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SHORT COMMUNICATION

Hypolipidaemic Activity of Methanol Extract of *Aleurites moluccana*

R. C. Pedrosa,[1]* C. Meyre-Silva,[2] V. Cechinel-Filho,[3] J. C. Benassi,[1] L. F. S. Oliveira,[1] V. Zancanaro,[1] J. Dal Magro[4] and R. A. Yunes[2]

[1]Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina - UFSC, Florianópolis - SC, Brazil
[2]Departamento de Química, Universidade Federal de Santa Catarina - UFSC, Florianópolis - SC, Brazil
[3]Núcleo de Investigações Químico-Farmacêuticas (NIQFAR)/CCS, Universidade do Vale do Itajaí - UNIVALI, Itajaí - SC, Brazil
[4]Departamento de Ciências Biológicas e da Saúde, Universidade do Oeste de Santa Catarina - UNOESC, Chapecó - SC, Brazil

The lipid-lowering action of the leaves of the *Aleurites moluccana* methanol extract was studied in Triton W-1339 and high-fat-diet fed rats. The serum lipids (total cholesterol, LDL- and HDL-cholesterol and triglycerides) and body weight were found to be lowered by *A. moluccana* (300 mg/kg, b.w.) in rats with Triton-induced hypercholesterolaemia and on a hyperlipaemic diet. The results suggest that the lipid lowering action of this natural product is mediated through inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine.

Keywords: Aleurites moluccana; cholesterol; flavonoids; phytosterols; lipid lowering.

INTRODUCTION

Coronary heart disease is an important cause of death in the Western world, and atherosclerosis accounts for the majority of these deaths. Since hypercholesterolaemia, characterized by an increase in serum cholesterol, has been generally recognized to contribute significantly to the progression of atherosclerosis, this fact points to the importance of reducing plasma cholesterol levels (Erkkila Arja *et al.*, 1999). In this regard, efforts to develop new and better hypolipaemic drugs have led to the discovery of many natural and synthetic agents.

Aleurites moluccana (L.) Wild (Euphorbiaceae) is native to Indonesia and has been introduced into southern Brazil. The fruits and leaves of this plant are used in traditional medicine for the treatment of headache, fever, inflammation and gonorrhoea, and also for the lowering of cholesterol (Meyre-Silva *et al.*, 1997). Phytochemical studies with this plant have revealed the presence of triterpenes, steroids, coumarins and flavonoid glycosides such moretenone, moretenol, acetil aleuritic acid, moluccanin, swertisin, α- and β-amyrin, stigmasterol, β-sitosterol and campesterol (Shamsuddin *et al.*, 1988; Meyre-Silva *et al.* 1998). Antibacterial activity and antiviral properties of this plant have also been reported (Locher *et al.*, 1995, 1996).

Previous pharmacological studies have shown that the hydroalcohol extract and some fractions obtained from *A. moluccana* leaves exhibit a potent antinociceptive action on mice, which is related to the presence of α- and β-amyrin and 2″-O-rhamnosylswertisin (Meyre-Silva *et al.* 1998, 1999). This fact probably accounts for the popular use of this plant in the treatment of headache.

* Correspondence to: R. C. Pedrosa, Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina - UFSC, Florianópolis - SC, Brazil.

The aim of the present work was to evaluate the influence of the methanol extract from *A. moluccana* leaves (MEAM) on serum lipid levels in experimental hypercholesterolaemia in rats, in order to confirm some of the reported popular lowering cholesterol property of this plant and to study its mechanism of action.

EXPERIMENTAL

Plant material. *A. moluccana* was collected in Tijucas, southern Brazil and a voucher specimen deposited at the Barbosa Rodrigues Herbarium, Itajaí (V.C. Filho, 001). Dried leaves (600 g) were cut into small pieces and the methanol extract was obtained by extraction with methanol, after maceration, at room temperature for 10 days. The extract was then concentrated (reduced pressure) to the desired volume and stored at −20 °C. The resulting extract was dissolved in 0.9% NaCl solution to the desired concentration just before use.

Animals. The experimental animals used in these studies were adult male Wistar rats (250–300 g, 70-days old) maintained under a 12 h/12 h light/dark cycle at an ambient temperature of 24° ± 2 °C with free access to standard commercial food and tap water *ad libitum*. After completing 1 week of acclimatization, the rats were divided into groups of six animals and used in the experiments described below. The animals were allowed free access to both food and drinking water throughout the experimental periods. The water and food consumption were measured each day.

Biochemical analysis. At the end of each experiment, the rats were anaesthetized with diethylether, and blood samples were collected by ocular puncture and centrifuged at 3000 rpm, 4 °C for 10 min. Serum samples were

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Table 1. Effect of the methanol extract of *A. moluccana* on serum lipid level in diet-induced hyperlipaemic rats

Parameter	Group I		Group II		Group III		Group IV	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
Total cholesterol[a]	88.09 ± 3.24[b]	74.68 ± 4.37[b]	192.06 ± 6.67	167.87 ± 3.63	77.64 ± 3.27	84.12 ± 6.58	72.14 ± 4.75	78.12 ± 5.68
HDL cholesterol[a]	87.60 ± 1.76	101.48 ± 4.55[b]	78.90 ± 2.23	81.56 ± 2.10	52.43 ± 0.99	45.24 ± 1.27	64.02 ± 0.72[c]	60.24 ± 0.99[c]
LDL cholesterol[a]	55.45 ± 1.21[b]	61.96 ± 3.15[b]	83.30 ± 2.00	113.76 ± 1.94	39.66 ± 1.25	34.80 ± 1.63	37.43 ± 1.23	34.80 ± 1.72
Triglyceride[a]	123.30 ± 4.15[b]	109.52 ± 6.76[b]	214.36 ± 6.63	231.83 ± 5.92	90.47 ± 4.16	93.45 ± 2.67	92.11 ± 3.89	94.43 ± 2.97

[a] mg/dL serum. Each value is the mean ± SEM for six animals.
[b] Significance of differences with respect to the hyperlipaemic diet group.
[c] Significance of differences with respect to the standard diet group evaluated by ANOVA and Tukey-Kramer multiple comparison tests ($p < 0.01$).

Table 2. Effect of the methanol extract of *A. moluccana* on serum lipid levels in Triton-induced hyperlipaemic rats (screening test, first-phase test and second-phase test)

	Control		Triton treated		Triton and *A. moluccana* treated	
Parameter	8 h	28 h	8 h	28 h	8 h	28 h
Total cholesterol[a]	69.06 ± 3.51	71.72 ± 2.91	190.69 ± 3.10	74.01 ± 1.41	74.01 ± 1.41[b]	59.00 ± 1.63
HDL cholesterol[a]	47.36 ± 3.29	48.65 ± 2.34	36.57 ± 0.54	35.35 ± 0.64	58.90 ± 2.23[b]	35.24 ± 1.27
LDL cholesterol[a]	25.97 ± 3.06	23.12 ± 2.34	40.80 ± 2.67	17.88 ± 0.29	23.14 ± 0.67[b]	22.96 ± 2.55
Triglycerides[a]	80.47 ± 4.16	83.65 ± 3.76	114.24 ± 5.97	94.24 ± 5.98	81.00 ± 1.95[b]	81.00 ± 1.95

[a] mg/dL serum.
[b] Values are mean ± SEM from six animals. Significant differences between the Triton plus *A. moluccana*-treated group and the Triton-alone group were evaluated by ANOVA and Tukey-Kramer multiple comparison tests, ($p < 0.01$).

stored at −20 °C until biochemical analysis. Total cholesterol, HDL-cholesterol, triglycerides and glucose were measured by colorimetric and enzymatic methods (Labtest kit Diagnostica) for human use and the results checked with the standard value proposed by Birchard and Sherding (1998). Low-density lipoprotein cholesterol (LDL) was estimated by the Friedewald formula (Friedewald *et al.*, 1972).

Diet-induced hypercholesterolaemia in rats. After the adaptation period, the rats were divided into four groups of six animals each so that the groups had a similar body weight distribution and plasma triglyceride concentration. Group I and II animals were maintained on a hyperlipaemic diet (cholesterol 1%, sodium cholate 2%, vitamin mixture 2%, oligoelements 0.2%, salt mixture 5.8%, com oil 20%, cellulose 4%, sucrose 44%, casein 5%, protein 15%) whereas groups III and IV were kept for 30 days on a standard commercial diet. Groups I and IV were kept fed with high-fat or standard diet plus MEAM (300 mg/kg, b.w.) administered by oral gavage for the same period. Blood samples for assays were collected on days 15 and 30 of treatment.

Triton-induced hypercholesterolemia in rats. To investigate the short-term effects on Triton-induced hyperlipaemic rats, heavy rats (290 ± 25 g) were used. The modified Garattini *et al.* test (1961), applicable to the first and second phases of Triton-induced hyperlipaemia and designed to elucidate the mechanism of action of hypolipaemic drugs, was carried out. For the first-phase test, corn oil or MEAM (300 mg/kg, b.w.) was administered at the same time as Triton WR-1339 (200 mg/kg dissolved in 0.9% NaCl solution, i.p.), and total cholesterol, HDL-cholesterol and triglyceride levels were measured 8 h later. For the second-phase test the extract was given 20 h after Triton administration, blood samples were collected 8 h later, and lipid analysis was performed.

Statistical analysis. The statistical significance of the data was analysed using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. Values corresponding to $p < 0.01$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The general effect of the methanol extract of *Aleuritis moluccana* (MEAM) on serum lipid levels was examined in rats treated with Triton or high-fat-diet-induced hyperlipaemia. As a first step, serum lipid levels were evaluated in normally fed rats. There was no significant reduction in the serum levels of total, LDL- or HDL-cholesterol at the doses tested. Then, the extract was assayed in induced-hyperlipaemic rats. The results obtained under our experimental conditions showed that *A. moluccana* influenced lipid metabolism.

A high-fat diet administered for 30 days increased serum cholesterol and triglyceride levels. This effect was reversed by simultaneous administration of *A. moluccana* (Table 1). After 15 days of the treatment there was a significant reduction in the levels of cholesterol, triglycerides and LDL-cholesterol (54%, 43% and 34%, respectively). After 30 days the plant extract had more marked effects on LDL-cholesterol levels with 46% reduction. Also, in all the experiments with MEAM, the HDL-cholesterol increased significantly compared with the hyperlipaemic diet or standard diet experiments. The induction of HDL-cholesterol by the therapeutic treatment of hypercholesterolaemia is generally considered to be of benefit because an inverse correlation between HDL-cholesterol level and coronary heart disease has been shown to exist (Rader, 1999).

The results clearly demonstrate that the active principles contained in the methanol extract have a significant hypocholesterolaemic activity and provide considerable protection against insurgence of high-fat-diet induced hyperlipaemia. These effects could be due to several mechanisms involving inhibition of intestinal absorption of lipids or cholesterol synthesis, enhancement of cholesterol degradation, or interference with lipoprotein distribution, and it is important that they should be analysed.

Thus, in order to understand the biochemical mechanism of the hypocholesterolaemic action of this extract, a second hyperlipaemic experimental model was used: the Triton assay. The systemic administration of Triton results in a rise in plasma cholesterol and triglyceride levels (Schurr *et al.*, 1972). This hypercholesterolaemia is biphasic. Initially, there is an increase in hepatic anabolism of cholesterol (first-phase), and in the following 28 h a fall-off in this sterol by catabolism occurs (second-phase). In our assays, the administration of Triton caused a marked increase in the serum levels of cholesterol and triglycerides. After treatment with MEAM, a significant reversal was noticed in the total cholesterol and triglycerides in the screening test. The effects of the crude extract were marked in the first-phase

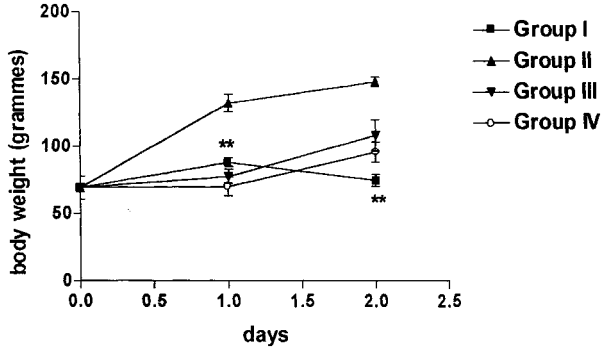

Figure 1. Effect of daily oral administration of *A. moluccana* extract plus diet hyperlipaemic (group I), hyperlipaemic diet (group II), standard diet (group III) and standard diet plus *A. moluccana* (group IV) on body weight in rats. All values are expressed as mean ± SEM, $n = 6$. (**) $p < 0.01$ compared with group II and III, ANOVA and Tukey-Kramer tests.

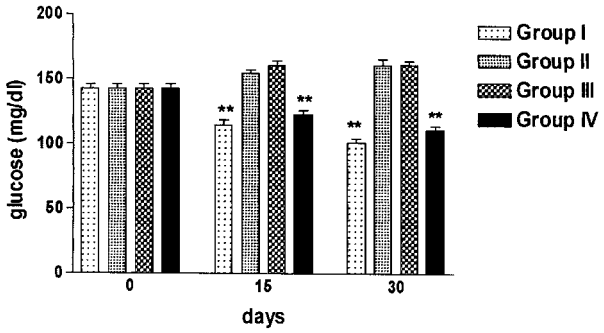

Figure 2. Glucose serum level in rats treated for 30 days with *A. moluccana* extract plus diet hyperlipaemic (group I), hyperlipaemic diet (group II), standard diet (group III) and standard diet plus *A. moluccana* (group IV). All values are expressed as mean ± SEM, $n = 6$. (**) $p < 0.01$ compared with group II and III, ANOVA and Tukey-Kramer tests.

of the Garattini test (8 h. There was a 61% reduction in total cholesterol and 30% in triglycerides (Table 2). However, there was no significant reduction in total cholesterol and triglycerides in the second phase of the screening (28 h). Since hepatic cholesterol synthesis is evaluated in the anabolic phase of Triton-induced hyperlipaemia and the catabolic phase was unchanged, the observed lipid-lowering effect of MEAM in these experiments may be at least partly attributed to the inhibition of hepatic cholesterol synthesis. We also suppose that the enhanced cholesterol excretion in the form of bile acids or other sterols was not affected.

Administration of MEAM caused a significant decrease in the body weight, without a reduction in food consumption in the rats (Fig. 1) and showed hypoglycaemic activity (Fig. 2). Therefore, it is suggested that this extract might also interfere with the nutrient absorption processes from the intestine. If this is the case, it could result in an inhibition of intestinal lipid resorption as an additional mechanism of cholesterol reduction.

The marked HDL-cholesterol increase and LDL-cholesterol decrease suggest an interference in cholesterol lipoprotein distribution with major uptake of this sterol in HDL and perhaps an up-regulation of LDL hepatic receptors which catabolize LDL (Krause and Hartman, 1984). It is then possible that active principles contained in MEAM, such as flavonoids, triterpenes and phytosterols, interfere simultaneously with various biochemical pathway lipids (Yugaran *et al.*, 1992; Ntanios and Jones, 1998).

In conclusion, our results indicate that *A. moluccana* has an anticholesterolaemic effect and suggest that the mechanism of action is constituted by an inhibition of hepatic biosynthesis plus a reduction of intestine lipid absorption and HDL uptake. The observed properties apparently validate the folk medicinal use of this plant, a fact that encourages the investigation of the phytochemical composition of the different extracts and fractions of *A. moluccana* in order to isolate the bioactive compounds and to elucidate the possible biochemical mechanisms of its antilipaemic action.

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