ABSTRACT

High fat or cholesterol have been implicated in the pathogenesis of atherosclerosis. The present study was designed to investigate the possible hypolipidemic effects of policosanol (Saccharum officinarum wax) on cholesterol fed rabbits and to compare with standard drug available in the market (statin). The rabbits were first made exogeneously 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 drugs like Policosanol and statin. In the hyperlipidemic rabbits there were an apparent reduction in the animal body weight and a significant increase in serum total cholesterol (78.63%), triglyceride (27.24%), LDL-c (78.56 %) and VLDL-c (38.04%) with a concomitant non significant increase in serum HDL-c (25.00%). Whereas oral treatment of animals with Policosanol for about 45 days in a dose of 0.50 mg/kg body weight lead to non significant change in the animal body weight but show significant reduction in serum total cholesterol (95%), triglyceride (82%), LDL-c (97%) and VLDL-c (82%) with a slightly non significant increase in serum HDL-c. The nearby same results were observed in statin treatment. Policosanol was found nearly equal in efficacy like statin standard drug in comparison with hyperlipidemic animals on all serum lipid parameters. Thus policosanol extract offers promising hypolipidemic effects with no drug related disturbances in safety medications that may be mainly attributed to its potent antiatherosclerotic potential.

KEYWORDS: Antiatherosclerotic potential, Policosanol, Hyperlipidemia, Statin, Cholesterol.
INTRODUCTION
The high fat diet and cholesterol feeding has been shown to cause significant elevation in serum cholesterol and LDL cholesterol level in various animal models. Various studies showed that oral administration of sugar cane policosanol reduced plasma total cholesterol and low-density lipoprotein (LDL)-cholesterol concentrations and increased high-density lipoprotein (HDL)-cholesterol concentrations in healthy subjects. Many of the wide-ranging health benefits conferred by statin therapy suggest patients to be wise to take statins, however, owing to the significant expense of statin therapy, as well as to the potential for dangerous side effects that mandates regular physician follow-up, this strategy appears impractical. However, policosanol, a mixture of long-chain aliphatic alcohols extractable from sugar cane wax, in healthy subjects as hypercholesterolemics, and type 2 diabetics as well as in a number of animal models showed its hypolipidemic efficacy.

Heartfelt, a drug used in the present study is a red coloured, heart shaped, biconvex, film coated tablet which contains purified *Saccharum officinarum* wax (Policosanol) which is a natural mixture of higher aliphatic primary alcohols isolated and purified from the wax of sugar cane (*Saccharum officinarum*). The main components of Policosanol include octacosanol (66%), followed by triacontanol (12%) and hexacosanol (7%). The other 15% of essential alcohols are dotriacontanol, eicosanol, tetracosanol, tetratriacontanol, heptacosanol and nonacosanol. Its chemical formula is CH3—(CH2)n—CH2OH with chain length varying from 24 to 34 carbon atoms. The efficacy of sugar cane policosanol in improving the plasma lipid profile is equal to or even better than that of statins such as lovastatin, simvastatin and pravastatin.

Policosanol has many effects analogous to those of statins that are not likely explained by reductions of LDL cholesterol. However, unlike statins, policosanol does not directly inhibit HMG-CoA reductase, and even in high concentrations it fails to down-regulate this enzyme by more than 50%, thus likely accounting for the safety of this nutraceutical. In light of the fact that policosanol is quite inexpensive and is becoming available as a non-prescription dietary supplement, it may represent a practical resource that could enable the general public to enjoy health benefits comparable to those conferred by statins. In the current investigation efforts were made to explore the antiatherosclerotic efficacy of policosanol in cholesterol fed rabbits.
1. 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.

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 ad libitum. 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. In addition animals were fed with atherogenic diet. The atherogenic diet comprised wheat flour base with addition of milk powder, dried egg yolk, hydrogenated fat, butter, dried yeast, salt, sugar and vitamin mixture to produce the following nutrients in the given proportion. The average consumption of diet was 200g/rabbit per day.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>High fat diet</th>
<th>Normal diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>15%</td>
<td>20%</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>60%</td>
<td>65%</td>
</tr>
<tr>
<td>Sugar</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Fats</td>
<td>15%</td>
<td>5%</td>
</tr>
<tr>
<td>Salt</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Vitamin</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>Fiber</td>
<td>2%</td>
<td>2%</td>
</tr>
</tbody>
</table>

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, 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.

1. **Total Cholesterol** - Total Cholesterol concentration was determined by an enzymatic method by using commercial total cholesterol test kit (CHOD-PAP ; Centronic GmbH GERMANY).

2. **Triglyceride** - Triglyceride was determined by an enzymatic method by using commercial triglyceride test kit (GPO-POD; Centronic GmbH GERMANY).

3. **HDL-Cholesterol** - HDL-Cholesterol was measured by using commercial Direct HDL test kit (Accurex pvt. Ltd. India.).

4. **LDL-Cholesterol** - LDL-Cholesterol was Calculated by the Friedewald Equation.

\[ \text{LDL} = \text{TC} - \text{HDL} - \text{VLDL} \]
Where \( \text{VLDL} = \frac{\text{TG}}{5} \)

**Statistical Analysis of Data**
All the values of body / organ weights and Biochemical estimation were expressed in terms of mean value ± 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 $p < 0.05$.

2. RESULTS

The animal model which were fed with atherodiet for complete 60 days (graph1) there serum cholesterol level was raised to its maximum limits. Simultaneously there was a highly significant increase in triglyceride, LDL-c, VLDL-c and nonsignificant change was observed in HDL-c when compared with control group. Organ weight (Table 1) 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 showed highly significant increase in the weight of liver while non significant increase was observed in weight of aorta.

Serum lipid parameters study (Table 2) showed a nine fold increase in serum cholesterol after 60 days of cholesterol feeding, while policosanol lowered serum cholesterol (95%), triglyceride (82%), LDL-c (97%) and VLDL-c (82%) and the Statin treated rabbits also showed reduction in serum cholesterol (94.75%), triglyceride (73%), LDL-c (97.1%) and VLDL-c (74%) with a non significant increase in HDL-c in both cases.

3. DISCUSSION

Policosanol has been reported to decrease total cholesterol and low density lipoprotein (LDL) cholesterol. Various agents like statin which affect hyperlipidemia are still not used for prevention of atherosclerosis because of their potential toxicity and intolerance. It is well known fact that elevated total cholesterol and low density lipoprotein cholesterol (LDL-c) levels promote atherosclerosis and cardiovascular complications. Oxidative modification of low density lipoprotein cholesterol (LDL-c) appears to have an important role in initiation and progression of atherogenic changes in aorta. The agents which can lower serum cholesterol and scavenge or inhibit free radicals formation have gained wide therapeutic value. Cholesterol feeding in rabbits caused a significant increase in the circulating total cholesterol, LDL-cholesterol, VLDL-cholesterol and also in the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol. These results are consistent with earlier reports which have clearly established a correlation between dietary lipids and serum lipid profile. Supplementation of cholesterol in diet rapidly results in a marked increase in the production of cholesteryl ester rich-VLDL by the liver and intestine and a reduced number as well as rate of cholesterol removal by the hepatic LDL receptors. Consequently serum
levels of LDL-cholesterol and VLDL-cholesterol is increased. A significant increase in the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol indicate increased risk of atherosclerosis and coronary heart disease. Simultaneous administration of policosanol caused a significant decrease in serum total cholesterol, LDL-cholesterol and VLDL-cholesterol suggesting beneficial modulatory influence on cholesterol metabolism and turnover. HDL-c alters the balance of unesterified cholesterol between plasma and cells by increasing its utilization in the lecithin cholesterol acyl transferase (LCAT) system to form cholesterol ester, which moves rapidly back into the cells. \[12\] Reports suggest that the plasma concentration of high density lipoprotein (HDL) increases at a slower rate than LDL decreases, so that the LDL: HDL ratio significantly improves with in 1 year. \[13\] Decline in the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol observed in the policosanol treated rabbits might be a consequence of higher proportion of HDL-cholesterol which reduced atherogenic risk by virtue of increased reverse cholesterol transport from peripheral organs to liver. \[14,15\] Animal studies showed that octacosanol may influence fatty acid metabolization in muscle, suppress lipid accumulation in adipose tissue and decrease serum triglyceride. Elevated serum triglycerides is considered as independent risk factor for cardiovascular disease. Reduction in LDL-cholesterol and increase in HDL-cholesterol concentration are significantly related to lipid-lowering therapy. \[16\] The investigation reveals that the policosanol lowers the serum total cholesterol and LDL-cholesterol levels significantly, which reduces the risk of coronary heart disease.

Regarding the mechanism of action by which policosanol affects cholesterol levels is not yet fully understood. In vitro studies indicate that cholesterol synthesis in the liver is inhibited at an earlier stage that is the case with the statin drugs. \[17\] It is possible that the hypocholesterolemic effect is associated with a decrease in intestinal absorption of cholesterol and with an increase in fecal excretion of steroids and bile acids. \[18\] An animal study provided evidence that policosanol also may increase liver binding of LDL cholesterol from the blood. \[19\] A significant reduction in serum total cholesterol, triglyceride and LDL-c may have been achieved by the alcoholic components and its derivatives, \[20\] may be these components 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. Because the overall absorption of policosanol is low but its effects are substantial, a lipid-
lowering effect on the level of the intestine should be considered, which would distinguish policosanol from other known lipid-lowering principles. It is assumed that its major constituent, octacosanol, is mainly responsible for its effects, but in some experimental models the specific mixture of the policosanol aliphatic alcohols is slightly superior to octacosanol alone. On the basis of the demonstration that fatty alcohol oxidation can be achieved in cell cultures and in intestinal mucosa through a reversible fatty alcohol cycle and that beta-oxidation chain shortening of very long chain fatty acids (VLCFA) occurs in rat liver peroxisomes \[16\] speculated that policosanol-induced changes in hepatic cholesterol metabolism may be caused by the presence not only of aliphatic alcohols but also of VLCFA and chain-shortened secondary metabolites. This hypothesis not yet been conclusively proven, studies supporting it had done by some researchers \[19\] and some recent studies had cast doubt on this hypothesis. \[21\]

As Policosanol seems to be a very promising phytochemical alternative to classic lipid lowering agents such as the statins \[22\] and thus deserves further evaluation. This study shows clearly that the policosanol was comparably effective to the standard drug statin, Policosanol showed the edge over the standard Drug statin in reducing TG Levels owing encouraging results of policosanol, but further investigation on different animal models and human beings to confirm its validity and efficacy is needed.

![Graph 1: Percentage deviation in concentration of lipid parameters of Atherodiet Treated Rabbits.](image-url)
Table 1. Body and Organ Weight of Drug Treated Intact Rabbits (mean of 5 values ± sem).

<table>
<thead>
<tr>
<th>TREATMENT GROUPS</th>
<th>Body Weight (Kg)</th>
<th>Liver (gm/Kg body weight)</th>
<th>Heart (gm/Kg body weight)</th>
<th>Kidney (gm/Kg body weight)</th>
<th>Aorta (gm/Kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL (Gr. 1)</td>
<td>1.52±0.17</td>
<td>1.33±0.02</td>
<td>25.98±1.13</td>
<td>2.43±0.13</td>
<td>6.72±0.33</td>
</tr>
<tr>
<td>HYPERLIPIDEMIC (Gr. 2)</td>
<td>1.74±0.13</td>
<td>1.34±0.03</td>
<td>39.00±1.67c</td>
<td>2.63±0.17d</td>
<td>6.62±0.34d</td>
</tr>
<tr>
<td>POLICOSINOL (Gr. 3)</td>
<td>1.40±0.13</td>
<td>1.35±0.02</td>
<td>24.70±1.35d,g</td>
<td>2.31±0.12d,h</td>
<td>7.06±0.44d,h</td>
</tr>
<tr>
<td>STATIN (Gr. 4)</td>
<td>1.44±0.15</td>
<td>1.35±0.02</td>
<td>28.02±1.58d,g</td>
<td>2.43±0.16d,h</td>
<td>6.65±0.35d,h</td>
</tr>
</tbody>
</table>

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

P ≤ 0.05 = a
P ≤ 0.01 = b
P ≤ 0.001 = c
Non significant = d

P ≤ 0.05 = e
P ≤ 0.01 = f
P ≤ 0.001 = g
Non significant = h
Table – 2. Serum Biochemistry of Drug Treated Intact Rabbits (mean of 5 values ± sem).

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>CHO. (mg/dL.)</th>
<th>TG. (mg/dL.)</th>
<th>HDL-C (mg/dL.)</th>
<th>LDL-C (mg/dL.)</th>
<th>VLDL (mg/dL.)</th>
<th>CHO/HDL</th>
<th>LDL/HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL (Gr. 1)</td>
<td>85.22±4.26</td>
<td>93.64±5.68</td>
<td>29.70±1.77</td>
<td>36.20±6.40</td>
<td>20.00±1.40</td>
<td>3.00±0.02</td>
<td>1.73±0.16</td>
</tr>
<tr>
<td>HYPERLIPIDEMIC (Gr. 2)</td>
<td>1610.00±89.40c</td>
<td>378.02±11.98c</td>
<td>30.00±1.7d</td>
<td>1504.50±5.74c</td>
<td>76.60±2.50c</td>
<td>53.00±1.00c</td>
<td>49.30±0.02c</td>
</tr>
<tr>
<td>POLICOSANOL (Gr. 3)</td>
<td>76.96±6.22d,g</td>
<td>66.5±6.58a,g</td>
<td>27.8±1.66d,h</td>
<td>35.86±6.12d,g</td>
<td>13.3±1.31a,g</td>
<td>2.79±0.32d,g</td>
<td>1.3±0.25d,g</td>
</tr>
<tr>
<td>STATIN (Gr. 4)</td>
<td>84.46±3.03d,g</td>
<td>98.43±11.01d,g</td>
<td>25.86±1.74d,h</td>
<td>41.94±5.46d,g</td>
<td>20.02±2.18d,g</td>
<td>2.70±0.13d,g</td>
<td>1.19±0.67d,g</td>
</tr>
</tbody>
</table>

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

P ≤ 0.05 = a
P ≤ 0.01 = b
P ≤ 0.001 = c
Non-significant = d

P ≤ 0.05 = e
P ≤ 0.01 = f
P ≤ 0.001 = g
Non-significant = h
ACKNOWLEDGMENTS
We are thankful for Pancea Biotec Pvt Ltd for providing drug assistance. This work is supported by Sera Care Lab and Research Centre for all biochemical analysis.

Appendix
LDL – Low density lipoprotein
HDL – High density lipoprotein
VLDL – Very low density lipoprotein
TC – Total cholesterol
TG – Triglyceride.
VLCFA-Very long chain fatty acids.
HMG-CoA - 3-hydroxy-3-methylglutaryl coenzyme A.

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