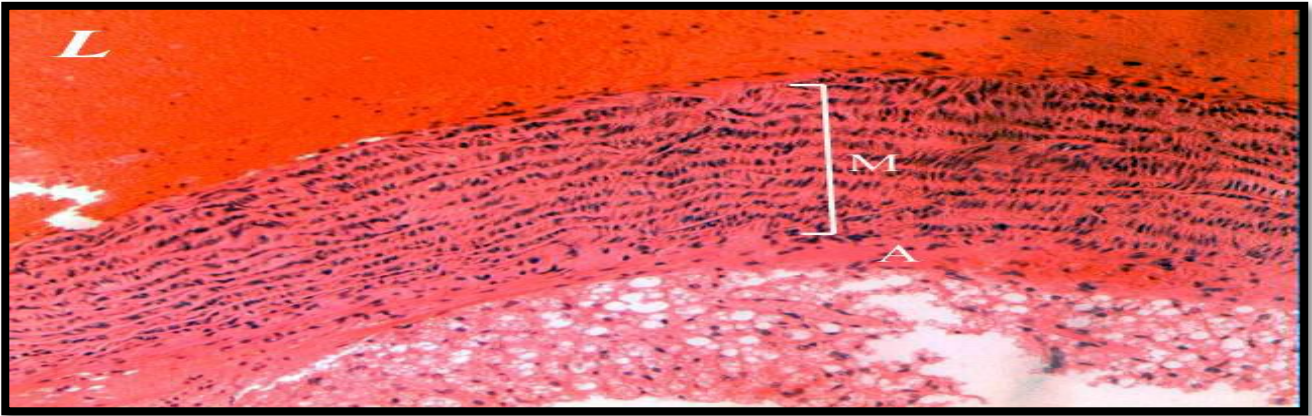


Figure 3: Histopathology of Aorta of group-III

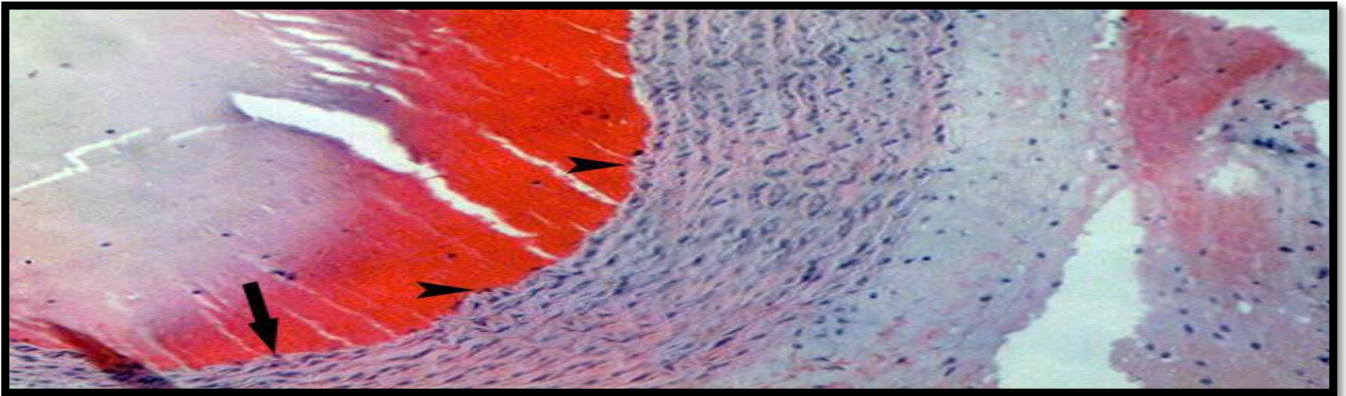


Figure 4: Histopathology of Aorta of group-IV

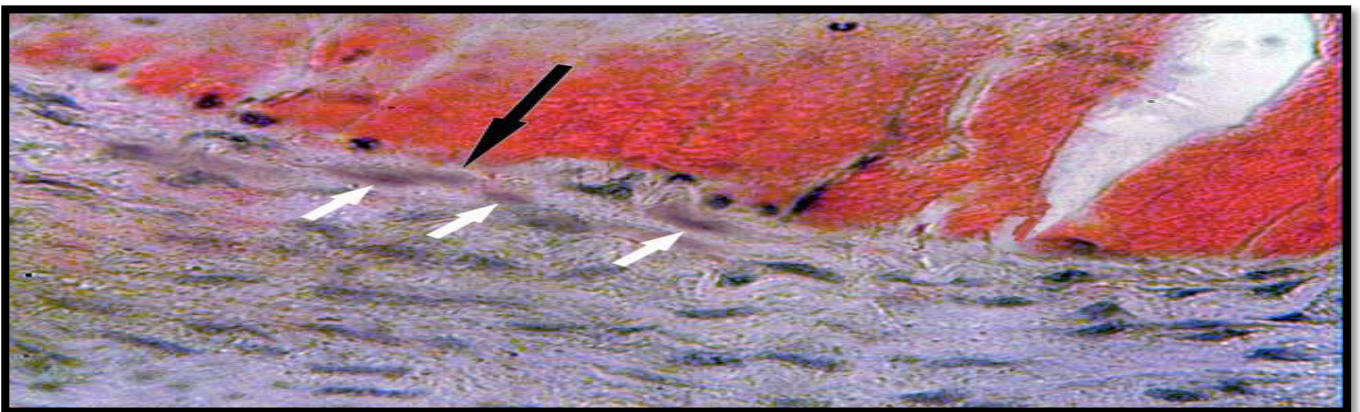


Figure 5: Histopathology of Aorta of group-V

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