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Effects Of Policosanol Plus Aspirin Therapy On The Neurological Recovery And Plasma Oxidative Markers Of Patients With Ischemic Stroke

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ABSTRACT

Background. *Oxidative stress (OS) contributes to brain reperfusion injury after ischemic stroke. Policosanol, a mixture of 8 higher aliphatic sugarcane wax alcohols, has shown to protect against experimental brain ischemia.*

Objectives. *To investigate the effect of policosanol plus aspirin (AS) therapy on the neurological outcome and plasma oxidative variables in patients post-ischemic stroke*

Methods. *Post-ischemic stroke (≤ 30 days evolution) sufferers with a modified Rankin Scale score (mRSs) ≥2 - ≤4 were randomized to placebo or policosanol (20 mg/day) + aspirin (AS) (125 mg/day) (pla + AS) or placebo + AS (pla + AS) for 24 weeks. The primary efficacy variable was to significantly increase the patients with a favourable stroke outcome (mRSs ≤1) versus pla +AS. Plasma oxidative markers (malondialdehyde –MDA-, sulphidril groups –SHG-, total antioxidant capacity –TAC-) were secondary variables.*

Results. *Sixty-one (61) patients (mean age: 64 years) were randomized. No significant changes occurred with pla +AS. After 6 weeks on treatment poli + AS reduced significantly the mean mRSs value ($p<0.001$ vs pla + AS), and this effect was not wear off, but enhanced, throughout the study. More ($p<0.0001$) poli + AS patients (29/31, 93.5%) achieved mRSs values ≤1 at study completion as compared to pla + AS patients (2/30, 6.7%). Also, poli + AS treatment produced significant reductions of plasma MDA and SHG, while increased plasma TAC. Treatments were well tolerated. There were not study withdrawals.*

Conclusions. *Poli + AS therapy improved the neurological recovery and favourably modified plasma oxidative markers versus pla + AS in patients post-ischemic stroke*

KEYWORDS- *Aspirin, Ischemic stroke, Oxidative stress, Policosanol, Rankin-modified scale, Stroke*

I. INTRODUCTION

Stroke is a consequence of the sudden interruption of blood flow to a brain region that impairs the energy supply to the central nervous system. Strokes can be ischemic (75-80%) or hemorrhagic (about 20%) [1]. Hypoxia is the main cause of central nervous system damage in stroke, and the ischemic gradient in brain ischemia involves the core and the penumbra. Although neurons and glial cells have functional changes in the penumbra, neurons are more vulnerable to hypoxia because they depend on the oxidative metabolism of glucose for energy [2]. Ischemic stroke is the third cause of adult mortality and the first one of long-term neurological disability worldwide. Patients that have had a transient ischemic attack (TIA) or stroke are at risk of stroke with stroke recurrence in about 6–15% of patients within 90 days and in up to 21% of cases by one year post-stroke. Control of modifiable stroke risk factors like hypertension, diabetes, dyslipidemia, cigarette smoking and obesity are key measures to prevent recurrent strokes [3]. Oxidative stress (OS) is a key factor that contributes to brain reperfusion injury after stroke [4]. The sources of increased reactive oxygen species (ROS) generation in the brain following injury include the impaired function of mitochondrial oxidative respiratory chain together with the activation of cytoplasmic oxidases that cannot be counteracted by the endogenous antioxidant mechanisms and contribute to the progression of injury over time. [5].

Although currently there are no clinically validated biomarkers of acute stroke, some studies have focused on

markers linked with distinct components of the ischemic cascade, including OS, microglial activation, inflammation, neuronal injury, hemostasis, and endothelial dysfunction [6]. In particular, assessment of serum lipoperoxide, hydroperoxide and conjugated diene levels in patients with acute ischaemic stroke found significantly higher values in early stages post-stroke than after 1 month of suffering the event, while plasma total antioxidant capacity (TAC) showed an opposite course, the values being higher 30 days after stroke [7]. Also, measurement of plasma lipid and redox markers in patients who had suffered a first-ever ischemic stroke found that stroke patients had higher values of total peroxidation by-products, NO stable metabolites and of total cholesterol, low density lipoprotein-cholesterol (LDL-C) and triglycerides, and lower plasma total antioxidant capacity (TAC) than aged-matched non stroke controls. Then, redox unbalance in stroke patients was seen as a potential early indicator of diffuse endothelial activation during which patients may be at increased risk for recurring stroke or other vascular events [8]. Also, intensive neurorehabilitation has been shown to lower redox plasma peroxidation by-products during the neurological recovery in post-acute ischemic stroke patients [9].Despite the promising effects of antioxidant substances on experimental stroke [10], their efficacy as neuroprotective on clinical ischemic stroke remains controversial, so that Vitamin C administration (500 mg/day, iv) to ischemic stroke patients since the first day post-ischemic stroke resulted in elevated serum levels of antioxidants, but failed to substantially improve the functional status of patients after 3 months [11].

Experimental and clinical studies have demonstrated that policosanol, a mixture of 8 high molecular weight sugarcane wax alcohols, produce antioxidant effects *in vivo*, not *in vitro* [12-19]. Also, policosanol has shown protective effects in experimental brain ischemia models [20,21], and long-term administered prevented the progression of carotid-vertebral atherosclerosis in humans [22]. Two open and long-term clinical studies found that the addition of policosanol to aspirin (AS) therapy was associated to very good neurological recovery [23,24], and a further double-blind, placebo-controlled study demonstrated that policosanol + AS treatment improved the neurological recovery and decreased platelet aggregation in patients with recent ischemic stroke [25].In light of this background, this study investigated the effects of the therapy with policosanol 20 mg/day + AS 125 mg/day (poli + AS) on the neurological outcome and plasma oxidative variables in patients who had suffered a recent ischemic stroke as compared to placebo + AS 125 mg/day (pla + AS).

II. MATERIALS AND METHODS

2.1 Study design

This study was conducted in the Institute of Neurology and Neurosurgery (Havana City, Cuba) after the approval of the Institutional Ethics Committee. The study enrolled patients who had had recent ischemic stroke within the 30 days before recruitment and gave their informed written consent (Visit 1). Participants underwent clinical history and full clinical examination, and were advised to start or continue on a low-sodium and low fat diet and strongly recommend stop smoking.Eligible patients were randomized to poli + AS or pla + AS (visit 2) for 6 months and attended to control visits at 6, 12, 18 and 24 weeks on treatment (visits 3 – 6). Patients underwent general examination and neurological assessment at each visit. Treatment compliance and adverse experiences (AE) were controlled from visits 3 to 6, and laboratory analyses at baseline and after 24 weeks on treatment.

2.2 Study patients

Enrolled patients were men and women over 40 years of age who had had an ischemic stroke (diagnosed by a neurologist) within the 30 days prior to enrolment. Stroke was defined as the occurrence of focal clinical signs of central nervous system dysfunction of vascular origin that lasted for at least 24 hours. Ischemic stroke was confirmed through clinical assessment and tomography scan performed within the 48 hours after stroke onset.Eligible patients fulfilled the enrolment criteria and had a modified Rankin Scale score (mRSs) of 2, 3 or 4 [26]. Exclusion criteria were to have had hemorrhagic stroke, atrial fibrillation, other cardiac sources of embolism, subarachnoid haemorrhage, diastolic hypertension ≥ 110 mm Hg, cardiac valve diseases, history of myocardial infarction, instable angina or revacularisation surgery within the 6 months prior to the trial and previous consumption of policosanol.

2.3 Treatment

Patients consumed policosanol (20 mg/day) + AS (125 mg/day) or placebo + AS (125 mg/day) once daily with the breakfast for 24 weeks. The AS dose was selected keeping in mind that daily doses of AS (75–150 mg) are recommended for the prevention of vascular events in high-risk patients [27-29].Good treatment compliance, assessed through counts of remainder tablets and patient's interviews, was to consume at least 85% of scheduled tablets per period. The consumption of other antioxidant or antiplatelet drugs was prohibited during the study. Likewise, patients who were taking other lipid-modifying drugs had to stop them 30 days before to enrolment.

2.4 Study outcomes

The primary efficacy variable was to obtain a significant increase of patients with a favourable stroke outcome (mRSs ≤1) at 24 weeks as compared to pla +AS.

Secondary efficacy variables were to obtain significant reductions of plasma MDA, SHG or significant increases of plasma TAC as compared to pla + AS. Effects on lipid profile values were collateral efficacy variables.

2.5 Laboratory analyses

Venous blood samples were taken following a fasting of 12 hours. Plasma was separated from red blood cells by centrifugation at 4°C and 2000 x *g* for 10 min, and aliquots were immediately taken. Lab analyses were performed within the next 8 hours after blood drawing.

2.6 Oxidative variables

Plasma MDA was measured by the thiobarbituric acid (TBA) assay [30]. Briefly, 0.5 mL of plasma were added to the TBA reagent (0.2 mL of 8.1% sodium dodecyl sulphate –SDS-, 1.5 mL of acetic acid 20% - pH 3.5- and 1.5 mL of TBA 0.8%), completing to 4 mL with distilled water. This mixture was incubated at 95^{o}C for 45 minutes. Butylated hydroxytoluene (BHT) (1 mmol/L) (50 L) were added and the mixture was cooled. Immediately after, 1 mL of distilled water and 5 mL of n-butanol:piridine (15:1 v/v) were added. The whole mixture was stirred and centrifuged (20 min at 1660 x g). The organic layer was taken and the absorbance was measured at 534 nm. The concentrations of TBA-reactive substances (TBARS) were calculated from a standard calibration curve generated with known amounts of freshly diluted malondialdehyde bis (dimethyl acetal). Values were expressed as MDA (μmol/mL).A modification of the Miao-Lin Hu method was used to assess plasma SHG [31]. Briefly, 950 μL de dithionitrobenzene (DTNB) 10 mmol/L were added to plasma aliquots of 50 μL, and this mixture was incubated for 20 min at 25^{o}C. A blank with DTNB was run in parallel. The absorbance of the supernatant was measured at 412 nm. The numbers of SHG were estimated using an absorptivity of 13,600 cm^{-1} mol^{-1} and expressed in nmoL.For TAC quantitation, 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 $ABTS^{\bullet+}$. This has a relatively stable blue-green colour that was measured at 600 nm. Based on their concentration, antioxidants will cause a suppression of the colour production. TAC was expressed in mmol/L.All assays were carried out in triplicate.

2.7 Lipid profile and blood safety indicators

Serum levels of total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C) and blood biochemistry indicators 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. Low-density lipoprotein-cholesterol (LDL-C) values were calculated using the Friedewald equation [32].

2.8 Safety and tolerability assessment

Safety and tolerability indicators included laboratory and physical examination data, and AE reports. Study protocol defined an AE as any undesirable experience, absent at hospital discharge or worsened thereafter, happening in a patient, independently if it could be or not related with the therapy. AE were classified as mild, moderate or serious according to their intensity. Mild AE should not require stopping of study medications or specific treatment of the AE, moderate AE should require the withdrawal of study medications and/or treatment of the AE, while serious AE should lead to patient hospitalization and/or to death.

2.9 Statistical Analysis

The study was designed to have a statistical power of 80 percent to detect a reduction of at least 30% in the frequency of poli + AS-treated cases with a favourable outcome as compared to the pla + AS group, with a two-sided significance level of $p<0.05$. Given the specified statistical power, we needed 90 eligible patients, and assuming a total dropout rate of 10 percent, we enrolled 99 patients. Data were analysed on an intention-to-treat basis, including those of all patients who underwent randomization. Analysis of Variance was used for repeated comparisons of continuous variables (mean mRSS values, bodyweight, pulse rate, arterial pressure). Laboratory variables were compared using the Wilcoxon test for paired matched samples (within group comparisons) and the Mann Whitney U test (between group comparisons). Categorical data were compared with the Fisher Exact Probability test. All p values were two-sided.

III. RESULTS

3.1 Population characteristics

Of 100 screened patients, 61 (mean age: 64 years) (26 men, 35 women) were eligible for randomization. All randomized patients (100%) completed the trial. 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 sedentary life (98.4%), intake of salt-rich food (96.7%), hypertension (91.8%), overweight plus obesity (73.8%) and smoking (36.1%). Concomitant therapy was also well balanced in the two groups, the most frequent being the angiotensin converting enzyme inhibitors (ACEI) (78.7%), fairly followed by diuretics (18.0%).

3.2 Effects on study outcomes

During the study drug compliance was ≥90% and similar in both groups. No patient had recurrent cerebrovascular event or any other major vascular event during the trial.The baseline distribution of patients into the different mRSs values was similar in both groups (Table 2). After 12 and 24 weeks on therapy, the frequency of poli + AS patients (11/31, 35.5% and 29/31, 93.5%, respectively) who achieved mRSs ≤1 was significantly ($p<0.001$ and $p<0.0001$, respectively) greater than in the pla + AS group (0/30, 0.0% and 2/30, 6.7%, respectively). At study completion, 2/31 (6.5%) poli + AS, 28/30 (93.3%) pla + AS patients had mRSs values ≥2 ($p<0.00001$). Table 3 shows mean mRSS changes during the study. After 6 weeks on therapy, poli + AS decreased significantly ($p<0.001$) mRSs values (28.6%) versus pla + AS, and this treatment effect did not wear off, but improved thereafter, so that significant decreases ($p<0.0001$) of 39.3% and 59.9% were found at weeks 12 and 24 weeks, respectively. Table 4 summarizes the effects on laboratory variables. All baseline variables were well balanced in both groups. No significant changes were found in pla + AS group. Treatment with poli + AS favourably modified all oxidative variables, since it lowered significantly plasma MDA ($p<0.01$ vs baseline, $p<0.05$ vs pla + AS) and SHG ($p<0.01$ vs baseline, while increased ($p<0.05$) plasma TAC. Poli + AS treatment decreased significantly serum LDL-C ($p<0.00001$ vs baseline, $p<0.01$ vs pla + AS) and total cholesterol ($p<0.00001$ vs baseline, $p<0.05$ vs pla + AS), while increased HDL-C ($p<0.05$ vs pla + AS).

3.3 Safety and tolerability

Treatments were safe and well tolerated. No patient discontinued from the study. Treatments did not impair safety physical or blood indicators and individual values were not out of normal limits. Treatment with poli + AS, not with pla + AS, significantly lowered serum ALT and GGT (Table 4); and systolic and diastolic blood pressure values as well ($p<0.001$) (Table 5). Two patients, one of each group, experienced mild AE: 1 from poli + AS group referred insomnia, 1 from pla + AS group had gastritis, without significant differences between the groups.

IV. DISCUSSION

This double-blind, randomized and controlled study confirms that poli + AS treatment significantly improved stroke functional outcomes in patients who had suffered a recent (≤30 days) moderate to severe ischemic stroke, consistent with the results of a previous double-blind, randomized, controlled study conducted in post-ischemic stroke sufferers [25]. In addition, for the first time, beneficial effects of poli + AS treatment on plasma oxidative variables of these patients were also found. It should be noted that prior long-term studies had also referred a favorable impact of poli + AS treatment on the neurological post-ischemic stroke recovery, but their open and uncontrolled designs imposed limitations for strong conclusions [23,24]. In contrast, this study was randomized, double-blinded and controlled with a group that received AS, the conventional therapy used to manage ischemic stroke, plus placebo. Since both groups were homogeneous at baseline the effects here described can be attributable to the combination therapy poli + AS, and more specifically to the addition of policosanol to the therapeutic scheme with AS alone. In addition, the fact that all randomized patients concluded the study and that treatment compliance was comparable in both groups supports the validity of our results.

Baseline characteristics of study patients, like the mean age of patients and the high frequency of stroke risk factors (salt intake, sedentary life, hypertension, overweight & obesity, hypercholesterolemia and smoking) are consistent with stroke epidemiological data [33]. Concomitant treatments, mostly ACEI, were well balanced in both groups, so that we discard the potential influence of concomitant therapy to the present results. Stroke scales, developed as tools for objectively assessing the degree of patient recovery and for comparing the data across stroke studies, have been used as efficacy variables for evaluating the neurological improvement in stroke studies [34]. We assessed the effects on stroke outcome by using the mRSs [19], a validated and reliable scale that reflects the global disability of the patients and that have been widely used as a clinical variable to assess post-stroke recovery [35,36]. In this study we included patients with mRSs values from 2 to 4 for reducing the influence of the variability of stroke severity on the results.

Nevertheless, since patients who suffered severe ischemic stroke were not included, our results should be extrapolated to patients to mild to moderate intensity ischemic stroke. Our results support the efficacy of poli + AS therapy in the neurological recovery of study patients over pla + AS. First, the rate of poli + AS-treated patients who achieved good stroke outcomes (29/31, 93.5%) was significantly greater than in pla + AS (2/30, 6.7%). Then, the difference between groups was over the pre-specified efficacy criterion (>30%) despite we selected a restrictive cut-off limit for qualifying a good stroke outcome (mRSs ≤1). Second, the significant decrease of mean mRSs values from 6 weeks after randomization to study completion. After 12 and 24 weeks on treatment, the differences of mRSs versus pla + AS were 39.3% and 59.9%, respectively. Since the NINDS rt-PA study, in which the patients were treated as soon as within the first hours of acute stroke, found a 11-13% reduction of mean mRSs at week 12 [35], we should consider the present results clinically meaningful.It should be noted that mRSs values were practically unchanged in the pla + AS group in spite of patients were advised to follow first-line conventional post-stroke therapy (life style changes + AS). The effects of poli + ASA treatment on oxidative variables were also beneficial. Meanwhile no changes were seen in the group treated with pla + AS, as expected, a significant reduction of plasma MDA (a marker of lipid peroxidation), SHG (a marker of protein oxidation) and a significant increase of plasma TAC (a marker of the overall antioxidant response of the body) were found. Since increased values of lipid peroxidation by-products and decreased levels of plasma TAC have been found in post-ischemic stroke patients [7,8], and neurorehabilitation, a common practice, has been able to lower plasma peroxidation by-products during the neurological recovery of these subjects [9], the present results argue for an additional advantage of poli + AS for helping in post-stroke rehabilitation. These results may be attributed to the specific addition of policosanol to the therapeutic scheme, as they are coherent with the antioxidant effects reported for policosanol previously[13-19].

We found that poli + AS produced beneficial effects on the lipid profile, as it reduced LDL-C, total cholesterol and increased HDL-C. Although some trials have failed to find lipid lowering effects of other policosanol tablets [37,38], the lipid-modifying effects here seen are consistent with previous experimental [16,40-42] and clinical [43-47] studies on policosanol, and may have contributed to the neurological recovery of study patients like happens with statins, which improve neurological outcomes in acute ischemic stroke [48].

There were not recurrent strokes or ATI among study participants, a result particularly good since the highest probability of recurrent events occurs within the first 12 weeks post-stroke [33]. This result confirms the usefulness of conventional therapy with AS in patients who had experienced an ischemic stroke.

Consistent with previous studies in stroke patients [17,18], poli + AS treatment was safe and well tolerated. No patient withdrew from the study, the frequency of AE was low and AE were mild. Systolic blood pressure decreased significantly with poli + AS versus pla + AS, consistent with previous results [23-25]. Nevertheless, since no patient had hypotension values and there was a high frequency of study patients with hypertension, this effect could be potentially beneficial, rather than adverse, for stroke recovery. In addition, the significant decrease of GGT observed with poli + AS was of particular interest since this variable has been considered as a marker of ischemic stroke risk in women and in older subjects, independent of established cardiovascular risk factors [49,50]. Nevertheless, further studies should expand more data on the effects of poli + AS treatment on such target in post-ischemic stroke patients.

V. CONCLUSIONS

Poli + AS treatment improved the neurological recovery as compared to pla + AS (conventional therapy), and favourably modify plasma oxidative variables and lipid profile in patients with a recent minor to moderate ischemic stroke, which suggests the usefulness of giving this therapy as soon as after hospital discharge due to a recent ischemic stroke.

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Table 1. Baseline characteristics of study population

Characteristics	Poli + AS (n= 31)	Pla + AS (n=30)	Total (n=61)
Age (years) (X ± SD)	65 ± 11	64 ± 11	64 ± 11
Body mass index (kg/m^2) (X ± SD)	26.7 ± 2.2	27.1 ± 3.6	26.9 ± 3.0
Women n (%)	19 (61.3%)	16 (53.3%)	35 (57.4%)
Men n (%)	12 (38.7%)	14 (46.7%)	26 (42.6%)
Modified Ranking Scale score	2.84 ± 0.52	2.53 ± 0.51	2.69 ± 0.53
Salt-rich die, n (%)	29 (93.5)	30 (100.0%)	59 (96.7%)
Sedentary life, n (%)	30 (96.8%)	30 (100.0%)	60 (98.4%)
Hypertension, n (%)	27 (87.1%)	29 (96.7%)	56 (91.8%)
Overweight & obesity, n (%)	25 (80.6%)	20 (66.7%)	45 (73.8%)
Smoking, n (%)	11 (35.5%)	11 (36.7%)	22 (36.1%)
Hypercholesterolemia, n (%)	6 (19.4%)	6 (20.0%)	12 (19.7%)
Diabetes, n (%)	4 (12.9%)	4 (13.3%)	8 (13.1%)
Coronary heart disease, n (%)	1 (3.2%)	2 (6.7%)	3 (4.9%)
Concomitant therapy (5%)			
ACEI, n (%)	23 (74.2%)	25 (83.3%)	48 (78.7%)
Diuretics, n (%)	6 (19.4%)	5 (16.7%)	11 (18.0%)
Oral hypoglycemic drugs, n (%)	3 (9.7%)	3 (10.0%)	6 (9.8%)

(X ± SD) mean ± standard deviation. ACEI angiotensing converting enzyme inhibitors
All comparisons were not significant

Table 2. Distribution of cases in accordance to the Modified Ranking Scale score (mRSs)

mRSs values	Baseline		3 months		6 months	
	Poli + AS	Pla + AS	Poli + AS	Pla + AS	Poli + AS	Pla + AS
0	0	0	0	0	5	0
1	0	0	11+	0	24++	2
0 - 1	**0**	**0**	**11+**	**0**	**29++**	**2**
2 - 3	29	30	20	30	**2+++**	28
4	2	0	0	0	0	0

Data presented as n(%), Poli policosanol, Pla placebo, AS aspirin
+ $p < 0.001$, ++ $p < 0.0001$, +++ $p < 0.00001$, comparison with pla + AS (Fischer´s Exact Probabilty test)

Table 3. Effects on neurological recovery assessed through the modified Rankin Scale score (X ± SD)

Treatment	Baseline	6 weeks	12 weeks	Percent changes	18 weeks	24 weeks	Percent changes
Poli + AS	2.8 ± 0.5	2.0 ± 0.6^{+}	1.7 ± 0.6^{++}	-39.3	1.0 ± 0.5^{++}	0.9 ± 0.5^{++}	-67.9
Pla + AS	2.5 ± 0.5	2.5 ± 0.5	2.5 ± 0.5	0	2.4 ± 0.6	2.3 ± 0.6	-8.0

(X ± SD) mean ± standard deviation. (%), Poli policosanol, Pla placebo, AS aspirin
$^{+}$ $p<0.001$, $^{++}p<0.0001$. Comparisons with pla + AS (Analysis of Variance)

Table 4. Effects on laboratory blood variables (X ± SD)

Treatment	Baseline	24 weeks
Plasma oxidative markers		
Malondialdehyde (MDA) (μmol/mL)		
Poli + AS	0.36 ± 0.11	0.31 ± 0.10 **+
Pla + AS	0.38 ± 0.07	0.37 ± 0.09
Sulfhydril groups (SHG) (nmoL)		
Poli + AS	0.52 ± 0.12	0.42 ± 0.12**
Pla + AS	0.52 ± 0.14	0.48 ± 0.16
Total antioxidant capacity (TAC))(mmol/L)		
Poli + AS	0.66 ± 0.18	0.72 ± 0.19*
Pla + AS	0.65 ± 0.22	0.67 ± 0.21
Serum lipid profile (mmol/L)		
Low-density lipoprotein-cholesterol (LDL-C)		
Poli + AS	3.79 ± 0.81	3.03 ± 0.83*****++
Pla + AS	3.68 ± 1.09	3.77 ± 1.16
Total cholesterol (TC)		
Poli + AS	5.77 ± 1.08	5.09 ± 0.98****+
Pla + AS	5.73 ± 1.30	5.63 ± 1.28
High-density lipoprotein-cholesterol (HDL-C)		
Poli + AS	1.35 ± 0.45	1.47 ± 0.43+
Pla + AS	1.37 ± 0.39	1.29 ± 0.31
Triglycerides		
Poli + AS	1.71 ± 0.61	1.58 ± 0.49
Pla + AS	1.72 ± 0.97	1.71 ± 0.92
Other serum biochemical variables		
Aspartate amino transferase (AST) (U/L)		
Poli + AS	24.84 ± 6.04	25.39 ± 8.75
Pla + AS	27.87 ± 14.89	28.33 ± 13.79
Alanine amino transferase (ALT) (U/L)		
Poli + AS	20.68 ± 4.87	18.16 ± 6.12*
Pla + AS	27.60 ± 22.11	23.43 ± 18.16
Gamma-glutamyl transpeptidase (GGT) (U/L)		
Poli + AS	43.22 ± 23.35	32.84 ± 21.17***
Pla + AS	44.03 ± 55.80	44.17 ± 70.44
Glucose (mmol/L)		
Poli + AS	4.91 ± 1.37	4.79 ± 0.86
Pla + AS	4.59 ± 0.92	4.56 ± 1.30
Creatinine (μmol/L)		
Poli + AS	85.39 ± 28.01	90.06 ± 35.08
Pla + AS	92.50 ± 22.37	99.43 ± 28.98

X mean, SD standard deviation, Poli policosanol, Pla placebo, AS aspirin
*p<0.05, **p<0.01,***p<0.001, ****p<0.0001,*****p<0.00001. Comparison with baseline (Wilcoxon test for matched samples)
+ p<0.05, ++ p<0.01 Comparison with Pla + AS (Mann Whitney U test)

Table 5. Effects on blood arterial pressure (X ± SD)

Treatments	Baseline	6 weeks	12 weeks	18 weeks	24 weeks
Diastolic blood pressure (mm Hg)					
Poli + AS	81.45 ± 4.86	79.68 ± 3.40^{+}	78.07 ± 4.22	76.45 ± 4.69^{+++}	76.45 ± 4.86^{++}
Pla + AS	83.67 ± 4.54	81.83 ± 2.78	80.67 ± 3.41	80.33 ± 1.83	80.17 ± 2.45
Systolic blood pressure (mm Hg)					
Poli + AS	131.94 ± 7.03	125.00 ± 5.92^{++}	122.90 ± 3.82^{+++}	120.81 ± 4.67^{++++}	120.00 ± 3.16^{+++}
Pla + AS	134.33 ± 6.12	130.17 ± 4.82	128.17 ± 5.17	127.50 ± 4.69	127.50 ± 5.04

(X ± SD) mean ± standard deviation. (%), Poli policosanol, Pla placebo, AS aspirin
$^{+}$ $p<0.01$, $^{++}$ $p<0.001$, $^{+++}$ $p<0.0001$, $^{++++}$$p<0.00001$, Comparisons with pla + AS (Analysis of Variance)