

Pharmacological Research of *VasoCleaner*TM on Hyperlipidemia and Atherosclerosis

Part I: Abstract

Part II: Clinical Trial of *VasoCleaner*TM on Hyperlipidemia and Atherosclerosis

Part III: Inhibitory effect of *VasoCleaner*TM on Dietary-induced Hyperlipidemia and Atherosclerosis in Rabbits

Part I Abstract***Background and Purpose***

*VasoCleaner*TM, which aims at cleaning blood by expelling harmful lipids and free radicals, is a patented formulation of Chinese herbs (patent number: ZL 98 1 17984.3) that has been used to treat hyperlipidemia and atherosclerosis (AS) in clinic at two hospitals. The purpose of this study is to provide a systematic research on its pharmacological effects and to dissect its functional mechanisms on AS and hyperlipidemia both in patients and dietary induced rabbits.

Methods and Results

In clinical studies, 187 patients were randomly arranged into two groups: *VasoCleaner*TM group (84 cases treated with herb *VasoCleaner*TM) and Gemfibrozil group (control group, 83 cases treated with Gemfibrozil). B-mode ultrasound examination was applied to the carotid arteries to get the intima-media thickness (IMT), the size of atherosclerotic plaque, the carotid blood flow, the resistant index (RI) and carotid pulse index (PI). Plasma lipid and lipoprotein assays were performed to detect total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), apoB, and apoA. The oxidation of LDL-Cholesterol (LDL-C), blood endothelin (ET) and calcitonin gene related peptide (CGRP) were also assayed by routine laboratory methods

Clinical studies indicate that *VasoCleaner*TM improved AS-related symptom in general. It reduced the level of TC by 24 %, LDL-C by 34%, the area of carotid atherosclerotic plaque by 34.13%, and the IMT of carotid artery by 14.9%. The resistant index (RI) and pulse index (PI) were also reduced. Studies also indicate that *VasoCleaner*TM can reduce the blood levels of oxidized LDL (oxLDL) I and ET, but increase carotid flow and the level of CGRP.

In animal studies, white male rabbits were chosen to develop dietary-induced atherosclerosis and hyperlipidemia. *VasoCleaner*TM was administrated to one group for treatment. As a control, Gemfibrozil was administrated to another group. Image analysis was applied to reveal the artery fat stripes and atherosclerotic plaques. The pathogenic changes in heart were determined by microscopic analysis of the histochemically stained slices. Serum lipid and lipoprotein assays were also performed to get the corresponding data.

Animal studies suggest that *VasoCleaner*TM can reduce the occurrence of artery atherosclerotic plaques and the level of fat-induced high TC and TG. It can also stabilize succinct dehydrogenase (SDH), lactate dehydrogenase (LDH) and adenosine triphosphate (ATPase) in heart tissue, bring a balance between 6-Keto-PGI₂ and XA₂, reduce the viscosity of plasma and the level of lipid peroxides (LPO).

Conclusions

Pharmacological studies of *VasoCleaner*TM indicate that this particular formulation can improve AS-related symptoms in patients and prevent dietary-induced AS in experimental animals. Its functional mechanism may lie in the following facts: (1) Adjusting the blood lipid profile, such as decreasing the level of TC, TG, LDL-C, whereas increasing the level of HDL-C; (2) Preventing free radical-induced oxidative damage, such as reducing the formation of LPO and increasing the activity of SOD; (3) Balancing coagulation-related factors and improving the function of blood vessel, such as decreasing the levels of ET, TXB₂, whereas increasing the level of CGRP and 6-keto-PGF₁.

Part II

Clinical Trial of *VasoCleaner*TM on Hyperlipidemia and Atherosclerosis

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Key Words: *VasoCleaner*TM, hyperlipidemia, atherosclerosis, serum lipid, Carotid ultrasonography examinations

1. Introduction

In the United States and most other Western countries, atherosclerosis is the leading cause of morbidity and mortality. Despite significant medical advances, coronary artery disease (which results from atherosclerosis and causes heart attacks) and atherosclerotic stroke are responsible for more deaths than all other causes combined. Twelve million people worldwide die of diseases associated with atherosclerosis each year. It is estimated that atherosclerosis costs the world approximately 100 billion dollars each year. In the United States alone, it caused almost 1 million deaths in 1992, twice as many as from cancer and 10 times as many as from accidents.

Atherosclerosis (AS) is characterized by a narrowing of the arteries caused by cholesterol-rich plaques. Genetic and/or environmental risk factors include: elevated blood cholesterol and triglyceride, high blood pressure, smoking, among which abnormal lipid metabolism is the main factor. Atherosclerosis can affect the arteries of the brain, heart, kidneys, other vital organs, and extremities. When atherosclerosis develops in the arteries, blood supply may be compromised and the patient is at the risk of plaque rupture and infarction.

Chinese Medicine has more than 2000-year history of treating various diseases. Although atherosclerosis and hyperlipidemia are not described in Chinese medicine, the characteristics of these two diseases are similar to "Xinbi", "Wind stroke" and "Wu xue", etc, described in Chinese medicine. There are a substantial number of animal studies of Chinese medicine on AS and hyperlipidemia during the last 50 years, only recently have few therapies applied to clinical trials.

2. Subjects and methods

2.1 Subjects

Beginning in March 1995 and running through December 1997, 167 patients were recruited from two hospitals, First Hospital, an affiliate of Beijing Medical University of Chinese Medicine and Pharmacy, and the Chaoyang Hospital, affiliate of Chinese Capital Medical University.

Patients were diagnosed with hyperlipidemia based on the criteria established by the American National Cholesterol Education Program. Patients were randomly arranged into two groups: *VasoCleaner*TM group (84 cases treated with herb *VasoCleaner*TM) and Gemfibrozil group (control group, 83 cases treated with Gemfibrozil). There were 85 men and 82 women whose ages ranged from 37 to 70 years, with an average of 56.6. There were no significant differences between two groups in the distribution of age, sex, and baseline lipid level characteristics ($P>0.05$, Table 1)

Table 1 Different hyperlipidemia distribution of the two groups before treatment

	TC	LDL-C	TG	TC, TG *	HDL-C
<i>VasoCleaner</i>	74	71	58	46	46
Control	73	65	53	42	54

TC: total cholesterol; LDL-C: LDL-cholesterol; HDL-C, HDL-cholesterol; and TG, triglyceride.

2.2 Methods

The following procedures were applied to patients during the study.

Step 1: Two weeks before treatment, the patients were requested to stop using: cholesterol-lowering drugs, hormones, anticoagulants (for example aspirin), and anticontraceptive drugs. The ultrasonography of carotid was performed. Fasting blood lipid and lipoprotein levels and other biochemical items, such as ox-LDL, ET, CGRP etc, were examined.

Step 2: *VasoCleaner*TM used in this study was manufactured by Jinghai Pharmaceutical Company (Lot# 950088). Gemfibrozil was manufactured by China Chemical Pharmaceutical limited Company (Taiwan). *VasoCleaner*TM group was orally administered 6g of *VasoCleaner*TM twice per day. Control group was orally administered 600mg of Gemfibrozil twice per day. All patients were treated for two months. During the treatment, hypertensive patients were not allowed to use depressor drugs unless the blood pressure rose up suddenly. The coronary heart disease patients were not allowed to use analgesics unless they were suffering from angina pectoris. All patients were on low-cholesterol diet throughout the whole treatment. Carotid ultrasound examination and plasma biochemical assays were performed at the end of the treatment.

2.3 Clinical observations

2.3.1 Observation of clinical symptoms and signs

Clinical symptoms and signs were recorded before and after two months of treatment. The signs and symptoms evaluated: Oppressed feeling in the chest, palpitation, dizziness, listlessness, and spontaneous perspiration.

2.3.2 Measurement of serum lipid and lipoprotein levels

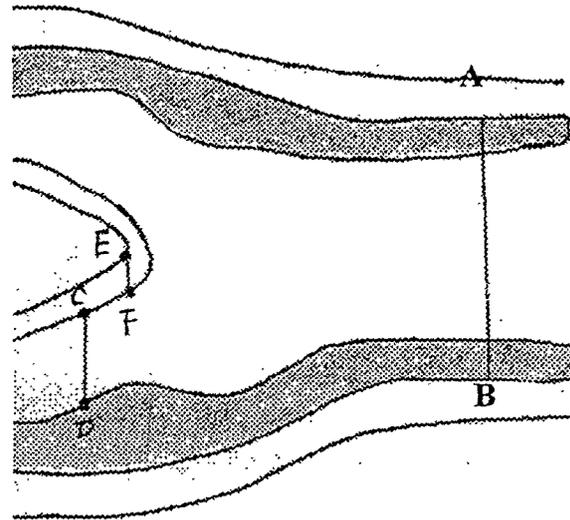
Blood samples were drawn from arm veins between 8 and 9 AM after a 12-hour fast. The plasma lipid profile was determined. Total serum cholesterol and triglyceride assays were assayed by enzymatic methods with commercial kits. HDL cholesterol was measured enzymatically after precipitation of apolipoprotein B-containing lipoproteins. ApoA-1 and apoB-100 were separated by

ultra-centrifugation (CCX auto-chemical analysis instrument U.S.A). The level of low-density lipoprotein cholesterol (LDL-C) was determined by the Friedewald's method.

2.3.3 Examination of carotid arteries

Ultrasound examinations were performed by trained technicians using ATL Ultramar's 9-ultrasound system following the Cemirovic method (*Jasenka Demirovic, Azmi Nabnlsi, and Aaron R Folsom. Alcohol consumption and ultrasonographically assessed carotid artery wall thickness and distensibility. Circulation, 1993; 88 (6): 2782-2793*). Transducer frequency was 7.5 MHz. All arterial segments, including the common carotid arteries, the carotid bifurcations, and the origin (first 2cm) of the internal carotid arteries, were scanned longitudinally and transversely to assess the occurrence of plaques.

Fig 1 Schematic overview of B-mode ultrasound examination of the carotid artery. RI, PI, and blood flow rate were measured at both common carotid artery (site AB in the picture, which is 2 cm away from bifurcation) and internal carotid artery (site CD in the picture, which is 1 cm away from bifurcation). The IMT was measured at site EF in the picture, which is 0.2 cm away from bifurcation lumen.



2.3.4 Measurement of clinical relevant items

Oxidized LDL (oxLDL) was detected by ELISA. ET and CGRP were measured by radioimmunoassay (RIA) using SLT 210.1 V 32 mode enzymatic labeling instrument (KONTRON Australia) and ET-630 G mode multicapital scintillation spectrometer (Beijing Nuclear Instrument Factory, China).

2.4 Observation of side effects

Blood, urine and stool were assayed routinely. Blood biochemical analysis was performed before and after therapy to check the function of liver and kidney. Symptoms, such as gastrointestinal disorders, myopathy and skin rashes, were also recorded.

2.5 Statistical analysis

All results are shown as mean \pm S.D. The Student's t-test was performed to determine if significant differences existed after treatment, or between two groups. A $P < 0.05$ was considered statistically significant.

2.6 Diagnostic criteria and Effective Evaluation criteria

2.6.1 Inclusion criteria

Diagnostic criteria were based on *Xin Yao [Zhong Yao] Lin Chuang Yan Jiu Zhi Dao Yuan Ze* (Principles for the clinical studies of Chinese Medicine), authorized by the Chinese Ministry of Health in 1993. Patients who satisfied one of the following criteria were recruited.

- TC level: 250mg/dl (6.4mmol/L) or over
- TG level: 140mg/dl (1.57mmol/L) or over
- LDL-C: 130mg/dl (3.4mmol/L) or over
- HDL-C: less than 40mg/dl (1.04mmol/L) in male, 45mg/dl (1.17mmol/L) in female.

2.6.2 Exclusion criteria

Patients were excluded from the study, if,

- they have serious liver or kidney dysfunction;
- they were either pregnant or lactating females;
- they suffered from psychiatric disease;
- they were unable to take the medications consistently.

2.6.3 Effective evaluation criteria

- Markedly effective

One of the following criteria was met after treatment:

20% decrease in TC level;

40% decrease in TG level;

20 % decrease in AI, which equal $(TC-HDL-C)/HDL-C$ level;

10mg/dl increase in HDL-C level

- Some Effective

One of the following criteria was met after treatment:

10% to 20% decrease in TC level;

20% to 40% decrease in TG level;

10% and 20 % decrease in AI

4 to 10mg/dl increase in HDL-C level.

- Ineffective

Non-effective was defined as no above lipid changes are observed.

- Negatively effective

One of the following criteria was observed after treatment:

- 10% increase in TC level;
- 10% increase in TG level;
- 10% increase in AI;
- 4mg/dl decrease in HDL-C level.

3. Results

3.1 *VasoCleaner*TM improves symptoms in patients with hyperlipidemia and AS

The main symptoms and signs of 167 cases of patients essentially are following: Oppressed feeling in the chest, palpitation, dizziness, listlessness, and spontaneous perspiration. Compared with the Gemfibrozil group, these symptoms have been significantly improved after treatment in *VasoCleaner*TM group (P<0.05) according to Ridit analysis.

3.2. *VasoCleaner*TM beneficially regulates the blood-lipid profile

3.2.1 Effects of *VasoCleaner*TM on the levels of TC and TG

*VasoCleaner*TM reduced the TC level of the patients by 24 %, TG level by 19%, whereas Gemfibrozil only reduced TC level by 12 %, TG level by 38%, which indicate that *VasoCleaner*TM is more effective in lowering TC than Gemfibrozil group (Fig 2).

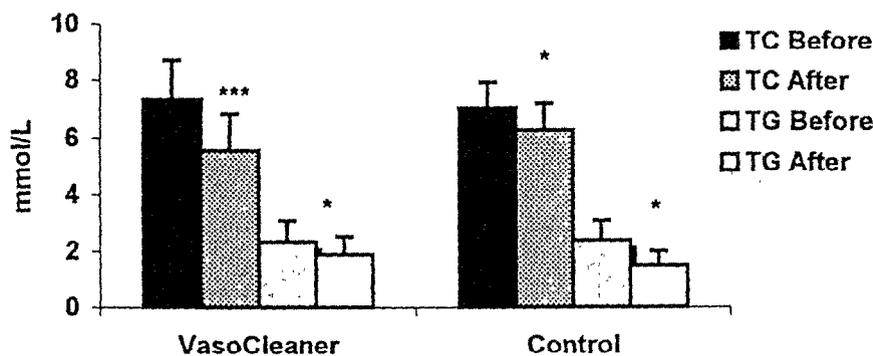


Fig 2. The change of TC and TG after treatment

Results are expressed as mean±SD. *P<0.05, ***P<0.001 vs. pre therapy. In TC assay, 74 cases from the *VasoCleaner*TM group; 73 cases from the control group. In the TG assay, 58 cases from the *VasoCleaner*TM group, 53 cases from the control group.

3.2.2 Effects of *VasoCleaner*TM on serum levels of LDL-C and HDL-C

*VasoCleaner*TM reduced the serum level of LDL-C by 34% in patients, whereas, no effect of Gemfibrozil on the serum level of LDL-C was observed, which indicates that *VasoCleaner*TM is more effective in lowering serum LDL-C level (Fig 3).

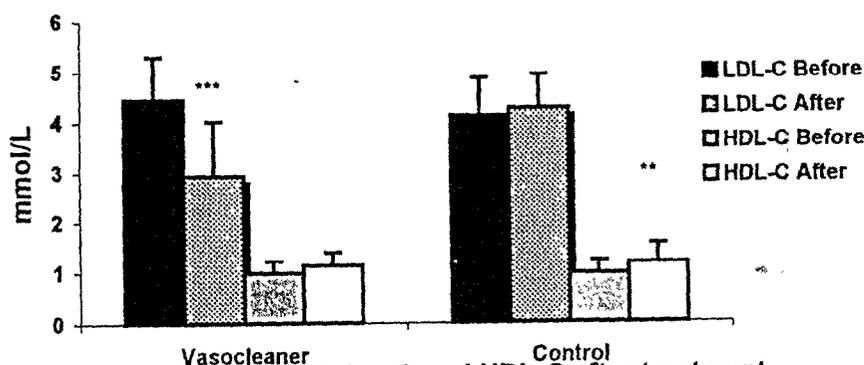


Fig 3. The change of LDL-C and HDL-C after treatment

Results are expressed as mean \pm SD. ** P<0.01, ***P<0.001 vs. pre therapy. In LDL-C assays, 71 cases from the *VasoCleaner*TM group, 65 cases from the control group. In HDL-C assays, 46 cases from the *VasoCleaner*TM group, 54 cases from the control group.

3.2.3. Effects of *VasoCleaner*TM on the levels of apoA-1 and apoB-100

The change of apoA-1 and apoB-100 before and after therapy in the two groups indicates *VasoCleaner*TM is more effective in increasing the level of apoA-1 than Gemfibrozil group (Table 2).

Table 2. ApoA-1 and apoB-100 (g/L) levels before and after therapy

	N	ApoA-1		ApoB-100	
		before	after	before	after
<i>VasoCleaner</i>	74	1.389 \pm 0.054	1.580 \pm 0.051** [#]	1.17 \pm 0.056	1.06 \pm 0.064
Control	70	1.407 \pm 0.040	1.460 \pm 0.041	1.21 \pm 0.073	1.19 \pm 0.067

Results are expressed as mean \pm SD. **P<0.01 vs. pre therapy, and [#] P<0.05 vs. the control group. 74 cases from the *VasoCleaner*TM group and 70 cases from the control group were analyzed in both ApoA-1 and ApoB-100 assays.

3.3. Effects of *VasoCleaner*TM on atherosclerotic carotid.

We are the first to apply ultrasonographical technique to evaluate the therapeutic efficacy of Chinese herbs on AS through measuring the size of carotid atherosclerotic plaques, the thickness of carotid artery walls, the rate of blood flow, RI and PI. In the *VasoCleaner*TM group, the size of carotid atherosclerotic plaques was reduced by 34.13%; and the thickness of carotid artery walls was reduced by 14.9%. RI and PI all markedly decreased (P<0.05-0.01) after therapy. Carotid atherosclerotic plaques even no longer detectable in three cases. Carotid blood flow rate increased in all cases. In the control group, neither the size of carotid atherosclerotic plaques nor the IMT was significantly changed post therapy.

3.3.1. Effects of *VasoCleaner*TM on the size of carotid atherosclerotic plaques

Quantitative comparison of the carotid atherosclerotic plaques area (cm²) before and after therapy indicates that *VasoCleaner*TM is more effective in reducing plaque size than Gemfibrozil group (Fig 4). Two patients with atherosclerotic plaque were treated with *Vasocleaner*TM. The plaques reduced and disappeared after two month therapy (Fig 5).

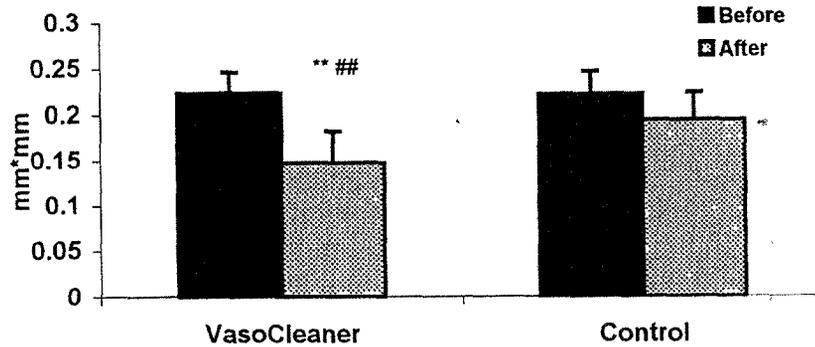


Fig 4 The change of atherogenic plaque after treatment

Results are expressed as mean±SD. **P<0.01 vs. pre therapy, and ##P<0.01 vs. the control group. 44 cases from the *VasoCleaner*TM group and 34 cases from the control group were analyzed.

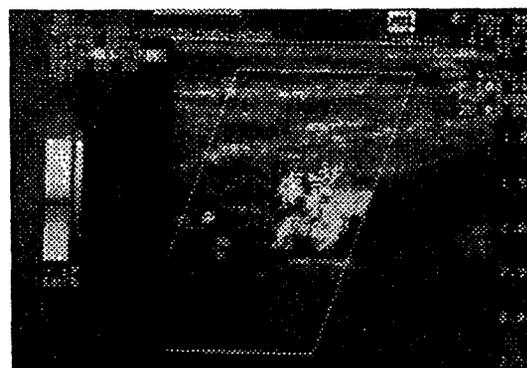
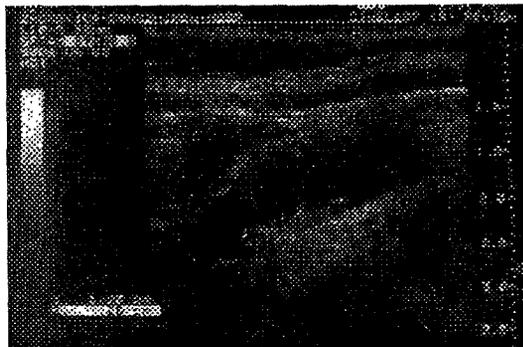


Fig 5 Two patients with atherosclerotic plaque were treated with *Vasocleaner*TM. The plaques reduced in patient Sun (A and B) and disappeared in patient Meng (C and D)

3.3.2 Effects of *VasoCleaner*TM on intimal-medial thickness (IMT) of carotid

Quantitative comparison of the IMT of the back carotid aorta wall (cm) before and after therapy indicates that *VasoCleaner*TM is more effective in reducing IMT than Gemfibrozil (Table 3).

Table 3. IMT change before and after therapy

	Arteries(N)	before	after
VasoCleaner	99	0.127±0.019	0.108±0.016* [#]
Control	83	0.126±0.017	0.121±0.016

Results are expressed as mean±SD. *P<0.05 vs. pre therapy, and [#] P<0.05 vs. the control group.

3.3.3 Effect of *VasoCleaner*TM on carotid flow rate

Quantitative comparison of the increment of blood flow rate (ml/min) in common and internal carotid before and after therapy indicates that *VasoCleaner*TM is more effective in increasing blood flow than Gemfibrozil control (Table 4).

Table 4. The blood flow rate (ml/min) in common and internal carotid before and after therapy

	N	Common carotid		Internal carotid	
		before	after	before	after
<i>VasoCleaner</i>	84	465.2±32.47	584.1±24.40 **	182.1±16.72	278.80±98.27 *
Control	83	473.5±25.55	590.3±39.16 *	168.4±36.47	268.18±35.57*

Results are expressed as mean±SD. *P<0.05, **P<0.01 vs. pre-therapy. N represents the number of analyzed cases.

3.3.4 Effect of *VasoCleaner*TM on PI and RI of common carotid artery

Quantitative comparison of the decrease in PI and RI of common carotid before and after therapy indicates that *VasoCleaner*TM is more effective in lowering both PI and RI than Gemfibrozil control (Table 5).

Table 5. Change of common carotid artery RI and PI before and after therapy

Arteries	N	RI		PI	
		before	after	before	after
VasoCleaner	99	0.793±0.010	0.76±0.009* [#]	1.998±0.114	1.659±0.095*
Control	83	0.803±0.013	0.79±0.018	2.102±0.13	1.965±0.093

Results are expressed as mean±SD. *P<0.05 vs. pre therapy, and [#] P<0.05 vs. the control group.

3.3.5 Effect of *VasoCleaner*TM on RI and PI of internal carotid artery

Quantitative comparison of the decrease in PI and RI of internal carotid artery before and after therapy indicates that *VasoCleaner*TM is more effective in lowering both internal carotid artery PI and RI than Gemfibrozil control (Table 6).

Table 6. Change of internal carotid artery RI and PI before and after therapy

	Arteries	RI		PI	
		before	after	before	after
<i>VasoCleaner</i>	98	0.866±0.052	0.743±0.021* [#]	2.055±0.130	1.377±0.082 **
Control	83	0.843±0.031	0.807±0.019	2.183±0.277	1.897±0.076

Results are expressed as mean±SD. *P<0.05, **P<0.01 vs. pre therapy, and [#] P<0.05 vs. the control group.

3.4. Effects of *VasoCleaner*TM on the level of oxLDL

OxLDL plays an important role in all stages of AS. Quantitative comparison of serum oxLDL level before and after therapy reveals a marked decrease in the *VasoCleaner*TM group, whereas no significant change was seen in the control group before and after therapy (P>0.05). These results indicate that *VasoCleaner*TM can inhibit the oxidation of LDL so that may help to reduce the injury on artery caused by oxidized LDL-C (Table 7).

Table 7. Level of oxLDL (ug/dl) before and after therapy

	N	before	after
<i>VasoCleaner</i>	84	44.43±5.95	28.83±4.35 *** [#]
Control	83	37.30±3.25	42.29±3.85

Results are expressed as mean±SD. **P<0.01 vs. pre-therapy, and [#] P<0.01 vs. the control group.

3.5. Effects of *VasoCleaner*TM on the serum levels of endothelin (ET) and calcitonin gene related peptide (CGRP).

ET-receptor is widely distributed in arteries, especially in constrictive arteries, where AS most often occurs. ET is a biologically active substance for vasoconstriction. The ET production was reduced in the *VasoCleaner*TM group (p<0.01), but not in the control group, suggesting that the artery consistent contractions in AS would be reduced eventually. CGRP can counteract the effects of ET by dilating blood vessels. Increased CGRP production may protect the blood vessel from over contraction induced by ET.

Table 8. Amount change of ET-1 and CGRP (pg/ml) before and after therapy

	N	ET-1		CGRP	
		Before	After	Before	After
<i>VasoCleaner</i>	84	55.76±6.33	36.87±4.06**##	39.92±7.33	59.83±5.45 **
Control	83	51.51±8.26	54.82±8.81	36.20±4.32	53.24±8.76 **

Results are expressed as mean±SD. **P<0.01 vs. pre-therapy, and ## P<0.01 vs. the control group.

3.6 Safety

In general, *VasoCleaner*TM is safe and well tolerated by patients. In the *VasoCleaner*TM group, at the very beginning of the therapy, 3 patients had loose stool, twice or three times a day. However, after three to five days, stools returned normal. No other side effects were found. In the control group, 8 patients suffered from heavy gastrointestinal disturbance and 3 patients had high GPT level in the blood. However, 2 weeks after stopping the Gemfibrozil, GPT levels returned to normal and GI disturbance subsided.

4. Conclusions

Clinical studies indicate that *VasoCleaner*TM is therapeutically effective in treating hyperlipidemia and AS. The mechanisms of acting may involve adjusting blood lipid profile, reducing the levels of oxLDL and ET, ameliorating blood flow, and decreasing the artery RI and PI.

Part III

Inhibitory effect of *VasoCleaner*TM on Dietary-induced Hyperlipidemia and Atherosclerosis in Rabbits

Report Outline

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2.5 Effect of *VasoCleaner*TM on the activity of succinate dehydrogenase (SDH) and adenosine triphosphate (ATPase) in cardiac tissue

2.6 Effect of *VasoCleaner*TM on the level of serum and brain endothelin (ET)

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2.9 Effect of *VasoCleaner*TM on the viscosity of plasma

3. Conclusions

Key Words: *VasoCleaner*TM, dietary-induced hyperlipidemia and atherosclerosis, rabbits

1. Materials and Methods

1.1 Materials

Animals: Twenty-eight four-month old male Japanese white rabbits (1.5-2.0Kg, China Agri. Sci. Inst.) were subjected to either normal diet or atherogenic diet. Animals were randomly assigned to four groups as follows: (I) normal group (5 rabbits, normal rabbit chow: refined rabbit chow 100g/day); (II) Cholesterol group (7 rabbits, cholesterol chow: normal rabbit chow 95g enriched with 1% cholesterol and 4% fat); (III) *VasoCleaner*TM group (6 rabbits, cholesterol chow supplemented with 5ml *VasoCleaner*TM in the drinking water); (IV) Control group (6 rabbits, cholesterol chow supplemented with 150mg of Gemfibrozil in the drinking water). The drinking water for all animals was ad libitum throughout the whole study.

After 2 months of treatment, all rabbits were anesthetized with xylazine/ketamine. The hearts and arteries were exposed by thoracotomy and further removed and fixed to formalin for atherogenic analysis.

1.2 Observation Items

1.2.1 Body weight: The rabbits were weighed every 20 days. A plot of body weight against time was made to show the animal weight gain vs. time

1.2.2 The levels of blood lipids, lipoprotein and Endothelin-1: All of them were determined by corresponding procedures listed in Part II.

1.2.3 The level of lipid peroxides (LPO) and the activity of superoxide dismutase (SOD): The level of LPO and activity of SOD in the serum, liver and brain were measured by the chemiluminescence immunoassay and radioimmunoassay respectively.

1.2.4 Amount of Thromboxane B₂ (TXB₂) and 6-Ketone-ProstaglandinF_{1a} (6-keto- PGF_{1a}): The amount of TXB₂ and 6-Keto-PGF₁ were measured by the radioimmunoassay.

1.2.5 Pathological examinations of atherogenic plaques: All rabbits were anesthetized with xylazine/ketamine after two months. Aortae were freed and removed from left heart-ear and opened longitudinally. The aortae were then stained with red oil O and the extent of the atherosclerotic plaque was estimated by a computer-assisted video imaging system. The aortae were divided into three regions: aortic arch, thoracic aorta and abdominal aorta. Paraffin sections were cut from each region and histochemically-stained. The vessel contours, the borders of atheromatous plaques, the surface area of the aorta, and the area occupied by atherosclerotic plaque were all measured.

1.2.6 Histochemical analysis: Heart slices were made from animals of each group sacrificed at the end of the experiment. After enzyme-linked histochemical staining, the level of ATPase and SDH were evaluated on each slice under microscope.

1.2.7 Hemorheological parameters: Hemorrheological parameters were measured by the commonly used procedures.

1.3 Statistical analysis

Results were expressed as mean±SD. Data representing the level of TC, TG, LDL-C, LPO and SOD were subjected to analysis of variance and Student T-test. These statistic analyses were also applied to the size of the atheromatous plaques. A value of P<0.05 was considered significant.

2. Results

2.1. Effect of *VasoCleaner*TM on the body weight

No significant difference in body weight was observed among all groups before and after 40-day treatment. The body weight of the cholesterol group is larger than that of the normal group (P<0.05) after 60-day treatment. Although the body weight of the *VasoCleaner*TM group and the control group are a little higher than that of the normal group, there is no statistically significant difference (P>0.05) (Fig. 1).

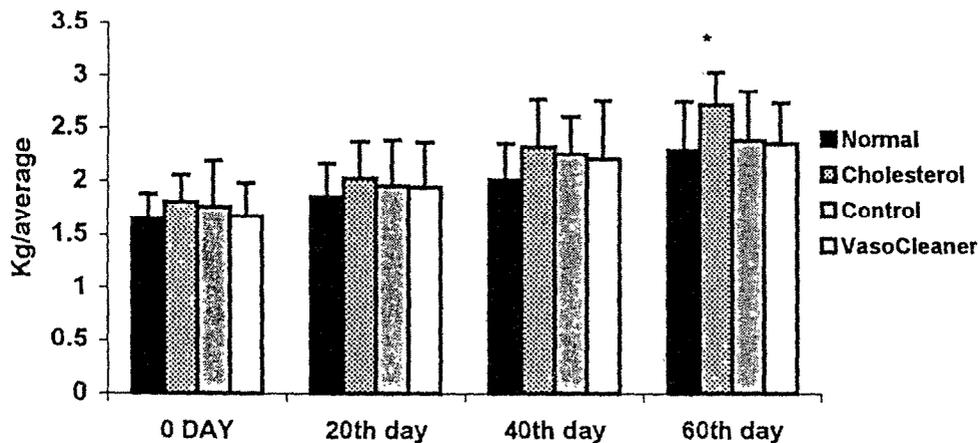


Fig 1. The change of body weight

Results are expressed as mean±SD. *p<0.05 vs. normal group.

2.2. Effect of *VasoCleaner*TM on the level of serum lipid and lipoprotein

TC level in the *VasoCleaner*TM group is lower than that in the control group (P<0.05) after 60-day treatment. TG level in both *VasoCleaner*TM and control group are lower than that in the cholesterol group (P<0.05&0.01). No significant difference in apoA-1 and apoB-100 level was observed between the *VasoCleaner*TM group and the control group. Fig 1-5.

Table 1 TC (mg/dL) level in the four experimental groups

Group	N	20th day	40th day	60th day
Normal	5	60.80±10.40	58.84±11.20	58.50±14.24
Cholesterol	7	533.57±120.20#	1313.29±143.21 ##	1304.86±110.92 ##
Control	6	504.83±88.29 #	1284.29±125.18 ##	1176.2±134.86 ##
<i>VasoCleaner</i>	6	443.57±10.78 #	931.33±154.25 ##	889.80±120.89 *##

Results are expressed as mean±SD. *p<0.05 vs. cholesterol group, # P<0.05, ## P<0.01 vs. normal group.

Table 2 TG (mg/dL) level in the four experimental groups

Group	N	20th day	40th day	60th day
Normal	5	71.67±13.70	77.60±13.57	58.20±14.07
Cholesterol	7	71.14±13.20	181.00±34.98 ##	167.29±37.16 ##
Control	6	73.00±15.74	128.43±23.13 ###	81.14±23.93 ###
VasoCleaner	6	69.00±15.76	120.50±23.27 ###	83.66±22.87 ###

Results are expressed as mean±SD. *p<0.05, **p<0.01 vs. cholesterol group, ## p<0.01 vs. normal group

Table 3 Serum HDL-C (mg/dL) level in the four experimental groups

	N	20th day	40th day	60th day
Normal	5	23.83±7.60	23.33±4.37	25.17±4.17
Cholesterol	7	25.71±5.53	37.43±7.43 ##	34.86±11.84 ##
Control	6	28.40±5.16	39.42±2.29 ##	38.18±5.48##
VasoCleaner	6	27.60±5.13	38.26±3.15 ##	38.40±5.17 ##

Results are expressed as mean±SD. ## P<0.01 vs. normal group.

Table 4 Serum apoA-1 (mg/dL) level in the four experimental groups

	n	40th day	60th day
Normal	5	3.87±0.98	3.75±0.52
Cholesterol	7	112.77±33.83 ##	108.89±25.18 #
Control	6	199.57±59.58 ###	156.57±28.29 ###
VasoCleaner	6	189.60±68.74 ###	160.52±22.26 ###

Results are expressed as mean±SD. * p<0.05 vs. the cholesterol group. # P<0.05, ##p<0.01 vs. normal group.

Table 5 Serum apoB-100 (mg/dL) level in the four experimental groups

	N	40th day	60th day
Normal	5	71.14±13.20	70.83±10.19
Cholesterol	7	464.43±138.91 ##	442.23±13.67 ##
Control	6	460.61±75.82 ##	449.29±146.34 ##
VasoCleaner	6	423.19±81.61 ###	423.20±106.34 ##

Results are expressed as mean±SD. *p<0.05 vs. cholesterol group, ## P<0.01 vs. normal group

2.3 Anti-atherogenic effect of *VasoCleaner*TM

Oil red O staining of atherogenic plaque: The extent of aortic lipid deposition (the formation of atherogenic plaque) was visualized by oil red O staining. Extensive lipid deposition was observed in the rabbits from the cholesterol group. Compared with the control group and cholesterol group, the occurrence of the aorta atherogenic lesions was significantly reduced in animals of the *VasoCleaner*TM group. Fig 2 and Table 6.

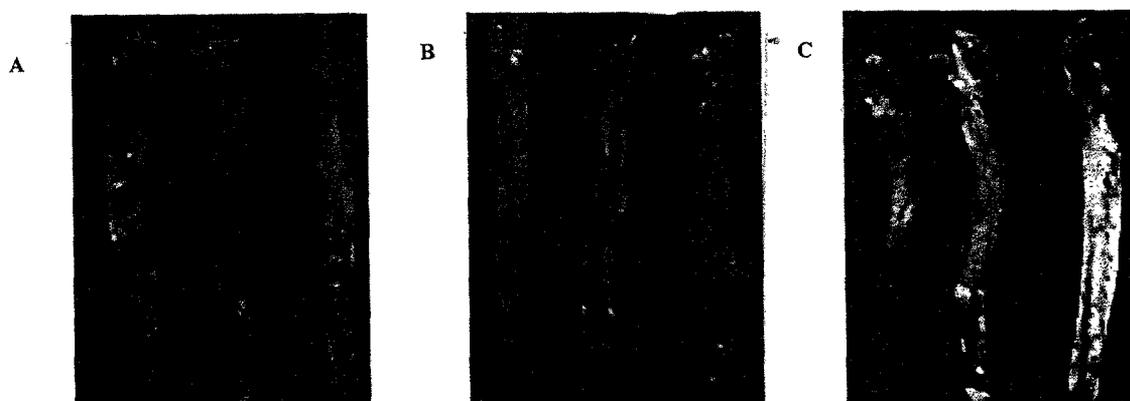


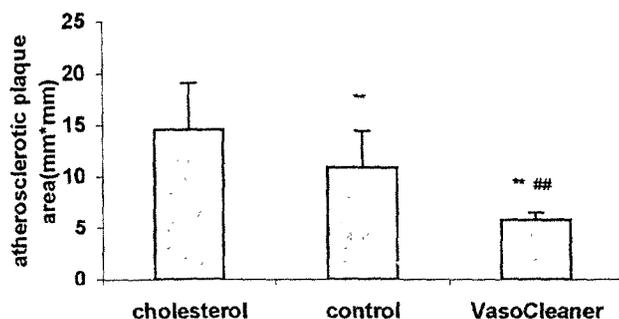
Fig 2 The atherogenic plaques formation in different treatment. A large number of plaques were formed under cholesterol diet (A). The control group can inhibit plaque formation (B). While *VasoCleaner*TM can inhibit plaque formation more effectively (C).

Table 6 Percentage of atherogenic plaques in aorta revealed by red-oil-O staining

Cholesterol group	Control group	<i>VasoCleaner</i> group
N=7	N=6	N=6
14.62±4.51	10.86±3.53*	5.87±0.67**ΔΔ

Results are expressed as mean±SD. *p<0.05 and **p<0.01 vs. cholesterol group. ΔΔ P<0.01 vs. control group.

Histological examination of lesion formation: Histological examination of the aorta: aortic arch, thoracic aorta and the abdominal aorta, in animals from the cholesterol group revealed apparent atheromatous deposits in all regions. The morphology of the aortic lesions was that of a typical atheroma with a well-developed lipid rich core, foam cell infiltrates, cholesterol crystals, and a thin fibrous cap. Animals from *VasoCleaner*TM group showed a marked reduction in the degree of lesion formation in all the above three examined regions compared with both the cholesterol and control group (Fig 3A and 3B).



N=7	N=6	N=6
2.649±3.03	0.795±0.56**	0.271±0.2**##

Fig 3 A. Total area of atherosclerotic plaques on pathogenic slices from aortic arch, thoracic aorta and abdominal aorta in the experimental groups under microscope ($cm \times cm/125$). Results are expressed as mean±SD. **p<0.01 vs. cholesterol group. ##P<0.01 vs. control group.

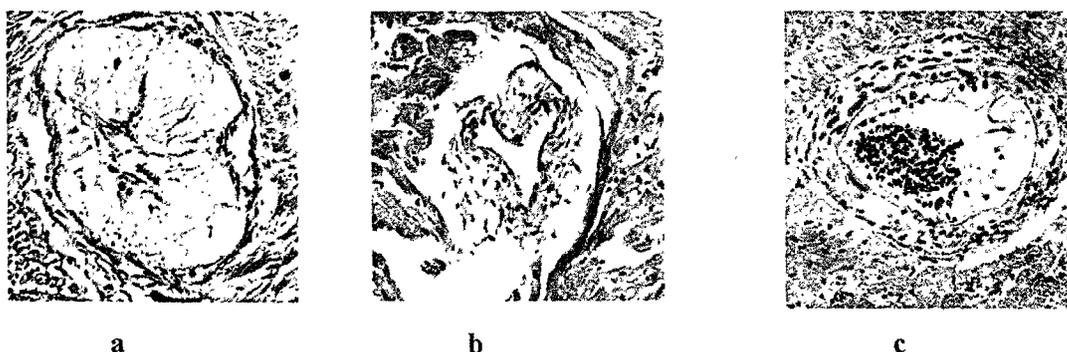


Fig 3 B Atherosclerotic plaques on pathogenic slices from aortic arch. a, Cholesterol group; b, Control group; c, *VasoCleaner*TM group.

2.4 Effect of *VasoCleaner*TM on the atherogenic tissue

The pathogenic slices revealed that the extent of aortic injury and smooth muscle hyperplasia in animals from the *VasoCleaner*TM group are smaller than those in the cholesterol and the control groups. Moreover in the control group, the muscle cell core was perpendicular to the endothelium and the migrating smooth muscle cells that have invaded the endothelium at the breach of the elastic membrane. However, in the *VasoCleaner*TM group, smooth muscle cells in the medium layer were parallel to the elastic membrane, suggesting that *VasoCleaner*TM can inhibit the movement of artery medium smooth muscle cells toward the endothelium. This is probably relevant to the inhibition of atherogenic plaque formation by *VasoCleaner*TM (Fig4).

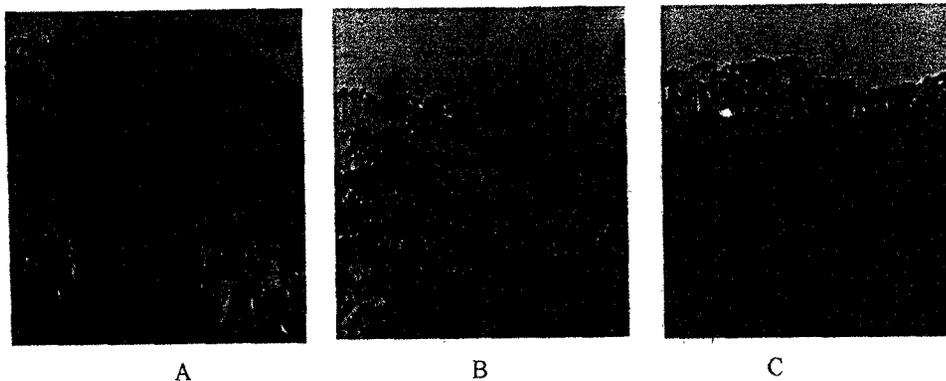


Fig 4 The effect of *VasoCleaner™* on atherogenic changes of artery. A, Cholesterol group; B, Control group; C, *VasoCleaner™* group .

2.5. Effect of *VasoCleaner™* on the activity of succinate dehydrogenase (SDH) and adenosine triphosphate (ATPase) in cardiac tissue.

SDH and ATPase locate in the mitochondria of the cardiac muscle cell. When myocardial ischemia occurs, SDH will be released and the activity of ATPase will be decreased. Positive correlation exists in the content of SDH and ATPase with the histochemistry staining. The degree of the staining of the slices from the *VasoCleaner™* group is similar to that from the normal group, but the staining is much lighter in the slices from the control group, suggesting that *VasoCleaner™* is effective in protecting the heart muscle cells from atherogenic injuries (Fig 5,6).



Fig 5 The effect of *VasoCleaner™* on ATPase. A, Normal group; B, Cholesterol group; C, Control group; D, *VasoCleaner™* group.

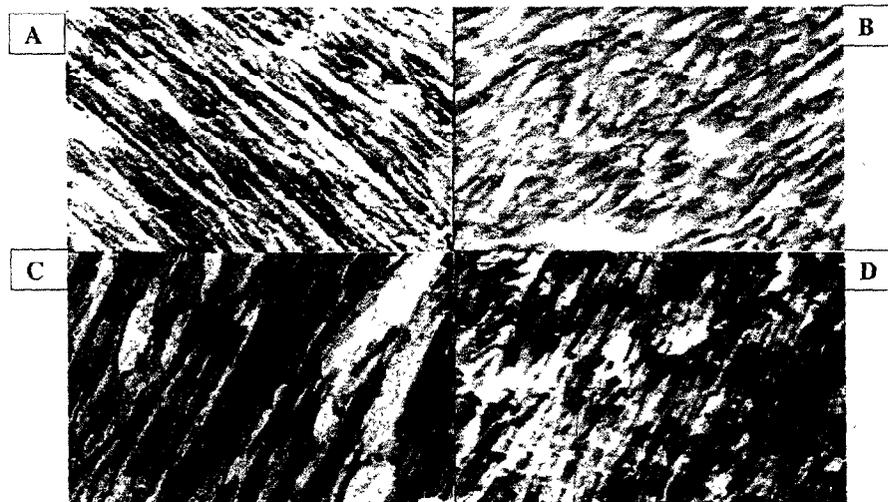


Fig 6 The effect of *VasoCleaner™* on SDH. A, Normal group; B, Cholesterol group; C, Control group; D, *VasoCleaner™* group.

2.6. Effect of *VasoCleaner*TM on the level of serum and brain endothelin (ET).

The level of ET in the *VasoCleaner*TM group is lower than control group and cholesterol control group, and similar to that in normal group. which indicates that *VasoCleaner*TM is more effective in preventing the production of ET than Gemfibrozil control.

Table 7 ET-1 (pg/ml) levels in blood plasma and brain tissue

	Normal (n=5)	Cholesterol (n=7)	Control (n=6)	<i>VasoCleaner</i> (n=6)
Plasma	372.69±57.60**Δ	646.40±107.27	468.71±88.52*	378.94±63.40** Δ
Brain	574.68±61.73 **	739.35±88.53	552.58±9.68 **	520.71±141.47**

Results are expressed as mean±SD. * P<0.05, ** P<0.01 vs. cholesterol group. Δ P<0.05 vs. the control group.

2.7. Effect of *VasoCleaner*TM on the balance between 6-Keto-PGF_{1α} and TXB₂.

TXA₂ is a biologically active substance released from platelets causing vasoconstriction and blood coagulation. PGI₂ is another biologically active substance synthesized by vascular endothelial cells preventing coagulate and dilating vasculature. The balance of these two bioactive molecules benefits normal vascular function. Because of their extremely short half-lives, TXA₂ and PGI₂ are measured via their immediate metabolite, TXB₂ and 6-keto-PGF₁ respectively. We observed that, in the *VasoCleaner*TM group, the level of 6-Keto-PGF₁ is higher than Cholesterol and Control groups (P<0.05), but no change in TXB₂ level was detected, so that the ratio of 6-keto-PGF_{1α} to TXB₂ is also higher than other two groups (P<0.05), indicating that as a result, *VasoCleaner*TM helps to maintain the function of blood vessels by balancing the ratio of 6-keto-PGF_{1α} to TXB₂

Table 8 Levels of TXB₂ (pg/ml) and 6-keto-PGF_{1α}(pg/ml) in blood plasma level of the experimental groups

	N	6-keto-PGF _{1α}	TXB ₂	6-keto-PGF _{1α} /TXB ₂
Normal group	5	654.77±127.53	24.88±3.88	26.68±5.77
Cholesterol group	7	276.71±78.05 ##	33.05±8.60	8.93±3.71 #
Control group	6	342.95±97.42 ##	33.10±8.64	10.62±2.95 #
<i>VasoCleaner</i> group	6	474.95±169.88* Δ	29.96±7.23	16.32±3.84 # ** Δ

Results are expressed as mean±SD. * P<0.05, ** P<0.01 vs. cholesterol group. ΔP<0.05 vs. control group. # P<0.05, ## P<0.05 vs. normal group.

2.8. Effects of *VasoCleaner*TM on the level of lipid peroxides (LPO) and the activity of superoxide dismutase (SOD) in serum, liver and brain.

The level of LPO in the serum indirectly reveals the amount of free radicals. Excessive production of free radicals can directly damage the membrane structure of blood vessel. SOD is a protective enzyme that can eliminate superoxide, a notorious free radical. The results show that the increasing activities of SOD is positively related to LPO levels. LPO amount in the *VasoCleaner*TM group is lower than that in the control and the cholesterol groups (P<0.01). The change of LPO and SOD in the brain and liver are similar to that in the serum.

Table 9 Serum LPO level (ng/ml) in the experimental groups

	Normal (N=5)	Cholesterol (N=7)	Control (N=6)	<i>VasoCleaner</i> (N=6)
30 th day	20.56±3.27	55.00±9.17##	65.94±10.24##	45.62±7.20*##
60 th day	21.39±4.65	51.87±6.29##	60.56±12.60##	34.20±5.04**##

Results are expressed as mean±SD. * P<0.05, ** P<0.01 vs. cholesterol group (P<0.05,0.01), ## P<0.01 vs. normal group.

Table 10 Serum SOD level (ng/ml) in the experimental groups

	Normal (N=5)	Cholesterol (N=7)	Control (N=6)	<i>VasoCleaner</i> (N=6)
30 th day	84.25±1.15	130.79±18.95##	119.19±20.59#	79.10±33.03**Δ
60 th day	92.82±42.55	142.52±30.98##	152.39±22.57##	119.07±46.82#

Results are expressed as mean±SD. ** P<0.01 vs. cholesterol group. ΔP<0.05 vs. control group. #P<0.05, ## P<0.01 vs. normal group.

Table 11 Liver and brain LPO (ng/ml) levels in the experimental groups

	Normal (N=5)	Cholesterol (N=7)	Control (N=6)	<i>VasoCleaner</i> (N=6)
Liver	2.28±0.58	4.02±1.81##	3.28±0.48#	1.59±0.26*Δ
Brain	9.04±2.59	16.14±3.55##	15.60±1.82##	11.47±1.27*

Results are expressed as mean±SD. * P<0.05 vs. cholesterol group. # P<0.05 and ## P<0.01 vs. normal group. ΔP<0.05 vs. control group.

Table 12 SOD (ng/ml) levels in the liver and brain tissues

	Normal (N=5)	Cholesterol (N=7)	Control (N=6)	<i>VasoCleaner</i> (N=6)
Liver	11.02±2.33	18.76±3.16##	14.10±4.94*#	13.49±2.05*#
Brain	5.87±3.19	8.73±2.94	6.04±1.86	5.03±2.21*

Results are shown as mean±SD. * P<0.05 vs cholesterol group. #P<0.05 and ## P<0.01 vs normal group.

2.9. Effect of *VasoCleaner*TM on the viscosity of plasma

Once the viscosity of either the blood plasma or the whole blood becomes too high, the concentrated blood cells can delay the blood circulation. Our results show that *VasoCleaner*TM reduces the viscosity of both blood plasma and the whole blood (P<0.01) compared with the control group. Thus the resistance is decreased.

Table13 Hemorrheological changes in the experimental groups

	Normal(n=6)	Cholesterol(n=7)	Control(n=7)	<i>VasoCleaner</i> (n=7)
230 ^{s-1}	4.75±0.58	4.98±0.39	4.10±0.51**	4.30±0.58*
5.75 ^{s-1}	5.52±0.43	6.33±0.69#	5.01±0.44*	5.53±0.53*
BV	1.72±0.30	2.20±0.29#	1.74±0.18*	1.58±0.13**
HCT (%)	39.00±2.39	37.29±3.49	35.17±3.24	36.19±4.03
TK	3.45±0.16	3.82±0.79	4.35±1.43*	4.03±1.43
RV	12.34±1.40	15.81±1.34##	12.25±1.00**	12.19±1.20**
EAI	1.27±0.15	1.27±0.17	1.32±0.20	1.27±0.19

Results are shown as mean±SD. *P<0.05, ** P<0.01 vs. cholesterol group, #P<0.05, ## P<0.01 vs. normal group. RV represents recovery viscosity, BV represents blood viscosity

3. Conclusions

The above data indicates that *VasoCleaner*TM is effective in treating and preventing dietary-induced hyperlipidemia and AS in rabbits. The mechanism may involve: reducing serum and tissue levels of lipid, LPO and ET, reducing the viscosity of the blood plasma and the whole blood, and balancing the ratio of 6-Keto PGF₁ to TXB₂.