

## Lipid-lowering and antioxidant effects of policosanol in diabetic patients: a pilot study

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**Palabras clave:** policosanol, hipolipemiente, antioxidante, diabetes mellitus.

**ABSTRACT.** Coronary artery disease is the major complication and leading cause of death among patients with diabetes mellitus. Oxidative stress and dyslipidemia plays an important role in the pathogenesis and the occurrence of complications in diabetic populations. Policosanol is a mixture of high molecular weight primary aliphatic alcohols isolated and purified from sugar cane wax. The objective of this randomized, double-blinded and placebo-controlled pilot study was to investigate the effect of policosanol treatment on lipid profile and plasma oxidative variables in diabetic patients with hypercholesterolemia. Thirty diabetic patients of both sexes, aged 50 to 70 years were enrolled in the study. Fifteen patients were treated with policosanol (10 mg/day) and 15 with placebo for 12 weeks. Changes in low-density lipoprotein cholesterol (LDL-C) values were considered the primary efficacy variable. Plasma oxidative markers were considered secondary efficacy variables. Baseline characteristics were well matched in both groups. After 12 weeks, policosanol produced significant reductions of LDL-C and total cholesterol, and increased high-density lipoprotein-cholesterol. Policosanol significantly decreased serum malondialdehyde and increased the plasma total antioxidant capacity. There were no significant changes in any of the variables in the placebo group. Treatments were safe and well tolerated. No patient withdrew from the study. It is concluded that policosanol treatment favorably modified lipid profile and plasma oxidative variables in diabetic patients. Further studies should expand more data on the effects of policosanol treatment in diabetic patients.

**RESUMEN.** La enfermedad coronaria es la más importante complicación y principal causa de muerte entre los pacientes con diabetes mellitus. El estrés oxidativo y la dislipidemia juegan un rol importante en la patogenia y la aparición de las complicaciones en la diabetes. El policosanol es una mezcla de alcoholes alifáticos primarios de alto peso molecular aislada y purificada de la cera de caña de azúcar. El objetivo del presente estudio aleatorizado, a doble ciegas y controlado con placebo consistió en investigar el efecto del tratamiento con policosanol sobre el perfil lipídico y las variables oxidativas plasmáticas en pacientes diabéticos con hipercolesterolemia. En el estudio participaron treinta pacientes diabéticos de ambos sexos, con edades entre 50 y 70 años. Quince pacientes fueron tratados con policosanol (10 mg/día) y 15 con placebo durante 12 semanas. La variable primaria de eficacia fue el cambio de los niveles de colesterol transportado por lipoproteínas de baja densidad (LDL-C) y los cambios en marcadores oxidativos del plasma fueron considerados como variables secundarias de eficacia. Las características basales de ambos grupos fueron similares. Después de 12 semanas el tratamiento con policosanol produjo una reducción significativa de las concentraciones de LDL-C y de colesterol total, así como incremento significativamente las concentraciones de colesterol transportado por lipoproteínas de alta densidad. Además, disminuyó significativamente las concentraciones de malondialdehído y aumentó la capacidad antioxidante total del plasma. No se observaron cambios significativos de ninguna de las variables en el grupo placebo. Los tratamientos fueron seguros y bien tolerados. Ningún paciente abandonó el estudio. Se concluye que el tratamiento con policosanol modifica favorablemente el perfil lipídico y las variables oxidativas del plasma en pacientes diabéticos. Futuros estudios podrán ampliar los resultados obtenidos acerca del efecto del tratamiento con policosanol en pacientes diabéticos.

## INTRODUCTION

Coronary heart disease (CHD) is the major complication and leading cause of death among patients with diabetes mellitus.<sup>1-3</sup> Hyperlipidemia is common in patients with diabetes mellitus and the high frequency of CHD in diabetics is partly a consequence of the abnormalities of lipid metabolism, as hypercholesterolemia, especially when a high level of low density lipoprotein cholesterol (LDL-C) is present.<sup>4,5</sup>

Although LDL-C levels are less affected by diabetes than other indicators, elevated levels of LDL-C represent as coronary risk factor in these patients. Thus, structural changes frequently present in diabetics, such as glycation and oxidation of LDL molecules, rendering LDL more atherogenic particles.<sup>6,7</sup>

Diabetes mellitus is a widespread and devastating disease, associated with several mechanisms of tissue damage, one of which is oxidative stress. Oxidative stress and oxidative tissues damage are common end points of chronic diseases such as atherosclerosis and, cardiovascular diseases.<sup>8-11</sup> Oxidative stress plays an important role in the pathogenesis and complications of diabetes. Hyperglycaemia results in overproduction of oxygen free radicals which contributes to the progression of diabetes.<sup>12,13</sup>

A combination of lifestyle changes, including glycemic control, is the first-choice therapy for dyslipidemia management in diabetes mellitus. Nevertheless, adherence to these measures alone is often not sufficient and lipid-lowering drugs must be prescribed.<sup>14-17</sup>

Policosanol is a mixture of high molecular weight primary aliphatic alcohols isolated and purified from sugar cane (*Sacharum officinarum*), wax with cholesterol-lowering effects.<sup>18</sup> Policosanol inhibits cholesterol synthesis, modulating the activity of hydroxymethyl glutaryl Coenzyme (HMG CoA) through the increase of AMP kinase activity,<sup>19-22</sup> increasing LDL receptor-dependent processing and catabolic rate of LDL.<sup>23-26</sup>

Previous studies conducted in diabetic patients with hypercholesterolemia treated with policosanol showed that policosanol was effective and well tolerated in these patients.<sup>27-31</sup>

The objective of the present pilot study was to investigate the effect of policosanol therapy on lipid profile and plasma oxidative variables in diabetic patients with hypercholesterolemia.

## MATERIALS AND METHODS

### Study Design

This randomized, double-blinded, comparative study was conducted in Medical Surgical Research Centre (Havana City, Cuba) in accordance with the Declaration of Helsinki, its protocol being approved by the Institutional ethics committee of the Centre.

After obtaining their informed written consent, diabetic patients with hypercholesterolemia, were enrolled. Patients underwent physical examination and their clinical history was recorded to screen their eligibility for randomization (visit 1). On the second visit, they were randomly allocated under double blind conditions to receive policosanol (10 mg) or placebo tablets, once daily for 12 weeks at dinner time and to continue on their recommended dietary habits. Interim check-up (visit 3) and final (visit 4) visits were done after complete 6 and 12 weeks on therapy, respectively.

Subjects underwent a physical examination at each visit. Treatment compliance and request for reporting adverse experiences were controlled at visits 3 and 4. Laboratory tests (lipid profile, oxidative variables, and blood safety indicators) were conducted at baseline and after completing 6 and 12 weeks on treatment.

### Study subjects

Patients of both sexes, aged 50 to 75 years were enrolled in the trial. To be eligible for randomization, the enrolled diabetic patients with hypercholesterolemia (Total cholesterol > 5.2 mmol/L) should not show any of the exclusion criteria summarised below.

Patients with active liver or renal diseases, diagnosed neoplasias, uncontrolled diabetes (glycosylate haemoglobin values > 9 %) or hypertension (arterial pressure  $\geq$  180/100 mm Hg), thyroid dysfunction, nephrotic syndrome, habitual alcoholic state, psychiatry problems, serious events (acute coronary syndromes, stroke, transient ischemic attacks, major surgery, among others) during the prior six months and/or receiving other cholesterol lowering drugs were also excluded.

Causes of premature discontinuations were any adverse experience (AE) justifying such a decision, unwillingness to continue on the trial and major violations (failure in taking study treatments for  $\geq$  20 d and/or to consume supplements or medicines with antioxidant effects).

### Treatments

The dosage (10 mg/d), was selected taken into account that short-term administration of policosanol reduced LDL-C values and favourably modified plasma oxidative variables in different clinical studies.

Policosanol and placebo tablets, identical on appearance and packaged in identical codified containers, were given to the subjects according to their serial progressive inclusion. Randomisation was computer-generated using balanced blocks and allocation ratio 1:1. Participants were advised to bring all unused treatment to each visit. At visits 3 and 4, treatment compliance was assessed by counting the remainder tablets and interviewing the subjects. Compliance was considered as good if the subjects had taken at least 80 % of the tablets scheduled from the previous visit, and very good if consumption was over 90 %.

Medications and/or supplements with known effects on cholesterol or/and antioxidant effects were not allowed during the study. Subjects who were taking some of them were eligible for randomisation, only if they discontinued consumption for at least three months prior to the trial.

### **Efficacy variables**

LDL-C change was the primary efficacy variable. Treatment was considered effective if LDL-C decreased by at least 15 %.<sup>32</sup> Decreases of MDA and increases of plasma total antioxidant capacity were secondary variables.

### **Assessment of oxidative variables**

Plasma was separated from red blood cells by centrifuging at 3000 x g for 10 min and suitable portions were taken to assess oxidative markers. Whole blood and serum samples were used for assessing different indicators. Determinations were conducted in an Utrospec-Plus LKB spectrophotometer (Pharmacia LKB Biotechnology, Uppsala, Sweden).

Oxidative markers were assessed in the same day of blood drawing. All assays were done in triplicate. Plasma samples were frozen at -70 °C for the other analyses, which were done within the next 48 hours.

Plasma malondialdehyde (MDA) concentrations were analysed with a reagent kit (NWK-MDA01, NWLSS™, Canada) based on the reaction of MDA with thiobarbituric acid (TBA) forming an MDA-TBA adduct that absorbs strongly at 532 nm.<sup>33</sup> The values of TBA-reactive substances (TBARS), expressed as MDA (µmol/L), were calculated from a standard calibration curve generated with known amounts of freshly diluted malondialdehyde bis (dimethyl acetal).

For Total antioxidant capacity (TAC) quantification, a commercial kit (NX2332; Randox, Ltd., Crumlin, United Kingdom) was used. Briefly, 2,2'-azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS) was incubated with metamyoglobin and hydrogen peroxide to produce the radical cation ABTS. This has a relatively stable blue-green color that was measured at 600 nm. Based on their concentration, antioxidants will cause a suppression of the color production. TAC was expressed in mmol/L. All assays were carried out in triplicate.

### **Lipid profile and blood safety indicators**

Serum levels of total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C) and, blood biochemistry safety indicators (alanine amino transferase -ALT-, aspartate amino transferase -AST-, glucose, creatinine) were determined by enzymatic methods using reagent kits (Roche, Basel, Switzerland) in a Hitachi 719 autoanalyzer (Tokyo, Japan) of the clinical laboratory of the Medical Surgical Research Centre (Havana City, Cuba). Systematic quality control assessments were made throughout the study.

### **Safety and tolerability**

Data from physical examination (body weight, pulse rate and blood arterial pressure), laboratory safety indicators and adverse experiences (AE) were analysed. All undesirable events occurred to a subject during the trial, disregarding the cause, should be considered as adverse experience, whenever they newly appeared during the trial.

In accordance with their intensity, AE were classified as mild, moderate or serious. Mild AE should not require suspension of study tablets and/or specific treatment of the AE, moderate AE should require stopping therapy and/or specific treatment of the AE, serious AE should lead to hospitalisation and/or deaths.<sup>34</sup>

### **Statistical analysis**

Data analyses were performed in accordance to intention to treat (ITT), including all randomized subjects, regardless of study treatment compliance. A sample size of 30 patients (15 subjects/treatment group) and, assuming a 10 % of premature withdrawals, then, approximately 35 patients should be enrolled.

Comparisons of continuous variables were performed using the Wilcoxon test (within group comparisons) and the Mann Whitney U test (between group comparisons). Categorical variables were compared with the two tailed Fisher's Exact Test. A value of  $\alpha = 0.05$  was assumed for statistical significance. Comparisons were done with the Statistics software for Windows (USA).

## **RESULTS**

### **Baseline characteristics of study subjects**

Of 35 enrolled patients, 30 (18 women, 12 men and mean age: 68 years) were randomised to policosanol (n = 15) or placebo (n = 15), while five were not eligible because uncontrolled diabetes values, since patients showed above mentioned exclusion criteria for glycosylated haemoglobin value.

Baseline characteristics were well matched in both groups, so that they were homogeneous for comparisons (Table 1). The most frequent (> 30 %) risk factors at baseline were hypercholesterolemia (100 %), hypertension (66.7 %), family history of diabetes mellitus (60 %) and smoking (36.7 %). Concomitant therapy was also well balanced in the two groups, the most frequent being the oral hypoglycemic drugs (83.3 %) angiotensin converting enzyme inhibitors (ACEI) (43.3 %), diuretics (40 %), calcium antagonist (13.3 %) and antiplatelet drugs (13.3 %).

All randomized patients (100 %) completed the study. Treatment compliance was very good (> 95 %) and similar in both groups.

**Table 1.** Mean baseline characteristics of study patients

	Policosanol (n=15)		Placebo (n=15)		Total (n=30)	
Age (years) (X±SD)	69 ± 9		67 ± 11		68 ± 10	
Body mass index (kg/m <sup>2</sup> ) (X±SD) (X±SD)	26.2 ± 1.5		25.8 ± 1.4		26.0 ± 1.5	
Glucose values (mmol/L) (X±SD)	5.81 ± 1.42		6.00 ± 1.77		5.90 ± 1.53	
Glycosylated hemoglobin (%) (X±SD)	5.30 ± 1.68		5.40 ± 1.57		5.35 ± 1.64	
	n	%	n	%	n	%
Women	10	66.7	8	53.3	18	60.0
Men	5	33.3	7	46.7	12	40.0
Personal history						
Hypercholesterolemia	15	100	15	100	30	100
Hypertension	9	60.0	11	73.3	20	66.7
Family history of diabetes	8	53.3	10	66.7	18	60.0
Smoking	5	33.3	6	40.0	11	36.7
Coronary artery disease	2	13.3	2	13.3	4	13.3
Ischaemic transient attack	1	6.7	2	13.3	3	10.0
Treatment for diabetes						
Diet only	3	20.0	2	13.3	5	16.7
Oral hypoglycaemic drugs	12	80.0	13	86.7	25	83.3
Concomitant therapy* oncomitantes (MC)						
ACEI	6	40.0	7	46.7	13	43.3
Diuretics	5	33.3	7	46.7	12	40.0
Calcium antagonist	2	13.3	2	13.3	4	13.3
Antiplatelet drugs	2	13.3	2	13.3	4	13.3

n numbers, X mean, SD standard deviation, ACEI angiotensin converting enzyme inhibitors\*Consumed by ≥ 2 patients. All comparisons were not significant Continuous variables (Mann Whitney U Test), categorical variables (Fisher Exact Probability Test)

### Effects on Lipid profile

After 12 weeks of therapy, policosanol significantly lowered LDL-C (16.7 %) compared to baseline (p < 0.01) and placebo (p < 0.05) group (Table 2). In addition, policosanol treatment decreased significantly (p < 0.05) serum total cholesterol (10.6 %) and increased significantly (p < 0.05) HDL-C (12.4 %). The placebo group did not show significant changes in any of the lipid profile variables.

**Table 2.** Effects on lipid profile (X±SD)

	Baseline	6 weeks	12 weeks	Changes (%)
	LDL-C (mmol/L)			
Placebo	3.87 ± 0.89	3.71 ± 0.78	3.68 ± 0.54	- 4.9
Policosanol	3.77 ± 0.98	3.58 ± 0.76*	3.14 ± 0.56***+	-16.7 <sup>+</sup>
	Total cholesterol (mmol/L)			
Placebo	6.01 ± 0.90	5.82 ± 0.83	5.74 ± 0.60	- 4.5
Policosanol	5.93 ± 0.77	5.44 ± 0.71*	5.30 ± 0.73**+	-10.6 <sup>+</sup>
	HDL-C (mmol/L)			
Placebo	1.23 ± 0.37	1.24 ± 0.37	1.25 ± 0.38	+ 1.6
Policosanol	1.21 ± 0.34	1.28 ± 0.60*	1.36 ± 0.36**+	+12.4 <sup>+</sup>
	Triglycerides (mmol/L)			
Placebo	1.94 ± 0.79	1.88 ± 0.88	1.89 ± 0.63	-2.6
Policosanol	1.82 ± 0.89	1.72 ± 0.61	1.68 ± 0.87 <sup>a</sup>	-7.7

X mean, SD standard deviation, LDL-C low-density lipoprotein-cholesterol, HDL-C high-density lipoprotein-cholesterol <sup>a</sup>p = 0.05, \*p < 0.05, \*\*p < 0.01, Comparison with baseline (Wilcoxon Test) <sup>+</sup>p < 0.05, Comparison between groups (Mann Whitney U Test)

### Effects on plasma oxidative variables

Table 3 shows the effects on plasma oxidative variables. After 12 weeks of treatment with policosanol, all oxidative variables were favorably modified, since it reduced significantly ( $p < 0.05$ ) plasma MDA (vs baseline and vs placebo), while increased ( $p < 0.05$ ) plasma total antioxidant capacity.

**Table 3.** Effects on oxidative variables (X±SD)

Treatment	Baseline	12 weeks
	Malondialdehyde (MDA) (µmol/L)	
Placebo	1.48 ± 0.65	1.55 ± 0.90
Policosanol	1.36 ± 0.46	0.88 ± 0.56**
	Total antioxidant capacity (mmol/L)	
Placebo	0.65 ± 0.24	0.67 ± 0.22
Policosanol	0.67 ± 0.20	0.73 ± 0.19**

X mean, SD standard deviation \* $p < 0.05$ , Comparison with baseline (Wilcoxon Test) \*\* $p < 0.05$ , Comparison between groups (Mann Whitney U Test)

### Safety and tolerability

Both treatments were safe and well tolerated. No patient discontinued from the study. No significant impairment of physical or blood safety indicators were found, all individual values remaining within normal range (Table 4).

Two patients, one of each group, experienced mild AE: one from policosanol group referred insomnia, one from placebo group had polyphagia, without significant differences between the groups.

**Table 4.** Effects on safety variables (X±SD).

Treatment	Baseline	6 weeks	12 weeks
Weight (kg)			
Policosanol	72.47 ± 6.39	72.10 ± 6.32	71.63 ± 6.17
Placebo	73.47 ± 5.06	73.47 ± 5.06	73.43 ± 5.03
Pulse (beat/min)			
Policosanol	80.60 ± 3.90	78.40 ± 4.68	78.93 ± 4.47
Placebo	79.77 ± 3.75	79.43 ± 4.80	78.99 ± 3.99
Diastolic arterial pressure (mm Hg)			
Policosanol	80.60 ± 3.90	79.50 ± 4.77	79.03 ± 4.23
Placebo	80.52 ± 3.63	80.43 ± 4.55	80.91 ± 4.09
Systolic arterial pressure (mm Hg)			
Policosanol	136.00 ± 7.00	135.83 ± 5.64	135.50 ± 3.54
Placebo	137.50 ± 5.53	136.00 ± 4.36	136.87 ± 5.08
AST (U/L)			
Placebo	29.92 ± 23.82	31.08 ± 23.39	22.00 ± 6.12
Policosanol	23.75 ± 5.53	24.75 ± 5.69	21.75 ± 3.74
ALT (U/L)			
Placebo	26.42 ± 17.96	28.33 ± 18.67	22.25 ± 9.55
Policosanol	20.67 ± 4.75	21.50 ± 9.10	23.58 ± 6.40
Glucose (mmol/L)			
Placebo	6.00 ± 1.77	6.01 ± 1.95	5.95 ± 2.29
Policosanol	5.81 ± 1.42	5.81 ± 1.28	5.61 ± 1.62
Creatinine (µmol/L)			
Placebo	90.83 ± 17.17	85.67 ± 14.12	86.17 ± 19.06
Policosanol	83.08 ± 18.29	86.83 ± 15.10	77.50 ± 13.56

X mean, SD standard deviation ALT alanine amino transferase, AST aspartate amino transferase

## DISCUSSION

The results of this study demonstrate that policosanol, administered at 10 mg/d for 12 weeks, produced beneficial changes on lipid profile and plasma oxidative variables of diabetic patients.

Both groups had similar baseline characteristics, so that, they were homogeneous for comparisons. Study patients (average age: 68 years) showed co-morbidities as diabetes, dyslipidemia, hypertension and smoking, all of them characteristic conditions of this population. Concomitant medications were consistent with the personal history of the patients.

Policosanol produced beneficial effects on the lipid profile, as it reduced LDL-C, total cholesterol and increased HDL-C. The lipid-modifying effects here seen are consistent with previous clinical studies on policosanol conducted in patients with hypercholesterolemia<sup>35-39</sup> and in diabetic patients with hypercholesterolemia.<sup>27-31</sup> Overall, the policosanol-induced lipid profile change favored a reduction in atherogenic risk.

Policosanol treatment also produced beneficial effects on oxidative variables, which can be considered relevant for these patients, because has been demonstrated that the antioxidant therapy is effective in the management of diabetes and diabetic complications.<sup>40,41</sup> Meanwhile no changes were seen in the group treated with placebo, as expected, a significant reduction of plasma MDA (a marker of lipid peroxidation), and a significant increase of total antioxidant capacity (a marker of the whole antioxidant response of the body) were found in the policosanol treated group. The present results are consistent with previous reports of the antioxidant effects of policosanol in diabetic patients.<sup>30</sup> Consistent with previous studies in diabetic patients, policosanol treatment was safe, well tolerated.<sup>27-31</sup> No patient withdrew from the study, the frequency of adverse events was low, and adverse events were mild.

## CONCLUSIONS

Policosanol treatment favorably modified lipid profile and plasma oxidative variables in diabetic patients. Further studies should expand more data on the effects of policosanol treatment in diabetic patients.

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