

Panax notoginseng (Burk.) Effects on Fibrinogen and Lipid Plasma Level in Rats Fed on a High-fat Diet

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Several studies have shown that notoginsenosides improve diastolic function in hypertensive subjects, induce the fibrinolytic system in *in vitro* models and act as antiproliferative agents on vessel leiomyocytes. Our aim was to evaluate their effect on fibrinogen and lipid plasma levels compared with a well-known HMGCoA reductase inhibitor. Seventy Wistar male adult rats on a fat-enriched diet were treated orally with *P. notoginseng* pulverized root (43 mg/kg/day or 86 mg/kg/day; 20 animals per group), fluvastatin (3 mg/kg/day; 20 animals) or physiological saline (5 mL/kg/day; 10 animals). The ten rats on a normocaloric diet were also treated with 5 mL/kg/day of physiological saline. After a 28-day treatment, the rats were killed and their blood analysed with standard procedures. Treatment with 43 mg/kg/day of *P. notoginseng* or 3 mg/kg/day of fluvastatin showed similar activity in decreasing total cholesterol (–23.70%, –19.29%, respectively) and triglycerides (–21.59%, –18.55%). The most evident effect of *P. notoginseng* was the reduction of fibrinogenaemia in treated rats compared with the control values (–38.10%; $p < 0.001$), no dose-relationship being shown in this effect. Moreover, no significant variation in HDL cholesterol and glucose levels was observed nor did relevant behavioural changes occur in association with the root intake. Besides a moderate, non dose-related decrease in the plasma lipid levels, *P. notoginseng* appeared to induce a significant reduction in the rat fibrinogenaemia. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: cholesterol; fibrinogen; *Panax notoginseng*; herbal remedies.

INTRODUCTION

The potential use of Araliaceae roots in cardiovascular disease prevention was first recognized by Japanese authors 20 years ago. In fact, *Panax ginseng* (Renshen) administration induced a decrease in low density lipoprotein cholesterol (LDL-C) triglycerides (TG) plasma and liver levels, as well as an increase in the high density lipoprotein cholesterol (HDL-C) plasma levels and a reduction of platelet adhesiveness in both rats (Yamamoto *et al.*, 1983a) and hyperlipidaemic patients (Yamamoto *et al.*, 1983b; Punnonen and Lukola, 1984). The proposed mechanisms of action were the following: an increase in faecal fat excretion, a decrease in the activities of β -hydroxy- β -methylglutaryl-CoA reductase, acyl-coenzyme-A-cholesterol acyltransferase and cholesteryl ester transfer protein (Qureshi *et al.*, 1983; Yamamoto *et al.*, 1983b; Punnonen and Lukola, 1984; Kwon *et al.*, 1996; Kwon *et al.*, 1999).

Panax notoginseng (Burk.) F. H. Chen (Sanqi) is a root, similar to the more well-known and studied *P. ginseng* (Ngan *et al.*, 1999). All the Araliaceae of the ginseng group have a similar composition, even if the chemical constituents have a different concentration, specificity and activity (Bruneton, 1999). Recent studies

demonstrated various specific pharmacological actions of notoginsenosides, especially on the cardiovascular system. The chronic administration of *P. notoginseng* with the ACE-inhibitor captopril improved the left ventricular diastolic function in hypertensive patients compared with treatment with captopril alone; this effect was partially related to an antagonism to selective Ca⁺⁺ receptor operating channels (Feng *et al.*, 1999). Among the haemorheological parameters, notoginsenosides appeared to increase the synthesis of the tissue-type plasminogen activator and to decrease the activity of plasminogen activator inhibitor-1 in cultured human endothelial and vascular smooth muscle cells from different vascular regions (Zhang *et al.*, 1997). Moreover, a 10-day oral administration of *P. notoginseng* increased prostacyclin synthesis in the carotid artery and decreased thromboxane A₂ synthesis in blood platelets; at the end of the experiment, aortic atherosclerotic plaque development was reduced (Shi *et al.*, 1990). In a previous study we evaluated the adaptogenic properties of the root (Cicero *et al.*, 2000). The aim of this study was to evaluate the effects of orally administered *P. notoginseng* (43 mg/kg and 86 mg/kg in a volume of 5 mL/kg) on plasma lipid levels and fibrinogenaemia in rats on a high-fat diet.

MATERIALS AND METHODS

Eighty adult male Wistar rats (Harlan Italy, Correzzana,

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Table 1. Components and chemical analysis of fat diet

Component	g/kg	Chemical analysis	%
Casein	220	Water	12.0
Maize starch	256		
Saccharose	100		
Cellulose	40		
Bacon	100	Raw proteins	19.0
Soybean oil	70	Raw lipids	25.5
Coconut oil	130	As-spun	4.0
Salt-mineral mixture ^a	52	Raw ash	7.0
Oligoelements ^a	8	Nitrogen-free extract	44.5
Vitamin mixture	20		
DL-Methionine	4		
Total	1000		100.0

^a Salt-mineral mixture and vitaminized starch were made according to Seetharamaiah *et al.* [23].

Milan, Italy) weighing 240 ± 20 g (3 months old on arrival) were used and housed 1 per cage in a temperature- ($22^\circ \pm 1\text{C}^\circ$) and humidity- (60%) controlled environment. The light/dark cycle was the natural one (the light phase of the cycle beginning at 6 a.m.). The animals were divided into two groups: ten rats (NC: normal diet controls) had free access to standard rat food in pellets (MIL, Morini, S. Polo D'Enza, Reggio nell'Emilia, Italy), 70 animals were fed with a high-fat diet containing 19% proteins and 25.5% lipids (10% cholesterol). Water was available *ad libitum*. This dietary regimen was adopted throughout the experiment. Housing conditions and experiments were in accordance with the European Community regulations on the care and use of animals for scientific purposes (CEE Council 86/609, included in the Italian writ DL 27/01/92 N. 116). The rats were acclimatized to our housing conditions for at least 1 week before being used.

Drugs and treatment. Rats were orally treated through a gastric tube for 28 days consecutively. The rats on the fat diet were divided into four subgroups: ten were treated with 5 mL/kg/day of saline (FC: fat diet controls), 20 were given 3 mg/kg/day of fluvastatin (FL: fluvastatin), 20 received 43 mg/kg/day of high quality *P. notoginseng* pulverized root (Ng) (Yunnan Medicines & Health Products Corp., Yunnan, R. P. China) and 20: 86 mg/kg/day of *P. notoginseng* (NG). The NC animals were also treated with 5 mL/kg/day of saline solution. At the end of the experiment, the animals were fasted overnight and killed under ether anaesthesia. Blood was collected by heart puncture. Plasma samples were stored at -20°C before being used for analysis.

Laboratory analysis. Lipid levels [total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C)] were analysed on plasma samples by enzymatic-colorimetric methods according to standard laboratory techniques. The concentration of LDL cholesterol (LDL-C) was estimated through the Friedewald formula: $\text{LDL-C} = \text{TC} - (\text{TG}/5 + \text{HDL-C})$ for TG value <400 mg/dL. Glycaemia was also determined by the enzymatic-colorimetric method, as described by Gaddi *et al.* (2001). Fibrinogen levels were measured in duplicate using the Clauss method (Data-Fi Fibrinogen determination Kit, Dade County, FL) (Clauss, 1957).

Microstructural analysis of unconditioned behaviour.

The test was carried out to detect any possible *P. notoginseng*-induced behavioural changes. Thirty minutes after the treatment, the observation started by keeping the animals in their individual home cages. The following parameters were separately registered on a specific schedule of 1 h periods: number and duration time dedicated to feeding, drinking, exploring, resting and grooming. Pre-weighed food pellets were provided at the beginning of the test and food intake was measured at the end by weighing the remaining food plus spillage. During the experiment the rats had free access to water.

Statistical analysis. Data were statistically processed by the Shapiro–Wilk normality test and analysis of variance (ANOVA-test) followed by Student's one-tail *t*-test (SPSS 8.0, Windows version, statistical software). A $p = 0.05$ significance level was chosen for all statistical tests (Normann and Streiner, 2000).

RESULTS

Before killing the rats' mean weight had increased by 20% (301 ± 19 g) with no differences between the NC and FC animals. This fat diet model produced a significant increase in TC ($F = 77.59$, $p < 0.001$), LDL-C ($F = 64.76$, $p < 0.001$) and TG ($F = 185.12$, $p < 0.001$) plasma levels, with no influence on HDL-C, glucose and fibrinogen levels ($p > 0.05$) compared with the NC values. Treatment with fluvastatin significantly lowered TC ($F = 39.94$, $p < 0.001$), LDL-C ($F = 9.98$, $p = 0.005$) and TG ($F = 17.99$, $p < 0.001$) levels compared with those of the FC group, as expected by the literature data (Langtry and Markham, 1999). No relevant differences were observed in the TC ($F = 4.31$, $p = 0.053$), LDL-C ($F = 4.25$, $p = 0.054$) and TG ($F = 0.76$, $p < 0.428$) levels between fluvastatin treated rats on a fat diet and controls on a standard diet. The administration of fluvastatin did not reduce fibrinogenaemia in comparison with NC and FC rats.

43 mg/kg/day of *P. notoginseng* pulverized root significantly decreased TC ($F = 20.84$, $p < 0.001$), LDL-C ($F = 19.42$, $p < 0.001$) and TG ($F = 9.58$, $p < 0.006$) levels compared with the FC group. No relevant differences between the lower dose *P. notogin-*

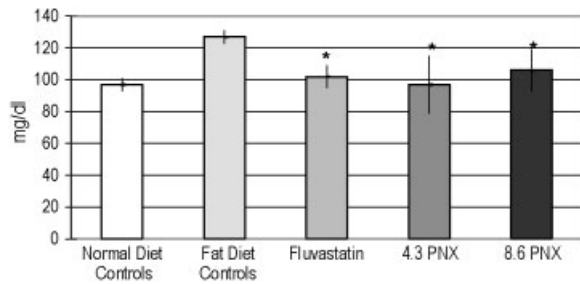


Figure 1. Effect of fat diet, fluvastatin 3 mg/kg and *P. notoginseng* (PNX) pulverized root (43 and 86 mg/kg) on rat plasma total cholesterol level. * $p < 0.01$ versus FC.

seng treated rats on a fat diet and controls on a standard diet were observed for TC ($F = 0.00$, $p = 0.987$), LDL-C ($F = 0.00$, $p = 0.975$) and TG ($F = 0.01$, $p < 0.936$) levels. The Ng treatment reduced fibrinogenemia compared with NC ($F = 36.60$, $p < 0.001$) and FC ($F = 51.95$, $p < 0.001$) rats.

86 mg/kg/day of *P. notoginseng* pulverized root significantly lowered TC ($F = 15.66$, $p < 0.001$), LDL-C ($F = 11.93$, $p = 0.003$) and TG ($F = 6.14$, $p < 0.023$) levels compared with the FC group. No relevant differences between the higher dose *P. notoginseng* treated rats on a fat diet and controls on a standard diet were observed concerning TC ($F = 4.16$, $p = 0.056$), LDL-C ($F = 0.00$, $p = 1.000$) and TG ($F = 4.40$, $p = 0.051$) levels. The NG treatment decreased fibrinogenemia compared with NC ($F = 20.12$, $p < 0.001$) and FC ($F = 57.51$, $p < 0.001$) rats.

No significant difference was found between fluvastatin and Ng treated groups in TC ($F = 0.81$, $p = 0.381$), LDL-C ($F = 2.46$, $p = 0.134$) and TG ($F = 0.14$, $p = 0.713$). Unlike fluvastatin, Ng significantly lowered the fibrinogen level. Similar results were found comparing fluvastatin and NG treated rats. Ng and NG showed similar activity in reducing TC ($F = 1.64$, $p = 0.217$), LDL-C ($F = 3.37$, $p = 0.083$) and TG ($F = 1.34$, $p = 0.263$), while Ng seemed to be more effective in reducing the fibrinogen level than NG ($F = 15.74$, $p < 0.001$).

Changes in TC, LDL-C, TG and fibrinogen plasma levels are reported in Figs 1 2 3–4. Microstructural analysis of the spontaneous behaviour revealed that both doses of orally administered *P. notoginseng* significantly increased the exploring time ($p < 0.03$) while reducing the grooming ($p < 0.001$) and resting times ($p < 0.03$). Once again, both *P. notoginseng* doses produced a greater number of exploring episodes ($p < 0.01$), and a lower number of grooming ($p < 0.03$) and resting episodes ($p < 0.05$) than the control rats. No significant differences in the duration and number of the episodes were observed between the 43 mg/kg and the 86 mg/kg *P. notoginseng*-treated rats ($p > 0.05$). Finally, no significant difference was found in either feeding or drinking behaviour ($p > 0.05$).

DISCUSSION

The utility of some natural products in the treatment and prevention of atherosclerosis-related cardiovascular disease has been rigorously tested on animal and human

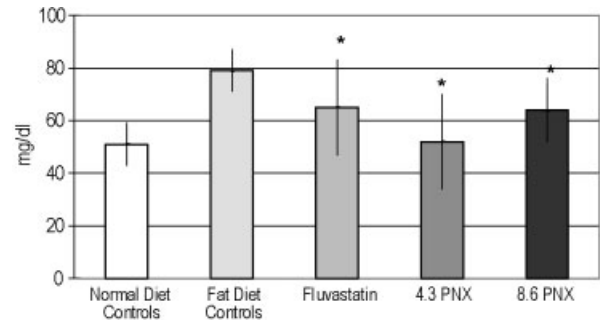


Figure 2. Effect of fat diet, fluvastatin 3 mg/kg and *P. notoginseng* (PNX) pulverized root (43 and 86 mg/kg) on rat plasma low density lipoprotein level. * $p < 0.01$ versus FC.

models. Currently the non-pharmaceutical aids in moderate dyslipidaemias are soy bean proteins for hypercholesterolaemias (Anderson *et al.*, 1995), fish oil for hypertriglyceridaemias and hypoHDLaeimias (GISSI, 1999), rice bran oil for moderate hypercholesterolaemias and hypertriglyceridaemias (Cicero and Gaddi, 2001). Recent studies show that high fibrinogen is an emerging independent risk factor for cardiovascular diseases (Harjai, 1999). In healthy non-smoking men, its level is associated with the expression of a more atherogenic LDL subfraction (small and dense LDL with $d > 1.044$ g/dL), and a lower HDL-C level, in a way that is independent of body mass index, age and insulin resistance (Halle *et al.*, 1996). In contrast, the only drugs that actually demonstrate a lowering effect on this parameter are the fibrates (bezafibrate, fenofibrate and ciprofibrate, but not gemfibrozil) that induced a fibrinogenemia decrease of -10% to -30% in different clinical trials (Pazzucconi *et al.*, 1992; Broijersen *et al.*, 1996). In contrast to fibrates, β -hydroxy- β -methylglutaryl-CoA reductase inhibitors (statins) generally induce only a slight effect on the fibrinogen blood level (Nair *et al.*, 1998; Jones *et al.*, 1998). However, recent reports indicate an association between atorvastatin administration, a very potent lipid lowering agent, and fibrinogenemia increase (ranging from $+20\%$ to $+50\%$) in some treated patients (Wierzbicki *et al.*, 1998; Sinzinger and Rodrigues, 1999). The aim of our study was to confirm the decreasing effect of *P. notoginseng* on plasma lipids and to evaluate its effect on the fibrinogen plasma levels in rats fed a new model of a fat diet compared with the activity of a well-known effective statin (Langtry and Markham, 1999). We know that, theoretically, we had to

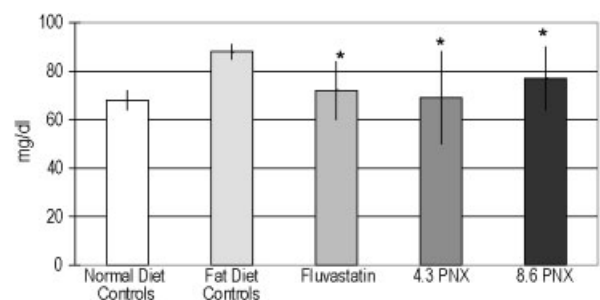


Figure 3. Effect of fat diet, fluvastatin 3 mg/kg and *P. notoginseng* (PNX) pulverized root (43 and 86 mg/kg) on rat plasma triglyceride level. * $p < 0.05$ versus FC.

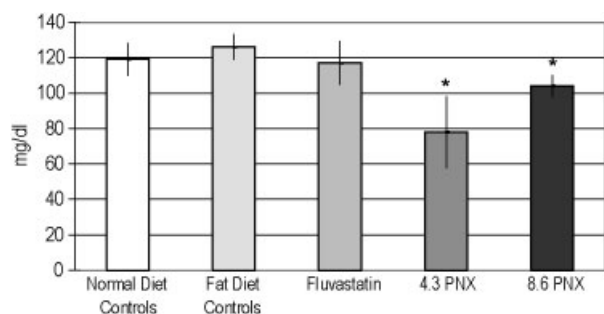


Figure 4. Effect of fat diet, fluvastatin 3 mg/kg and *P. notoginseng* (PNX) pulverized root (4.3 and 8.6 mg/kg) on rat plasma fibrinogen level. * $p < 0.01$ versus FC.

use a fibrate or pravastatin as a positive control to test the *P. notoginseng* efficacy to reduce the fibrinogenaemia, but we did not expect such a marked effect of the root on this parameter. The *P. notoginseng* doses were planned on the basis of the Traditional Chinese Medicine prescriptions for the treatment of angina pectoris and dyslipidaemias. The role of the root components (flavonoids, saponins, notoginsenosides and others) in cardiovascular disease management may be due to different properties: Ca^{++} -channel blocking activity, improvement of diastolic function in hypertensive patients (Feng *et al.*, 1999), increase in rTPA and

decrease in PAI-1 synthesis in cultured human endothelium cells (Zhang *et al.*, 1997) and correction of an imbalance between prostacyclin and thromboxane A_2 (Shi *et al.*, 1990). Our data confirm that orally administered *P. notoginseng* pulverized root produced a significant, non dose-related, lipid lowering effect compared with controls and a well-known effective treatment in hyperlipidaemic rats. 43 mg/kg/day of *P. notoginseng* and 3 mg/kg/day of fluvastatin showed a similar activity in decreasing plasma TC (-23.70% and -19.29% , respectively) and TG (-21.59% and -18.55%) levels, while no significant change in HDL-C and glucose levels was observed ($p > 0.05$). The most important *P. notoginseng* effect was the mean reduction in fibrinogenaemia compared with the control values (-38.10% ; $F = 51.95$, $p < 0.001$). No relevant behavioural modification was associated with the root intake. Just as all herbal remedies that consist of a mixture of many compounds, *P. notoginseng* root exerts its several pharmacological actions on different parameters. We suggest that further molecular studies are needed on the *P. notoginseng* root to identify the different components of this root in order to correlate molecular structure and effects. Since only a small fraction of the world is plant biodiversity has been tested for biological activity, it can be assumed that natural products will continue to offer new leads for innovative therapeutic agents.

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