JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 535–539

Received July 20, 2000
Accepted October 18, 2000
ISSN 0022-3573

The lipid-lowering effect of *trans*-dehydrocrotonin, a clerodane diterpene from *Croton cajucara* Benth. in mice fed on high-fat diet

Regilane M. Silva, Flávia A. Santos, Vietla S. N. Rao, Maria A. M. Maciel and Angelo C. Pinto

Abstract

The clerodane diterpene *trans*-dehydrocrotonin extracted and isolated from the stem bark of *Croton cajucara* Benth. was investigated for its lipid-lowering effect in mice fed on a high-fat diet. Mice fed on a high-fat diet for a two-week period demonstrated significantly increased blood levels of total cholesterol and triglycerides, compared with normal controls. Oral treatment with *trans*-dehydrocrotonin at a dose of 25 or 50 mg kg^{-1} daily markedly suppressed the high-fat-diet associated rise in total cholesterol and triglyceride levels. The hypocholesterolaemic effect of *trans*-dehydrocrotonin was more prominent at the dose of 50 mg kg^{-1} with significant decreases in high-density lipoprotein, very-low-density lipoprotein and low-density lipoprotein cholesterol levels. The lower atherogenic index of the *trans*-dehydrocrotonin-treated groups suggests the hypolipidaemic potential of this plant-based drug. These results indicate that orally administered *trans*-dehydrocrotonin is effective in suppressing high-fat-diet-induced hyperlipidaemia in mice and suggest its likely beneficial use as anti-atherogenic agent.

Department of Physiology and Pharmacology, Faculty of Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil

Regilane M. Silva, Flávia A. Santos, Vietla S. N. Rao

Department of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Maria A. M. Maciel, Angelo C. Pinto

Correspondence: V. S. N. Rao, Departamento de Fisiologia e Farmacologia, Faculdade de Medicina, Rua Cel. Nunes de Melo, 1127, Caixa Postal 3157, 60430–270, Fortaleza, CE, Brasil.

Funding: We thank Conselho Nacional de Desenvolvimento Ciêntifico e Tecnológico for the Research Grant (300108/86-9) and a Research Fellowship to R. M. Silva.

Introduction

Abnormalities in blood lipids and hypercholesterolaemia are well established as major risk factors for coronary heart disease and atherogenesis (Ross 1993; Superko 1996). Most lipid-lowering drugs currently used in the treatment of hyperlipidaemic patients can modify plasma high-density lipoprotein (HDL)-cholesterol and subfraction distribution (Sirtori & Franceschini 1990) and these drug-induced changes in the HDL system have been associated with a reduction in cardiovascular risk (Manninen et al 1992). Many traditional plants are claimed to be useful in the control of hyperlipidaemia and associated pathologies (Choi et al 1991a; La Cour et al 1995; Craig 1999; Wang & Ng 1999). One of these plants, *Croton cajucara* Benth. (Euphorbiaceae), commonly known as "Sacaca" in many parts of Brazil, is a traditional remedy for the treatment of diabetes, hypertension, and high blood cholesterol (Di Stasi et al 1989). Several clerodane diterpenes have been isolated from the bark extracts of this plant which include *trans*-dehydrocrotonin, *trans*-crotonin, *cis*-cajucarina B, cajucarina A and sacacarina (Maciel et al 1998). In recent years, the bioactivity of the principal constituent, *trans*-dehydrocrotonin (Figure 1) has been extensively studied and it has been found to possess anti-inflammatory and anti-nociceptive (Carvalho et al 1996), anti-ulcer (Brito et al 1998),

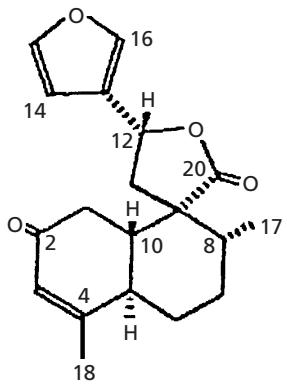

Figure 1 Molecular structure of *trans*-dehydrocrotonin, a 19-nor clerodane diterpene.

anti-tumour (Grynberg et al 1999) and anti-oestrogenic (Luna Costa et al 1999) properties. Further investigations confirmed that *trans*-dehydrocrotonin is neither genotoxic nor cytotoxic to mouse bone-marrow cells (Agner et al 1999). Our previous studies have demonstrated significant hypolipidaemic activity in the crude ethanolic extract of sacaca in rats fed a high-fat diet (Farias et al 1996) and a hypoglycaemic effect of *trans*-dehydrocrotonin in alloxan-induced diabetic rats (Farias et al 1997). This study extends our previous investigation to verify whether *trans*-dehydrocrotonin, the major diterpene present in *Croton cajucara* bark, can exert a hypolipidaemic effect in mice fed on a high-fat diet.

Materials and Methods

Animals

Mature male Swiss mice, 25–30 g, were used. The mice were housed in polypropylene cages at an ambient temperature of 22–24°C with a 12-h light-dark cycle. They were fed a standard pelletted diet (purina chow, Brazil) and were given free access to water. The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Federal University of Ceará, Fortaleza in accordance with internationally accepted principles.

Isolation of *trans*-dehydrocrotonin

trans-Dehydrocrotonin was extracted and isolated as described previously (Maciel et al 1998) from the bark of *Croton cajucara* Benth. collected from Jacunda-PA (Amazón region, Brazil) after its authentication by Dr Nelson A. Rosa, Museum Paraense Emilio Goeldi (voucher specimen No. 247). The isolated material was characterized by spectroscopy (IR, UV, MS and ^{1}H and ^{13}C NMR) as recently described (Maciel et al 1998).

Treatment of animals

Thirty-two male mice were divided at random into four groups of eight. The first group served as normal controls and received normal pelletted feed and free access to water. The mice in the other groups received, in addition to pelletted diet and water, a hyperlipidaemic diet which was a combination of sunflower oil (10 mL kg^{-1}), 5 % cholesterol and 0.5 % cholic acid daily by oral gavage (Arichi et al 1982) for two weeks.

trans-Dehydrocrotonin was administered orally once daily to mice in groups three and four at a dose of 25 or 50 mg kg^{-1}, respectively, 4 h after feeding. The body weights of animals were recorded initially, and also at the end of the second week. *trans*-Dehydrocrotonin was dissolved in dimethyl sulfoxide and further dilutions were made in distilled water. The final concentration of the solvent did not exceed 5 %. Control animals (second group) received the same volume of vehicle that contained no *trans*-dehydrocrotonin.

Analysis of serum lipids

At the end of two weeks, following an overnight fast, blood samples from the peri-orbital sinus were collected under ether anaesthesia in ice-chilled tubes, and allowed to clot before serum was obtained by subsequent centrifugation at 2500 *g* for 20 min. The separated serum was stored at −20°C before analysis of lipids. Serum total cholesterol (TC), HDL-cholesterol (HDL-c) and triglyceride (TG) were analysed by the colorimetric method in a semi-automatic analyser (RA-50, Bayer, Brazil) using diagnostic kits (Labtest, Brazil). The serum low-density lipoprotein-cholesterol (LDL-c) concentration was calculated using the Friedwald formula (Friedwald et al 1972), where LDL-c = TC − (HDL-c + VLDL-c) and VLDL-c = TG/5 (VLDL: very-low-density lipoprotein). The atherogenic index (AI) was expressed as LDL-c + VLDL-c/HDL-c.

Statistical analysis

Data are expressed as mean ± s.e.m for eight animals. Data on lipid parameters were analysed by analysis of variance. Values of $P < 0.05$ were considered to be statistically significant.

Results

Serum lipids

The serum lipid profiles of mice after a 2-weeks normal or high-fat diet are shown in Table 1. The normal group of mice fed on normal chow diet had serum total cholesterol and triglyceride levels of 122.21 ± 10.35 and 82.17 ± 4.80 mg dL^{-1}, respectively as against 197.75 ± 1.86 and 342.48 ± 13.76 mg dL^{-1}, respectively, in mice on the high-fat diet. Orally administered *trans*-dehydrocrotonin significantly ($P < 0.05$) decreased the high-fat-diet-induced increases in total cholesterol and triglyceride levels, respectively, by 20 and 30 % at a dose of 25 mg kg^{-1} and by 50 and 51 % at a dose of 50 mg kg^{-1}. The effect of *trans*-dehydrocrotonin on total cholesterol appeared to be dose-related but the effect on triglyceride did not. The cholesterol fractions HDL, LDL and VLDL were significantly increased by a high-fat diet. *trans*-Dehydrocrotonin had a significant inhibitory effect on these atherogenic lipoproteins, more so at a dose of 50 mg kg^{-1}. Although the level of HDL-c tended to decrease at this dose, the serum HDL-c level remained similar to that of control rats. *trans*-Dehydrocrotonin significantly lowered the atherogenic index at both doses employed.

Body weight

The initial mean body weight of the control group of mice fed on normal diet and that of the high-fat diet group that received vehicle were in the order of 30.50 ± 0.50 g and 25.75 ± 0.56 g, respectively. At the end of the experimental period, an increase of 30 % in mean body weight was observed in the high-fat-fed control group of animals (Figure 2). This increase in mean body weight was not observed in the groups of animals treated with 25 or 50 mg kg^{-1} of *trans*-dehydrocrotonin.

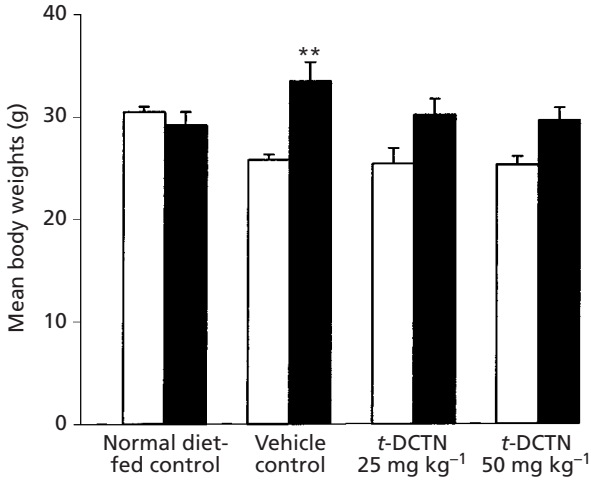

Figure 2 Initial (□) and final (■) mean body-weight of mice fed on normal pelletted-diet or high-fat diet with or without *trans*-dehydrocrotonin (*t*-DCTN) treatment (25 or 50 mg kg^{-1} daily). Body weights were recorded at the beginning and at the end of experiment period (2 weeks). Each column represents the mean ± s.e.m., n = 8. $^{**}P < 0.01$, compared with initial body weight.

Discussion

In this study, *trans*-dehydrocrotonin, a clerodane diterpene isolated from the stem bark of *Croton cajucara* Benth. markedly suppressed the high-fat-diet-associated

Table 1 Serum lipid parameters in mice fed or normal or high-fat diet and the effects of *trans*-dehydrocrotin treatment.

Lipid parameter	Normal diet (control)	High-fat diet and treated		
		Vehicle control	*trans*-Dehydrocrotin 25 mg kg^{-1} daily	*trans*-Dehydrocrotin 50 mg kg^{-1} daily
Total cholesterol (mg dL^{-1})	122.21 ± 10.35	197.75 ± 12.86*	158.94 ± 7.64†	120.87 ± 6.40†
Triglyceride (mg dL^{-1})	82.17 ± 4.80	342.48 ± 13.76*	171.33 ± 18.16†	166.80 ± 13.16†
HDL-c (mg dL^{-1})	43.83 ± 4.75	58.38 ± 1.75*	61.40 ± 4.96	47.24 ± 1.56†
VLDL-c (mg dL^{-1})	16.42 ± 0.97	68.50 ± 2.75*	34.26 ± 3.63†	33.34 ± 2.63†
LDL-c (mg dL^{-1})	52.32 ± 6.60	72.53 ± 9.13*	70.36 ± 7.68	43.50 ± 4.38†
Atherogenic index (AI)	1.88 ± 0.25	2.38 ± 0.18*	1.50 ± 0.14†	1.52 ± 0.10†

Serum lipids were measured enzymatically two weeks after feeding mice a normal pelletted diet or high-fat diet with or without *trans*-dehydrocrotin treatment. Each value represents the mean ± s.e.m., n = 8. The atherogenic index (AI) was expressed as LDL-c/HDL-c. $^{*}P < 0.05$, compared with normal diet-fed control value. †$P < 0.05$, compared with vehicle control value.

rise in total cholesterol and triglyceride levels in mice at oral doses of 25 or 50 mg kg^{-1} daily. Most lipid-lowering drugs currently used in the treatment of hyperlipidaemic patients can modify plasma HDL-c levels and subfraction distribution (Sirtori & Franceshini 1990). The effect of *trans*-dehydrocrotonin was therefore analysed on cholesterol subfractions. The hypocholesterolaemic effect of *trans*-dehydrocrotonin was more prominent at the dose of 50 mg kg^{-1} with significant decreases in HDL-, VLDL- and LDL-c levels. Although the level of HDL-c tended to decrease at this dose, its level remained similar to that seen in the control group. Our study is the first to demonstrate that *trans*-dehydrocrotonin can affect the serum lipid parameters of diet-induced hyperlipidaemia in mice. Our data also show that the AI was significantly lowered in the *trans*-dehydrocrotonin-treated group of mice compared with the vehicle-treated control group. A decrease in AI is believed to be beneficial, since the HDL level is inversely correlated with coronary heart disease and its elevation is considered to be an anti-atherosclerotic factor (Marx 1979; Choi et al 1991b; Manninen et al 1992). The lower AI of the *trans*-dehydrocrotonin-treated groups suggested the hypolipidaemic potential of this plant-based drug.

It is interesting that *trans*-dehydrocrotonin markedly lowered (50%) the levels of serum triglycerides and VLDL-c. The decrease in triglycerides may indicate increased oxidation of mobilized fatty acids or inhibition of lipolysis. Nicotinic acid and fibric acids, widely used for the treatment of various forms of hyperlipidaemia (Fattore & Sirtori 1991), similarly significantly reduce serum triglycerides and VLDL-c. Disturbance of lipid metabolism is also a common complication of diabetes mellitus. Thus, increased mobilization of triglycerides and release of unesterified fatty acids from adipose tissue into the blood stream is common, especially in type II diabetes (Kunjathoor et al 1996). The hypolipidaemic action of *trans*-dehydrocrotonin is advantageous since it lowers blood glucose in diabetic rats (Farias et al 1997). The decreased free fatty acid levels brought about by *trans*-dehydrocrotonin treatment may suggest the inhibition of lipolysis which is further indicated by the maintenance of body weight.

Different species of *Croton* are noted for their tumourogenic and pro-inflammatory effects due to their phorbol ester diterpene content (Schmidt 1986). Interestingly, *C. cajucara* presents only clerodane diterpenes that possess anti-tumour and anti-inflammatory effects (Ichihara et al 1992; Carvalho et al 1996; Grynberg et al 1999). The established lethal dose (oral LD50) in mice is 555 mg kg^{-1} (Carvalho et al 1996), 10–20 times the doses employed in this study. Thus, the absence of phorbol ester activity and overt toxicity is advantageous in its therapeutic utility as a hypercholesterolaemic agent.

In conclusion, the data indicate that orally administered *trans*-dehydrocrotonin is effective in suppressing high-fat-diet-induced hyperlipidaemia in mice and suggest its likely beneficial use as anti-atherogenic agent.

References

Agner, A. R., Maciel, M. A. M., Pinto, A. C., Pamplona S. G. S. R., Cólus, I. M. S. (1999) Investigation of genotoxic activity of *trans*-dehydrocrotonin, a clerodane diterpene from *Croton cajucara*. *Teratog. Carcinog. Mutagen.* **19**: 377–384

Arichi, H., Kimura, Y., Okuda, H., Baba, K., Kozawa, M., Arichi, S. (1982) Effects of stilbene components of the roots of *Polygonum cuspidatum* Sieb.et Zucc. on lipid metabolism. *Chem. Pharm. Bull.* **30**: 1766–1770

Brito, A. R., Rodriguez, J. A., Hiruma-Lima, C. A., Haun, M., Nunes D. S. (1998) Antiulcerogenic activity of *trans*-dehydrocrotonin from *Croton cajucara*. *Planta Med.* **64**: 126–129

Carvalho, J. C., Silva, M. F., Maciel, M. A. M., Pinto, A . C., Nunes, D. S., Lima, R. M., Bastos, J. K., Sarti, S. J. (1996) Investigation of anti-inflammatory and antinociceptive activities of *trans*-dehydrocrotonin, a 19-*nor*-clerodane diterpene from *Croton cajucara*. Part 1. *Planta Med.* **62**: 402–404

Choi, J. S., Suh, S. S., Young, H. S., Park, H. J. (1991a) Hypolipemic and hypoglycemic activities of *Prunus davidiana* in high fat-fed rats. *Arch. Pharm. Res.* **14**: 44–47

Choi, J. S., Yokozawa, T., Oura, H. (1991b) Antihyperlipidaemic effect of flavonoids from *Prunus davidiana*. *J. Nat. Prod.* **54**: 218–224

Craig, W. J. (1999) Health promoting properties of common herbs. *Am. J. Clin. Nutr.* **70** (Suppl 3): 491S–499S

Di Stasi, L. C., Santos, E. M. G., Santos, C. M., Hiruima, C. A. (1989) *Plantas Medicinais da Amazônia*. Ed UNESP, São Paulo, pp 127–128

Farias, R. A. F., Neto, M. F. O., Viana, G. S. B., Rao, V. S. N. (1996) Effects of *Croton cajucarae* extract on serum lipids of rats fed a high fat diet. *Phytother. Res.* **10**: 697–699

Farias, R. A. F., Rao, V. S. N., Viana, G. S. B., Silveira, E. R., Maciel, M. A. M., Pinto, A. C. (1997) Hypoglycemic effect of *trans*-dehydrocrotonin, a *nor*-clerodane diterpene from *Croton cajucara*. *Planta Med.* **66**: 558–560

Fattore, P. C., Sirtori, C. R. (1991) Nicotinic acid and derivatives. *Curr. Opin. Lipidol.* **2**: 43–47

Friedwald, W. T., Levy, R. I., Fredrickson, D. S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin. Chem.* **18**: 499–502

Grynberg, N. F., Echavarria, A., Lima, J. E., Pamplona, S. S., Pinto A. C., Maciel M. A. (1999) Antitumor activity of two 19-*nor*-clerodane diterpenes, *trans*-dehydrocrotonin and *trans*-crotonin, from *Croton cajucara*. *Planta Med.* **65**: 687–699

Ichihara, Y., Takeya, K., Hitotsuyanagi, Y., Morita, H., Okuyama, S., Suganuma, M., Fujiki, H., Motidome, M., Itokawa, H. (1992) Cajucarinolide and isocajucarinolide: anti-inflammatory diterpenes from *Croton cajucara*. *Planta Med.* **58**: 549–551

Kunjathoor, V. V., Wilson, D. L., LeBoeuf, R. C. (1996) Increased atherosclerosis in streptozotocin-induced diabetic mice. *J. Clin. Invest*. **97**: 1767–1773

La Cour, B., Molgaard, P., Yi, Z. (1995) Traditional Chinese Medicine in treatment of hyperlipidaemia. *J. Ethnopharmacol*. **46**: 125–129

Luna Costa, A. M., Silva, J. C., Campos, A. R., Rao, V. S., Maciel, M. A., Pinto, A. C. (1999) Antioestrogenic effect of *trans*-dehydrocrotonin, a *nor*-clerodane diterpene from *Croton cajucara* Benth. in rats. *Phytother. Res*. **13**: 689–691

Maciel, M. A. M., Pinto, A. C., Brabo, S. N., Silva, M. N. (1998) Terpenoids from *Croton cajucara*. *Phytochemistry* **49**: 823–828

Manninen, V., Tenkanen, L., Koskinen, P., Huttunen, J. K., Mänttäri, M., Heinonen, O. P., Frick, M. H. (1992) Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentration on coronary heart disease risk in the Helsinki heart study. *Circulation* **85**: 37–45

Marx, J. L. (1979) The HDL: the good cholesterol carriers? *Science* **205**: 677–679

Ross, R. (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* **362**: 801–809

Schmidt, R. J. (1986) Biosynthetic and chemosystematic aspects of the Euphorbiaceae and Thymelaeaceae. In: Evans, F. J. (ed.) *Naturally Occurring Phorbol Esters*. CRC Press, Boca Raton, FL, pp 87–106

Sirtori, C. R., Franceschini, G. (1990) Pharmacological modifications of high-density lipoproteins. In: Carlson, L. A. (ed.) *Disorders of HDL*. Smith-Gordan Co., London. pp 197–202

Superko, H. R. (1996) Lipid disorders contributing to coronary heart disease. An update. *Curr. Probl. Cardiol*. **21**: 736–780

Wang, H. X., Ng, T. B. (1999) Natural products with hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. *Life Sci*. **65**: 2663–2677