

ANTIATHEROSCLEROTIC EFFECT OF POLICOSANOL ON HISTOPATHOLOGY OF CHOLESTEROL INDUCED MALE ALBINO RABBITS.

¹Purohit A, ²Kotru B* and ³Joshi K

¹Department of Zoology, JNVU University, Jodhpur 342 001, India.

²Department of Biochemistry, Sera Care lab and research centre, Jodhpur 342001, India.

Article Received on
16 Nov 2014,

Revised on 11 Dec 2014,
Accepted on 05 Jan 2015

***Correspondence for
Author**

Dr. Kotru, B.

Department of Zoology,
JNVU University,
Jodhpur 342 001, India.

ABSTRACT

The rabbits were first made exogenously hyperlipidemic by giving them high fat diet and cholesterol powder (500mg/kg body weight) in 5ml of coconut oil orally for 15 days and then were administered with policosanol and is compared with the standard drug currently used in the market i.e statin. Hyperlipidemic control group is provided with cholesterol for complete 60 days and the result of plaque size is maximum (16.93% of total wall area) and that of lumen size is reduced upto minimum (28.96% of total wall area). While on the administration of policosanol the plaque size is reduced upto minimum of (8.26% of total wall area) and the lumen area is increased upto (35.45% of total

wall area), which was concordant with statin that had showed the plaque size reduced upto (8.17% of total wall area) and increase of lumen area of about (48.93% of total wall area). The variation of body and organ weight was also studied and a non significant changes were observed in them. A slight significant variation were observed in case of organ weight of liver and aorta. Indicating that weight of individual is independent by its cholesterol intake and is widely dependent on the metabolic activity of the body organs. Thus a definite regression of atheroma and hindered plaque formation in aorta after policosanol treatment concluded that it possesses antiatherosclerotic potential in cholesterol induced male albino rabbits.

KEYWORDS: Antiatherosclerotic potential, *Saccharum officinarum*, plaque, lumen, policosanol.

INTRODUCTION

Atherosclerosis is a complex multifactorial inflammatory disease, characterized by the presence of lesions due to the accumulation of lipids in the walls of large and medium-sized arteries (Ross, 1999). The earliest event is the formation of atherosclerotic lesion or "fatty streak" due to endothelial dysfunction, characterized by inflammatory cellular infiltration, mainly of monocyte derived macrophages and T-lymphocytes. Fatty streaks are usually present in humans in several major vessels, namely aorta, coronary artery and cerebral artery during different stages of life (Lusis, 2000). The clinical manifestation of atherosclerotic plaque formation is acute vascular occlusion due to the formation of a thrombus or clot, which can lead to ischemia of vital organs, such as heart causing myocardial infarction, brain resulting in strokes and lower extremities causing peripheral artery disease (Kruth, 2001). Due to the plaque formation the lumen area of the aorta gets reduced.

Since the incidence of CHD peaks in elderly people (Manolio *et al.*, 1992), thus primary prevention in the elderly patients is subject of great clinical importance nevertheless, special precautions have to be taken in these patients because of their increased susceptibility to drug related adverse reactions, their deteriorating general health status (frequently characterized by concomitant diseases) and treatment cost considerations. The various studies demonstrate that the long chain aliphatic alcohols extractable from sugarcane wax (*Saccharum officinarum*) can markedly reduce serum cholesterol in animals (Menendez *et al.*, 1997). Policosanol is a natural mixture of long chain primary aliphatic saturated alcohols that is isolated from sugarcane wax (*Saccharum officinarum*) (Hargrove *et al.*, 2004). Subsequent toxicological studies determined that policosanol had no discernible toxicity, carcinogenicity, teratogenicity and indeed inhibited spontaneous atherogenesis in the macaques (Rodriguez *et al.*, 1998). In particular there were no signs of damage to the liver or skeletal muscles and no histological abnormalities such as one would expect with high doses of statins.

In the present study, an attempt was made to elucidate hypolipidemic and antiatherosclerotic efficacy of policosanol in cholesterol fed rabbits.

MATERIALS AND METHODS

Collection of Policosanol

Policosanol used in the present study was provided by Panacea Biotec Pvt.Ltd. India with the name Heartfelt. All the other used chemicals were of the highest analytical grades commercially available.

Experimental design

Animals

Healthy adult male New Zealand rabbits were procured from Forest Department, Jodhpur (Rajasthan). Weights and age of animals were 1.25-1.75 kg and 10-12 month respectively. Animals were housed in well-lighted air-conditioned room in metallic wire gauge cages, under controlled environmental conditions with 12 hours illumination and 12 hours darkness cycle. Animals were fed on standard rabbit chow supplied by Hindustan liver ltd., India. The food was supplemented with green leafy and seasonal vegetables and water *adlibitum*. Ethical approval to conduct the study was obtained from the ethical committee of the university.

Induction of hyperlipidemia

The hyperlipidemic condition was induced by cholesterol feeding to rabbits. The cholesterol powder (500 mg/kg body weight) was mixed in 5ml of oil mixture and administered to the animals orally.

Standard drugs

Atorvastatin was used as standard hypolipidemic drug, and it was given to the animals at the dose of 0.25mg/kg body weight dissolved in 5ml distilled water.

Feeding of Policosanol

For administration to the animals, the policosanol (0.5mg/kg body weight) was suspended in 5ml of distilled water. The dose of the drug was determined by LD50 test.

Experimental groups

Twenty four male albino rabbits were divided into four groups the control and experimental groups, usually consisted of six animals each.

Group 1 - Vehicle treated control or intact control (60 days)

Group 2 - Atherodiet + cholesterol feeding (500mg/kg body weight) for 60 days

Group 3- Cholesterol feeding (500mg/kg body weight) for 15 days + policosanol (0.5mg/kg body weight) for 45 days

Group 4- Cholesterol feeding (500mg/kg body weight) for 15 days + statin (0.25mg/kg body weight) for 45 days.

Criteria of observation

At the end of experimental period, final weight were taken and all animals of the group were sacrificed under prolonged ether anesthesia. Blood was collected through cardiac puncture, and serum was separated by centrifugation for 10 minutes at 3000 rpm and was divided into 4 to 5 portions for different determinations. Aorta tissues were collected in 10% formalin and thin sections were made by microtomy technique. Planimetric study was done by Camera Lucida.

Statistical analysis of data

All the values of body / organ weights and planimetric observation were expressed in terms of mean value \pm standard error by using SPSS- statistical data analysis software. The different groups were compared among each other using *post hoc* Sheffe's test. the level of significance was set at $p < 0.05$.

RESULTS

A non-significant reduction in final body weight was observed in rabbits fed with atherodiet (Gr. 2). Simultaneously administration of drug did not cause any appreciable change in the final body weight (Gr. 3-4). The weight of aorta and liver was increased in atherodiet fed rabbits, This could be due to lipid deposition after continuous atherodiet administration. The weight of heart and kidney in all the groups remain unaltered. Policosanol or statin feeding did not change the weight of liver and aorta (Table 1).

Surface area studies (Planimetric table 2) of ascending aorta showed significant increase in total wall area of aorta in atherodiet fed rabbits (Gr. 2) when compared to intact control group (Gr.1). The simultaneous feeding of atherodiet (15 days.) and drugs (Gr.3) and (Gr.4) showed significant increase in total wall area, when compared with Group 1 while on comparing with Group 2 slightly significant decrease was observed in both the 2 cases.

The lumen area of aorta was reduced significantly after atherodiet feeding for complete 60 days (Gr.2). The drug treatment (Gr.3-4) lead to significant decrease in lumen area of policosanol and statin treated groups.

The 40% of total wall area was occupied by plaque in rabbits fed with atherodiet (Gr.2) when compared to intact control group. Plaque size was reduced significantly after administration of policosanol (Gr.3) & statin (Gr. 4) when compared to hyperlipidemic control group 2.

The histological observation of aorta (Plate 1 to 3) of intact control animals shows three layers i.e., tunica intima, tunica media and tunica adventitia. The tunica intima is composed of collagenous connective tissue with few elastic fibres. The media is particularly broad and extremely elastic and consist of fenestrated sheets of elastin separated by collagenaeous connective tissue and few smooth muscle fibres. The collagenous tunica adventitia contains small vasa vasorum. The atherodiet feeding to rabbits (Gr.2) resulted into formation of plaques in aorta. This is characterized by thickened intima, cell proliferation, collagen and lipid deposition. Calcification of tunica media was conspicuous with abundance of foam cells. Lumen of aorta became narrow. The tissues lining the aorta consist of large macrophages with abundant foamy cytoplasm accompanied by strands of fibrous tissue. The policosanol treatment reduced the size to greater extent but plaque persists till the end of the experiment, the rabbits treated with statin showed negligible plaque structure.

Table–1: Body and organ weight of drug treated intact rabbits (mean of 5 values \pm sem).

TREATMENT GROUPS	Body Weight (Kg)		Liver	Heart	Kidney	Aorta
	Initial	Final	gm /Kg body weight			
CONTROL (Gr. 1)	1.52 \pm 0.17	1.33 \pm 0.02	25.98 \pm 1.13	2.43 \pm 0.13	6.72 \pm 0.33	0.21 \pm 0.07
HYPERLIPIDEMIC (Gr. 2)	1.74 \pm 0.13	1.34 \pm 0.03	39.00 \pm 1.67 ^c	2.63 \pm 0.17 ^d	6.62 \pm 0.34 ^d	0.35 \pm 0.12 ^c
POLICOSINOL (Gr.3)	1.40 \pm 0.13	1.35 \pm 0.02	24.70 \pm 1.35 ^{d,g}	2.31 \pm 0.12 ^{d,h}	7.06 \pm 0.44 ^{d,h}	0.26 \pm 0.12 ^{d,h}
STATIN (Gr. 4)	1.44 \pm 0.15	1.35 \pm 0.02	28.02 \pm 1.58 ^{d,g}	2.43 \pm 0.16 ^{d,h}	6.65 \pm 0.35 ^{d,h}	0.28 \pm 0.13 ^{d,h}

Gr. 2, 3 and 4 were compared with Gr. 1

Gr. 3 and 4 were compared with Gr. 2

P \leq 0.05 = a

P \leq 0.05 = e

P \leq 0.01 = b

P \leq 0.01 = f

P \leq 0.001 = c

P \leq 0.001 = g

Non significant = d

Non significant = h

Table 2: Planimetric Dimensions of Ascending Aorta of Various Drug Treated Intact Rabbits (Mean of 5 Values \pm Sem).

Treatment Groups	Total Wall Area	Lumen	Intima	Plaque	Media	Adventitia
	% of Total Area					
Control (Gr.1)	48.26 \pm 2.08	51.50 \pm 1.07	8.93 \pm 0.15	Nil	29.0 \pm 0.8	10.32 \pm 0.05
Hyperlipidaemic (Gr.2)	70.11 \pm 3.52 ^b	28.96 \pm 2.21 ^b	11.01 \pm 0.32 ^a	16.93 \pm 1.07 ^c	31.77 \pm 0.64 ^d	10.92 \pm 0.12 ^d
Policosanol (Gr.3)	59.16 \pm 2.41 ^{b,e}	35.45 \pm 4.02 ^{b,f}	10.21 \pm 0.64 ^{d,h}	8.26 \pm 1.06 ^{a,f}	29.63 \pm 3.02 ^{d,h}	11.26 \pm 0.85 ^{d,h}
Statin (Gr.4)	58.63 \pm 1.06 ^{b,e}	48.93 \pm 2.46 ^{d,f}	10.01 \pm 0.32 ^{d,h}	8.17 \pm 1.04 ^{a,f}	28.91 \pm 1.63 ^{d,h}	11.42 \pm 1.06 ^{d,h}

Gr. 2, 3 and 4 were compared with Gr.1

P \leq 0.05 = a

P \leq 0.01 = b

P \leq 0.001 = c

Non significant = d

Gr. 3 and 4 were compared with Gr.2

P \leq 0.05 = e

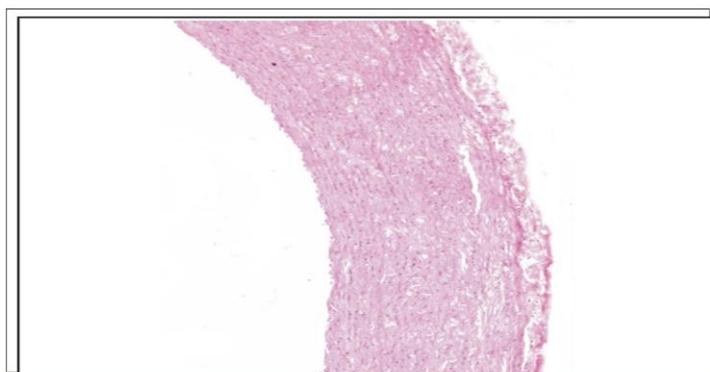
P \leq 0.01 = f

P \leq 0.001 = g

Non significant = h

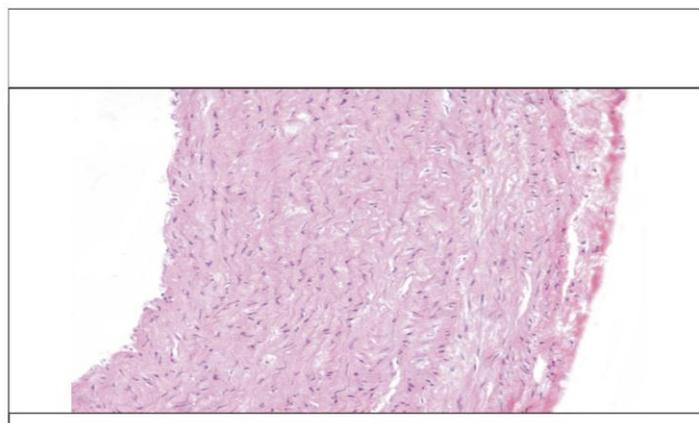
AORTA (x100 & x200 HE)

PLATE : 1



INTACT CONTROL (X100)

Fig. 1

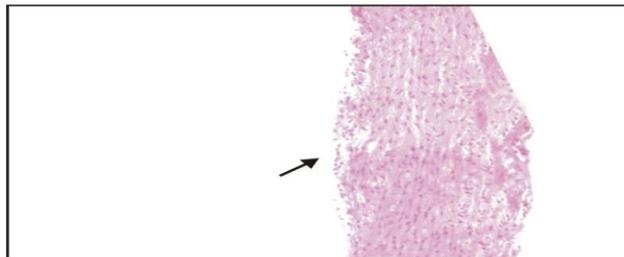


INTACT CONTROL (X200)

Fig. 2

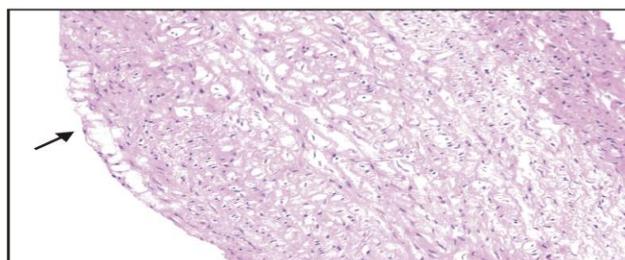
AORTA (x100 & x 200 HE)

PLATE : 2



HYPERLIPIDEMIC CONTROL (X100)

Fig.3



HYPERLIPIDEMIC CONTROL (X 200)

Fig.4

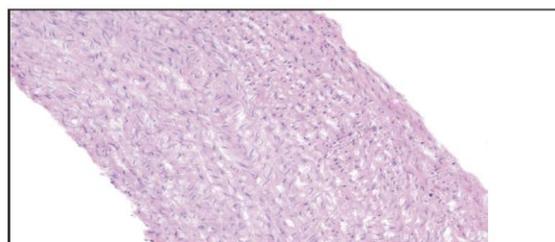
AORTA (x100 & x 200 HE)

PLATE : 3



POLICOSANOL (X 100)

Fig. 5

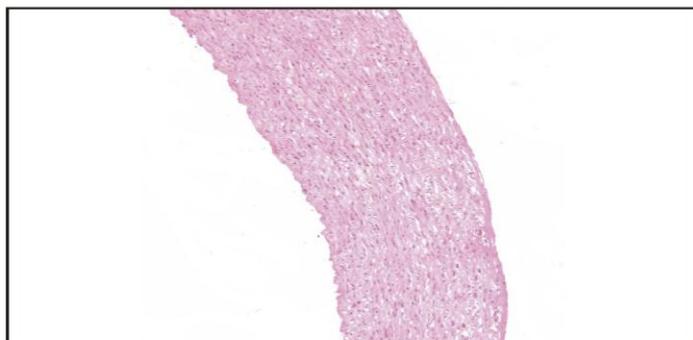
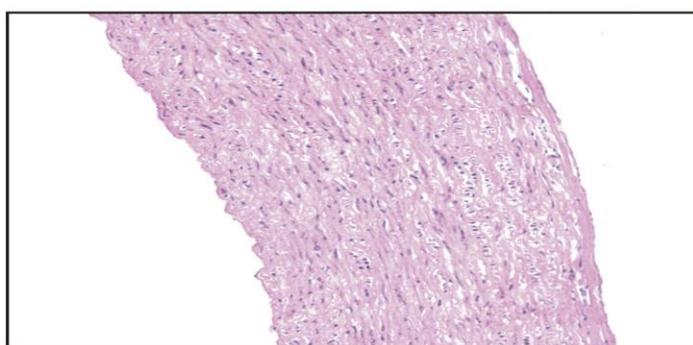


POLICOSANOL (X 200)

Fig. 6

AORTA (x100 & x 200 HE)

PLATE : 4

**STATIN
(X 100)***Fig. 7***STATIN
(X 200)***Fig. 8***DISCUSSION**

The effect on body weight after the treatment of the test substance was an important yardstick of the anabolic or catabolic mode of action of the test substance and the general metabolic status of the animal. There was natural, marginal increase in the final body weight after the simultaneous administration of atherodiet and policosanol and in vehicle treated control group, as compare to their respective mean initial body weight. But it was indisputable that there was no statistical correlation between elevation of the body weight and administration of the test substance, as the elevation recorded in all experimental groups was nonsignificant statistically. It suggests the general metabolic well being of the experimental animals.

A marginal increase in weight of liver was observed after administration of high fat diet and cholesterol. This may be due to deposition of lipid in the liver, causing fatty liver (Sharma *et al.*, 1990; Purohit and Daradka, 1999; Purohit, 1999). Purohit and Vyas (2006) also showed significant increase in liver weights in cholesterol fed rabbits. The weight of aorta was increased in atherodiet fed rabbits. This could be due to accumulation of lipid in the intima.

In drug treated groups nonsignificant reduction was observed in weight of liver and aorta when compared with vehicle treated group.

The results from planimetric studies of ascending aorta were coinciding with the results of histology of aorta. A significant increase in the total wall area of aorta was observed in rabbits fed with atherodiet. The lumen of aorta was reduced and of total area of aorta was occupied by atherosclerotic plaque (plate-2). Similar type of results were also observed by several researchers in different plant products (Dixit and Joshi, 1985; Gusain *et al.*,1995; Purohit and Vyas, 2005 and 2006; and Sharma *et al.*,1996). In policosanol and statin treated groups the plaque size in reduced significantly increasing the lumen area and decreasing total wall area, thus both the drugs are equal in efficacy and can diminish plaque deposition (plate 3-4).

Regarding the mechanism of action, it is possible that the hypocholestrolemic effect is associated with a decrease in intestinal absorption of cholesterol and with an increase in fecal excretion of steroids and bile acids (Mehta *et al.*,2003). Hypolipidemic effect of policosanol may be achieved due to its interference in the biosynthetic pathway of cholesterol. May be policosanol put some effect on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate controlling enzyme in cholesterol biosynthesis. The inhibition of this enzyme depresses the *de novo* synthesis of HMG-CoA reductase or stimulates its degradation. This hypothesis not yet been conclusively proven, studies supporting it had done by some researchers (Menendez *et al.*,1997) and some recent studies had cast doubt on this hypothesis (Wang *et al.*,2003). These results of histology and planimetric studies indicates that policosanol is nearly equal in action with that of statin with no drug related disturbances and reduces plaque or lipid deposition in blood. Therefore, it may be concluded that policosanol possesses pronounced hypolipidemic and antiatherosclerotic potential in cholesterol fed rabbits.

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