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TOXICOLOGICAL STUDY OF POLICOSANOL IN EXPERIMENTALLY INDUCED HYPERLPIDEMIC MALE ALBINO RABBITS

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ABSTRACT

Policosanol has been reported to decrease total cholesterol and low density lipoprotein (LDL) cholesterol. The comparative hematological study was conducted in policosanol treated animals to evaluate its safety potential in white male albino rabbits. Four groups of 6 male albino rabbits each were used for study. To Intact control group no drug was given, to hyperlipdemic control group atherogenic diet with cholesterol powder (500 mg/kg body weight) mixed in 5ml coconut oil was given. To group 3 & 4 policosanol and statin was given as drug by oral administration; the drug treatment was carried out for complete 60 days. Animals were sacrificed by prolonged ether anesthesia after the 24 hours of last dose of drug, blood and serum samples were collected for the hematological and biochemical assays. The platelet count showed reduction from its normal range showing anti platelet activity while other hematological parameters like erythrocyte count, leucocyte count, hemoglobin count etc. were found to be in normal range. Other parameters like urea, creatinine etc, remain normal owing to nontoxic nature of drug.

Keywords: Antiplatelet, Hyperlipidemia, Atherosclerosis, Policosanol, Cholesterol.

INTRODUCTION

Atherosclerosis disease causes inflammation and damage of arterial wall through several cell types i.e. smooth muscle cells, monocyte derived macrophages, T-lymphocytes and platelet [1]. The denudation of arterial wall leads to endothelial cell dysfunction and platelet clumping [2]. Platelets are anucleate cells having a limited life span. Platelets are a rich source of mediators and lead to prominent release of mediators diffusing into the wall; these mediators cause continuous recruitment and activation of monocytes mainly through activation of the monocyte chemoattractant protein-1 (MCP-1) pathway which have a central role in atherogenesis [3]. Thus the platelet, once thought to be solely involved in clot formation, is now known to be a key mediator in various other processes such as inflammation, thrombosis and atherosclerosis. Supported by the wealth of evidence from clinical trials demonstrating their benefits in patient outcomes, antiplatelet agents have become paramount in the prevention and management of various diseases involving the cardiovascular, cerebrovascular and peripheral arterial systems. Despite being among the most widely used and studied classes of medical therapies, new discoveries regarding important clinical aspects and properties of these agents continue to be made [4]. Hence assessment of hematological profile becomes a prerequisite to understand the normal functioning of the system and to confirm the toxic nature of the administered drug policosanol.

Policosanol, a mixture of long-chain aliphatic alcohols extractable from sugar cane wax, in healthy

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subjects as hypercholesterolemics and type 2 diabetics as well as in a number of animal models showed its hypolipidemic efficacy [5]. 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 CH_3-$(CH2)_n$-CH_2OH with chain length varying from 24 to 34 carbon atoms [6]. 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 [7].

The present study was designed to investigate the antiplatelet activity and toxic nature of policosanol and its comparative status with that of synthetic drug statin (atorvastatin), currently present in market as drug treatment of hypercholesterolemia having certain side effects if used for a long period of time like rhabdomylysis, muscle weakness etc.

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 lever ltd., India. The food was supplemented with green leafy and seasonal vegetables and water *ad libitum.*

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 coconut oil mixture and administered to the animals orally. In addition animals were fed with atherogenic diet. The atherogenic diet was comprised of 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 as recommended by WHO protocol. The average consumption of diet was 200g/rabbit per day. Standard drug - 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 LD_{50} 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, animals were autopsized under prolonged ether anesthesia. Blood was collected through cardiac puncture in E.D.T.A vial for blood parameters determinations and centrifuged in plane vials for serum analysis.

Observation

Hematological observation- Hemoglobin concentration, WBC, RBC, Platelet Counts, MCV, MCH, MCHC, Lymphocytes, Monocytes, Granulocytes, RDW, PCT, MPV, PDW and Hematocrit were all determined on a Celltac- α Hematology analyzer (NIHON KOHDEN JAPAN).

Other Biochemical Parameters- The plasma activities of AST (SGOT) And ALT (SGPT) were determined by using commercial test kits SGM-Italia. Blood Urea (UV-GLDH), Serum Creatinine (Jaffe-Picrate) were determined by using commercial test kits Randox UK and Anamol pvt. Ltd. India. Blood Sugar (GOD-POD) was determined by using commercial kits of Anamol India. All Serum Biochemical analysis were Performed on Nex-Gen Auto-Chem Analyser (USA Awareness Technology).

RESULTS

Hematology

The results of all the haematological prameters except platelet count of Vehicle treated control (Gr. 1) and all other experimental groups (Gr.2-4) were found to be within the normal range.Slightly significant reduction was observed in a drug treated groups when compared with group 1 and group 2 (Table 1).

Other Parameters

Significant (P≤0.05) increase of 40 % was observed in atherodiet fed rabbits (Gr.2) when compared with control group (Gr.1). When compared with group 2 blood sugar showed significant decrease of 23.53% and 22.50% in policosanol and statin treated groups .Non significant change was observed in blood urea and creatinine. SGOT increases with increase in cholesterol. Policosanol reduces it to some limit but statin did not showed much impact. In SGPT level, no change was showed by statin while policosanol reduces it. The serum protein level in all the treated groups did not show any significant change when compared to intact control group (Gr.1) and hyperlipidemic control group (Gr.2) (Table 2).

Table 1. Hematology of drug treated intact rabbits (Mean of 5 Values ± SEM)

Hematology Parameters	Control (GR.1)	Hyperlipidemia (GR.2)	Policosanol (GR.3)	Statin (GR.4)
TLC/CU.MM.	7290.0 ± 380.0	7360.3 ± 390.0^{D}	7106.6± 233..3^{D,H}	7200.00 ±209.18^{D,H}
RBC.ML/DL	6.44 ± 0.40	5.82 ± 0.39^{D}	5.50 ± 0.26^{D,H}	5.09 ± 0.34^{D,H}
HGB.GM/DL	11.26± 0.44	10.29 ± 0.28^{D}	10.96 ± 0.54^{D,H}	10.43 ± 0.30^{D,H}
HCT.%	35.33± 0.598	36.00±3.732^{D}	34.600± 3.021^{D,H}	30.00± 3.08^{D,H}
MCV.FL.	64.19± 2.70	64.466± 2.90^{D}	63.16 ± 2.66^{D,H}	66.26± 2.74^{D,H}
MCH.PG.	17.62±0.434	18.666±0.66^{D}	17.16 ± 0.48^{D,H}	17.76± 0.47^{D,H}
MCHC.GM/DL	28.61±2.309	28.00±2.08^{D}	27.13± 2.64^{D,H}	27.43± 2.41^{D,H}
PLT.LACS/CU.MM.	2.80±0.980	3.85±0.20^{C}	1.90± 0.4^{B,F}	1.82± 0.82^{B,F}
LYM.%	39.290±3.057	39.680±3.48^{D}	42.26 ± 3.63^{D,H}	44.23± 2.98^{D,H}
MO.%	10.70±.0.651	11.666±0.88^{D}	10.23± 0.80^{D,H}	9.99 ± 1.10^{D,H}
GRN.%	50.71±3.315	48.666±3.05^{D}	47.50±4.83^{D,H}	45.78 ± 4.76^{D,H}
RDW.%	14.84 ± 0.59	14.26 ± 0.40^{D}	13.53± 0.32^{D,H}	12.66± 0.24^{D,H}
PCT.	0.02± 0.002	0.02± 0.003^{D}	0.02± 0.003^{D,H}	0.02 ± 0.00^{D,H}
MPV.FL.	7.12 ± 0.23	7.76± 0.05^{D}	7..37± 0.3^{D,H}	7.00 ± 0.5^{D,H}
PDW.%	18.22 ± 0.47	17.66 ± 0.88^{D}	17.40± 1.00^{D,H}	18.93± 0.29^{D,H}

GR. 2, 3 and 4 were compared with GR.1; GR. 3 and 4 were compared with GR.2; P ≤ 0.05 = A; P ≤ 0.05 = E; P ≤ 0.01 = B; P ≤ 0.01 = F; P ≤ 0.001 = C; P ≤ 0.001 = G; Non-Significant = D; Non-Significant = H.

Table 2. Other parameters of drug treated intact rabbits (Mean of 5 Values ± SEM)

Treatment Groups	B.Sugar mg/dl	B.Urea mg/dl	S. Creatinine mg/dl	SGOT IU/L	SGPT IU/L	T.Protein mg/dl
Control (GR. 1)	110.00±13.46	33.00±3.84	1.16±0.01	63.15±6.96	88.02±9.86	7.45±0.02
Hyperlipidemic (GR. 2)	154.86±5.73^{B}	30.10±1.00^{D}	1.20±0.03^{D}	105.13±10.62^{C}	114.40±7.06^{A}	7.50±0.15^{D}
Policosinol (GR.3)	118.33±4.02^{A,F}	26.63±2.22^{D,H}	0.98±0.02^{D,H}	86.36±5.63^{A,F}	82.30±4.32^{A,F}	7.53±0.02^{D,H}
Statin (GR. 4)	120.00±7.63^{A,F}	32.06±4.01^{D,H}	1.21±0.23^{D,H}	109.06±11.98^{B,H}	95.48±1.86^{B,H}	7.62±0.02^{D,H}

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; P ≤ 0.05 = E; P ≤ 0.01 = F; P ≤ 0.001 = G; Non-Significant = D; Non-Significant = H

DISCUSSION

Atherodiet or high cholesterol leads to increase in blood sugar level in blood. It was reported that there is a strong correlation between glucose and lipid profile. Glucose derived from diet or endogenous sources stimulates insulin secretion. Insulin promotes glucose uptake by skeletal muscle and fat, opposes hepatic glucogenolysis and gluconeogensis and inhibits fatty lipolysis. Free fatty acids (FFA) Liberated from adipose tissue contribute to insulin resistance in skeletal muscle and liver. Additional fat derived signals including TNF-α, renastin and adiponectin modulate insulin sensitivity and fatty acid metabolism in muscle and liver. The hypoglycemic efficacy of policosanol as well as hypolipidemic effect makes this natural drug beneficial to diabetic dyslipidemic patients. Increase in SGOT and decrease in SGPT level showed the hepatoprotective efficacy of policosanol. Policosanol does not impair glycemic control in diabetic patients as assessed through the evaluation of its effects on blood glucose and glycosylated hemoglobin (HbAlc) values.

In the present investigation the hematological parameters in all experimental groups remain unaltered except platelet count. Normal unperturbed endothelial cells exhibit anticoagulant properties that include the release of the inhibition of platelet aggregation prostacyclin, however, exposure to inflammation and atherogenic factors induces procoagulant activity [8]. More over apoptosis of endothelial cells increases the expression of phosphatidylserine and the loss of anticoagulant components of the endothelial cell membrane, as phosphatidylserine exposure enhances tissue factor activity, which is highly thrombogenic. Certainly, extra-cellular tissue factor expression is increased in and around apoptotic monocyte/lymphocyte

cells in necrotic basis for the generation of microparticles within the circulation, which act as potent pro-coagulant substrates both locally and systemically [9].

These particles are increased in patients with unstable coronary disease and account for the vast proportion of the procoagulant activity of the plaque. Thus prostacyclin reduces atherogenic cholesteryl ester accumulation in macrophages and vessel cell, inhibits platelet activation and mitogen release. Studies have demonstrated that policosanol induces increased prostacyclin levels [10].

CONCLUSION

In the present investigation the hematological parameters in all experimental groups remain unaltered except platelet count. The normal range of hematological and other parameters (sugar, urea, creatinin etc.) suggests non-toxic nature of the drug and indicates no drug related side effects on the animal models and the reduction in platelet count show some sort of antiplatelet activity of the policosanol similar to that of statin.

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