

## Effect of an Aqueous Extract of Annatto (*Bixa orellana*) Seeds on Lipid Profile and Biochemical Markers of Renal and Hepatic Function in Hipercholesterolemic Rats

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### ABSTRACT

*Annatto extract is a natural food color obtained from the outer coatings of the seeds of the Annatto tree (Bixa orellana L.). This is the first report in the literature that shows the relationship between the aqueous annatto extract and its influence on lipid profile in animals. Male Fisher rats were divided into three groups (n=12): C group, fed standard diet and water; H group, fed high-lipid diet and water and; HU group, with high-lipid diet and aqueous annatto extract for 60 days. The treatment with annatto extract in animals fed with the high-lipid diet lowered the LDL- and total cholesterol and raised the HDL-cholesterol, suggesting a hypocholesterolemic effect. Neither high-fat diet nor aqueous annatto extract had any significant effect on serum levels of albumin or serum activities of transaminases which suggested that no liver injury was induced.*

**Key words:** Annatto, *Bixa orellana*, cholesterol, rat, aqueous extract, lipid profile

### INTRODUCTION

Annatto extract is a natural food color, which is obtained from the outer coatings of the seeds of the Annatto tree (*Bixa orellana* L.) (Hagiwara et al., 2003). The use of annatto by the New World man dates back to ancient times. The indigenous Amazon people have used "urucum" for body painting for centuries. Seeds and leaves of the annatto tree were used by the Aztecs to prepare remedies for a variety of illnesses such as tonsillitis, asthma, pleurisy, rectal disorders, headache, jaundice, sunstroke, and burns (De Oliveira et al., 2003). Although the plant species originated in northern South America, it is now

widely cultivated in tropical areas for commercial production (Hagiwara et al., 2003). In 2003, the average annual production of annatto seeds was 10,000 tons. Two third of this are traded as seeds and the rest as seed extracts. Latin America produces 60% of the world's annatto, followed by Africa (27%) and Asia (12%). Annatto is used primarily for coloring cheese (50%) and butter, other products like margarine, snacks, etc, and body care products (Giuliano et al., 2003). Annatto was classified by the Food and Drug Administration of the U.S.A. as a "color additive exempt of certification" (Hallagan et al., 1995). Lifetime toxicity studies revealed that annatto did not produce toxic effects in rats or mice if

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administered orally (26 mg/day for rats, and one drop of 10% annatto in soya oil/day for mice). It has been considered safe for human consumption. Indeed it has been reported that annatto is not carcinogenic (Engelbreth-Holm and Iversen, 1955), neither maternally toxic nor embryotoxic (Paumgartten et al., 2002). Shilpi et al. (2006) showed no lethal effects within 24h after the administration of annatto extract, even at the highest dose tested (4,000 mg/kg) for mice. Approximately 80% of the pigments present in annatto seeds correspond to bixin (Preston and Rickard, 1980) but several other minor carotenoids have been isolated and identified, such as norbixin (Mercadante et al., 1997b; Mercadante et al., 1997a; Mercadante et al., 1999). There are not much literature data about annatto effects over cholesterolemia and atherosclerosis, but Silva et al. (2001) showed that bixin inhibited lipid peroxidation induced by cisplatin, suggesting that annatto infusion, might have effect on factors that influenced the development of cardiovascular disease.

The present study was undertaken to investigate the lowering effect on lipid profile of the aqueous extract of seeds of *Bixa orellana*.

## MATERIAL AND METHODS

*Bixa orellana* seeds were collected in October 2005 at Ouro Preto (MG–Brazil). A voucher specimen (No. OUPR 20097) was identified and deposited in the Professor José Badini Herbarium of the Federal University of Ouro Preto, Brazil.

To prepare the aqueous extract, *Bixa orellana* seeds (8 g) were soaked overnight in 1,000 mL of water and the obtained extract was stored at 2-8°C until being used.

Thirty-six Fisher male rats, weighing about 160 g were used. They were housed under standard conditions of temperature, humidity and dark–light cycle. Diet and water or the annatto extract were available *ad libitum*. The HU group received the extract instead of water. All the animals were carefully monitored and maintained in accordance with the ethical recommendation of Cardoso (2002) and the Canadian Council on Animal Care (1984). They were divided into three groups (n = 12): C group, fed standard diet and water; H group, fed high-lipid diet (1% cholesterol) and water and; HU group, fed with high-lipid diet (1% cholesterol) and aqueous annatto extract.

The standard diet contained (g/kg) casein (Isofar, Duque de Caxias, Brazil), 120; salt mixture, 50; vitamin mixture, 10; soybean oil, 80 (Sadia, São Paulo, Brazil); cellulose 10 (Merck, Darmstadt, Germany); cornstarch, 730.0 (Unilever Bestfoods, Mogi-Guaçu, Brazil). Total energy was 17.18 kJ/kg. The high-lipid diet was the same, except for soybean oil, 250; cornstarch, 546; cholesterol, 10, with total energy 20.57 kJ/g (Matos et al., 2005).

After 60 days, animals were fasted overnight and killed by decapitation. Blood samples were collected from brachial plexus, immediately centrifuged and assayed. Liver and abdominal fat were removed and weighed. Total cholesterol, HDL-cholesterol, triacylglycerol, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, uric acid, glucose and iron were assayed with colorimetric or enzymatic methods using kits (Labtest Diagnóstica, Belo Horizonte, Brazil). The VLDL- and LDL-cholesterol concentrations were calculated using the Friedewald equation: LDL-C = total cholesterol – (HDL-C + VLDL-C), and VLDL-C = triglycerides/2.175.

All results values are expressed as means ± SD. Data were tested by one-way ANOVA. When  $p < 0.05$ , Tukey's test was done to determine the specific differences between the means. A difference of  $P < 0.05$  was considered significant.

## RESULTS

Animals that received the hypercholesterolemic diet had a higher body weight at the end of the experiment. Group H showed lower weight of abdominal fat as compared to group C, but this difference disappeared when the value was divided by that of body weight. Group HU presented a higher liver weight as compared to group C and this difference was maintained when this value is related to that of body weight (LW/BW). The same pattern was found in group H for the LW/BW ratio, but not for LW itself (Table 1).

No differences were found for serum levels of triacylglycerol and VLDL-cholesterol. In relation to C, H animals had higher total and LDL-cholesterol but lower HDL-cholesterol. When comparing with H, we observed that HU reduced the serum levels of total and LDL-cholesterol and increased those of HDL-cholesterol (Table 2).

No differences were found in both serum transaminases: neither in alanine- and aspartate-

aminotransferases activities, nor in both serum levels of urea and uric acid. The animals fed a high-lipid diet (H and HU groups) had reduced serum levels of total proteins, but not serum albumin when comparing with C group. The animals fed with a high-lipid diet (H and HU

groups) showed increased activity of alkaline phosphatase when compared with C group. This increase was also observed for the plasma levels of glucose. No differences were found for serum levels of iron (Table 3).

**Table 1** - Body weight (BW), adipose tissue weight (ATW) from abdominal fat, liver weight (LW), liver- and body-weight ratio, and adipose tissue- and body-weight ratio in rats fed standard (C) or high-lipid (H) diets plus water or aqueous extract of annatto seeds (HU) after 60 days<sup>1</sup>

	C	H	HU
BW (g)	222.62 ± 20.56 <sup>a</sup>	173.12 ± 15.38 <sup>b</sup>	187.06 ± 17.95 <sup>b</sup>
ATW (g)	5.61 ± 1.74 <sup>a</sup>	3.31 ± 0.70 <sup>b</sup>	4.77 ± 0.79 <sup>a,b</sup>
LW (g)	7.01 ± 0.40 <sup>b</sup>	8.05 ± 0.89 <sup>a,b</sup>	9.78 ± 1.09 <sup>a</sup>
LW/BW (x100)	3.15 ± 0.20 <sup>a</sup>	4.65 ± 0.58 <sup>b</sup>	5.23 ± 0.77 <sup>b</sup>
ATW/BW(x100)	2.52 ± 0.68 <sup>a</sup>	1.91 ± 0.26 <sup>a</sup>	2.55 ± 0.44 <sup>a</sup>

<sup>1</sup>Data are means ± SD, *n* = 10. Means with different letters differ, *P* < 0.05 (one-way ANOVA). **C**: Rats fed standard diet and water; **H**: rats fed a high-lipid diet and water; **HU**: rats fed a high-lipid diet and aqueous extract of annatto seeds.

**Table 2** – Serum levels of cholesterol, triacylglycerol, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and VLDL-cholesterol (VLDL-C) in rats fed control (C) or high-lipid (H) diets plus water or annatto extract (HU)<sup>1</sup>

	C	H	HU
Cholesterol (mmol/L)	2.07 ± 0.25 <sup>c</sup>	9.44 ± 4.17 <sup>a</sup>	5.67 ± 2.44 <sup>b</sup>
Triacylglycerol (mmol/L)	0.91 ± 0.58 <sup>a</sup>	0.45 ± 0.25 <sup>a</sup>	0.64 ± 0.61 <sup>a</sup>
LDL-C (mmol/L)	0.23 ± 0.40 <sup>c</sup>	7.92 ± 4.95 <sup>a</sup>	4.64 ± 2.56 <sup>b</sup>
HDL-C (mmol/L)	1.43 ± 0.39 <sup>a</sup>	0.39 ± 0.20 <sup>c</sup>	0.73 ± 0.41 <sup>b</sup>
VLDL-C (mmol/L)	0.42 ± 0.27 <sup>a</sup>	0.21 ± 0.11 <sup>a</sup>	0.30 ± 0.28 <sup>a</sup>

<sup>1</sup>Data are means ± SD, *n* = 10. Means with different letters differ, *P* < 0.05 (one-way ANOVA). **C**: Rats fed standard diet and water; **H**: rats fed a high-lipid diet and water; **HU**: rats fed a high-lipid diet and aqueous annatto extract.

**Table 3** - Serum levels of total proteins, albumin, Aspartate- (AST) and Alanine Aminotransferase (ALT), Urea, Uric Acid, Alkaline Phosphatase (ALP), Glucose and Iron in rats fed control (C) or high-lipid (H) diets plus water or annatto extract (HU)<sup>1</sup>

	C	H	HU
Total Protein (g/dL)	45.50 ± 9.90 <sup>a</sup>	32.00 ± 6.70 <sup>b</sup>	27.00 ± 4.30 <sup>b</sup>
Albumin (mg/dL)	505.87 ± 60.73 <sup>a</sup>	449.28 ± 73.84 <sup>a</sup>	461.01 ± 64.87 <sup>a</sup>
AST (U/L)	80.27 ± 17.84 <sup>a</sup>	74.93 ± 15.58 <sup>a</sup>	67.62 ± 11.11 <sup>a</sup>
ALT (U/L)	17.27 ± 3.11 <sup>a</sup>	23.93 ± 8.67 <sup>a</sup>	20.61 ± 3.77 <sup>a</sup>
Urea (mmol/L)	33.52 ± 3.25 <sup>a</sup>	34.84 ± 5.81 <sup>a</sup>	34.19 ± 9.40 <sup>a</sup>
Uric Acid (µmol/L)	1.34 ± 0.46 <sup>a</sup>	1.37 ± 0.42 <sup>a</sup>	1.13 ± 0.32 <sup>a</sup>
ALP (U/L)	26.88 ± 7.72 <sup>a</sup>	41.26 ± 7.47 <sup>b</sup>	46.49 ± 5.95 <sup>b</sup>
Glucose (mmol/L)	142.13 ± 36.62 <sup>a</sup>	98.65 ± 14.50 <sup>b</sup>	104.47 ± 21.62 <sup>b</sup>
Iron (mmol/L)	174.6 ± 56.46 <sup>a</sup>	149.86 ± 36.86 <sup>a</sup>	200.29 ± 54.50 <sup>a</sup>

<sup>1</sup>Data are means ± SD, *n* = 10. Means with different letters differ, *P* < 0.05 (one-way ANOVA). **C**: Rats fed standard diet and water; **H**: rats fed a high-lipid diet and water; **HU**: rats fed a high-lipid diet and aqueous annatto extract.

## DISCUSSION

Hypercholesterolemia plays an important role in the initiation and progression of atherosclerosis and has a positive correlation with cardiovascular disease, largely depending on the oxidation of

low-density lipoprotein (LDL), the main cholesterol carrier in plasma (Hsu 2003). Many different animal models have been used to study the effects of high-cholesterol levels *in vivo* (Moghadasian 2002) and how this concentration could be reduced by chemical substances, such as

statins (Shah 2003) or plants extracts (Lans 2006). High-lipid diet (25% soybean oil and 1% cholesterol) is one among those accepted for promoting hypercholesterolemia in rats. In the present study, this diet increased the total cholesterol and LDL-cholesterol and reduced HDL-cholesterol. This was consistent with earlier reports (Gonçalves et al., 2006; Matos et al., 2005; Turbino-Ribeiro et al., 2003). The treatment with annatto extract in animals fed with the high-lipid diet lowered LDL- and total cholesterol and raised HDL-cholesterol, suggesting its hypocholesterolemic effect. HDL-cholesterol has been shown to have direct anti-atherogenic properties, while LDL-cholesterol has direct atherogenic properties, in animals (Barter et al., 2003). This is the first report in the literature showing the relationship between the aqueous annatto extract and its influence on lipid profile in animals. Haggiwara et al. (2003) worked with organic and alkaline solution extracts and did not find any effects on serum levels of total cholesterol. This suggested that the substance responsible for this hypocholesterolemic effect could be found in the aqueous fraction of annatto. Earlier reports (Engelbreth-Holm and Iversen, 1955; Paumgartten et al., 2002) showed that annatto did not produce any toxic effect, hence it was decided to evaluate the effect of an aqueous extract of annatto seeds over liver and biochemical parameters of its function and also over renal function. At the end of the experiment, the animals fed with high-lipid diet had lower body weight (BW), adipose tissue weight (ATW) and the same liver weight (LW) than those fed with the control diet. However, an increased LW/BW ratio of the animals fed with high-lipid diet was observed. The animals fed with high-lipid diet showed fatty-looking (data not shown) and relative heavier livers. Silva et al. (1999) reported that rats fed high-lipid diet had similar results for liver weight. Mattos *et al.* (2005) reported that rats fed various high-lipid diets had similar results for body weight ATW/BW ratio and relative liver weight as reported in the present work. They suggested that increased relative liver weight was due to increased levels of fat in this organ. The treatment with annatto extract in animals fed with the high-lipid diet did not alter these observations. However, De-Oliveira et al. (2003) showed that oral annatto intake induced liver cytochrome P450

monooxygenase system, which was a collection of isoenzymes which catalyze different types of oxidation reactions (Jewell and O'Brien, 1999).

Liver is the organ responsible for glucose homeostasis, thus observed hepatic injury could explain the glucose levels reduction in high-lipid fed animals. Pedersen et al. (1991) observed that Sprague-Dawley rats fed with high-fat diet also showed a reduction in glucose level. Neither high-fat diet nor aqueous annatto extract had any significant effect on serum levels of albumin or serum activities of ALT and AST. This suggested that no liver injury was found. The elevation in the activity of either alanine aminotransferase or aspartate aminotransferase in the serum is often useful as an index of liver cell damage. Furthermore, serum ALT might be a more specific index of liver cell damage than AST because of its selective concentration in the liver tissue (He and Aoyama, 2003). The high-fat diet and the aqueous annatto extract reduced the serum total proteins. Kroes et al. (2003) treated rats with a polyunsaturated fatty acid (docosahexaenoic acid) and showed that high-lipid diet increased albumin/globulin ratio.

The animals fed with high-lipid diet with or without aqueous annatto extract increased the activity of alkaline phosphatase. ALP is a sensitive marker of cholestasis, which is associated with a marked increase in the release of canalicular membrane enzymes into bile (Bdel Salam et al., 2005). The increase in alkaline phosphatase activity in several high-fat groups including the corn-oil controls is also attributed to the administration of extra fat in the diet. Similar increases in alkaline phosphatase activity have been reported previously with high-fat diets (corn oil, canola oil as well as PUFA-containing oils) (Burns et al., 1999; Hempenius et al., 1997; Kroes et al., 2003; Lina et al., 2006).

In conclusion, present data suggested that aqueous annatto extract had a hypocholesterolemic effect and more studies would be necessary to clarify which compound present in this extract was responsible for these effects.

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## RESUMO

Extrato de urucum é um corante alimentar natural que é obtido da casca das sementes do urucueiro (*Bixa orellana* L.). Este é o primeiro trabalho da literatura que mostra a relação entre o extrato aquoso de urucum e sua influência no perfil lipídico de animais. Durante 60 dias, 36 ratos machos Fisher foram divididos em 3 grupos: Grupo C que recebeu uma dieta controle e água; Grupo H que recebeu uma dieta rica em lipídios e água e; Grupo HU que recebeu uma dieta rica em lipídios e extrato aquoso de semente de urucum. O tratamento com extrato de urucum nos animais alimentados com a dieta rica em lipídios abaixou o colesterol total e a fração LDL e aumentou a fração HDL, sugerindo um efeito hipocolesterolemizante. Nem a dieta rica em lipídios, nem o extrato de urucum tiveram algum efeito sobre os níveis séricos de albumina ou sobre a atividade de alanina aminotransferase e aspartato aminotransferase. Este fato sugere que o consumo do extrato não provocou injúria hepática nos animais.

## REFERENCES

- Canadian Council on animal Care. (1984) Guide to the care and use of experimental animals. Ottawa.
- Barter P, Kastelein J, Nunn A, Hobbs R (2003) High density lipoproteins (HDLs) and atherosclerosis; the unanswered questions. *Atherosclerosis* 168:195-211
- Bdel Salam OM, Baiuomy AR, El-Shenawy SM (2005) Effect of cysteamine on bile secretion in the rat. *Pharmacol Rep* 57:345-351.
- Burns RA, Wibert GJ, ersen-Schade DA, Kelly CM (1999) Evaluation of single-cell sources of docosahexaenoic acid and arachidonic acid: 3-month rat oral safety study with an in utero phase. *Food and Chemical Toxicology* 37:23-36
- Cardoso, CVP (2002) Eutanásia. *In-Animais de laboratório – Criação e experimentação*, ed. Andrade A, Pinto SC, Oliveira RS. Editora Fiocruz, Rio de Janeiro, pp. 275-279.
- De Oliveira AC, Silva IB, Manhaes-Rocha DA, Paumgarten FJ (2003) Induction of liver monooxygenases by annatto and bixin in female rats. *Braz J Med Biol Res* 36:113-118.
- Engelbreth-Holm J, Iversen S (1955) Is vegetable annatto butter colour cancerogenic? *Acta Pathol Microbiol Scand* 37:483-491.
- Giuliano G, Rosati C, Bramley PM (2003) To dye or not to dye: biochemistry of annatto unveiled. *Trends Biotechnol* 21:513-516.
- Gonçalves AC, Oliveira EC, Pedrosa ML, Silva ME (2006) Effect of physical exercise on lipid metabolism in rats submitted to hypercholesterolemic diet. *The FIEP Bulletin* 76:186-189.
- Hagiwara A, Imai N, Ichihara T, Sano M, Tamano S, Aoki H, Yasuhara K, Koda T, Nakamura M, Shirai T (2003) A thirteen-week oral toxicity study of annatto extract (norbixin), a natural food color extracted from the seed coat of annatto (*Bixa orellana* L.), in Sprague-Dawley rats. *Food Chem Toxicol* 41:1157-1164.
- Hallagan JB, Allen DC, Borzelleca JF (1995) The safety and regulatory status of food, drug and cosmetics colour additives exempt from certification. *Food Chem Toxicol* 33:515-528.
- He G, Aoyama Y (2003) Effects of Adding Some Dietary Fibers to a Cystine Diet on the Activities of Liver Antioxidant Enzymes and Serum Enzymes in Rats. *Biosci Biotechnol Biochem* 67:617-621.
- Hempenius RA, Van Delft JMH, Prinsen M, Lina BAR (1997) Preliminary safety assessment of an arachidonic acid-enriched oil derived from *Mortierella alpina*: summary of toxicological data. *Food and Chemical Toxicology* 35:573-581.
- Hsu HHT (2003) In vitro effect of cholesterol on calcifying activity of vesicles isolated from rabbit aortas. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1638:235-240.
- Jewell C, O'Brien NM (1999) Effect of dietary supplementation with carotenoids on xenobiotic metabolizing enzymes in the liver, lung, kidney and small intestine of the rat. *Br J Nutr* 81:235-242.
- Kroes R, Schaefer EJ, Squire RA, Williams GM (2003) A review of the safety of DHA45-oil. *Food and Chemical Toxicology* 41:1433-1446.
- Lans CA (2006) Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *J Ethnobiol Ethnomedicine* 2:45.
- Lina BAR, Wolterbeek APM, Suwa Y, Fujikawa S, Ishikura Y, Tsuda S, Dohnalek M (2006) Subchronic (13-week) oral toxicity study, preceded by an in utero exposure phase, with arachidonate-enriched triglyceride oil (SUNTGA40S) in rats. *Food and Chemical Toxicology* 44:326-335.
- Matos SL, de Paula H, Pedrosa ML, Santos RC, Oliveira EL, Silva ME (2005) Dietary models for inducing hypercholesterolemia in rats. *Braz Arch Biol Technol* 48:203-209.
- Mercadante AZ, Steck A, Pfander H (1997a) Isolation and Identification of New Apocarotenoids from Annatto (*Bixa orellana*) Seeds. *J Agric Food Chem* 45:1050-1054.

- Mercadante AZ, Steck A, Pfander H (1997b) Isolation and structure elucidation of minor carotenoids from annatto (*Bixa orellana* L.) seeds. *Phytochemistry* **46**:1379-1383.
- Mercadante AZ, Steck A, Pfander H (1999) Three minor carotenoids from annatto (*Bixa orellana*) seeds. *Phytochemistry* **52**:135-139.
- Moghadasian MH (2002) Experimental atherosclerosis: a historical overview. *Life Sci* **70**:855-865
- Paumgartten FJR, De Carvalho RR, Araujo IB, Pinto FM, Borges OO, Souza CAM, Kuriyama SN (2002) Evaluation of the developmental toxicity of annatto in the rat. *Food and Chemical Toxicology* **40**:1595-1601.
- Pedersen O, Kahn CR, Flier JS, Kahn BB (1991) High fat feeding causes insulin resistance and a marked decrease in the expression of glucose transporters (Glut 4) in fat cells of rats. *Endocrinology* **129**:771-777.
- Preston H, Rickard MD (1980) Extraction and chemistry of annatto. *Food Chemistry* **5**:47-56
- Shah PK (2003) Emerging non-statin LDL-lowering therapies for dyslipidemia and atherosclerosis. *Rev Cardiovasc Med* **4**:136-141.
- Shilpi JA, Taufiq-Ur-Rahman M, Uddin SJ, Alam MS, Sadhu SK, Seidel V (2006) Preliminary pharmacological screening of *Bixa orellana* L. leaves. *J Ethnopharmacol* **108**:264-271.
- Silva ME, Santos RC, O'Leary MC, Santos RS (1999) Effect of Aubergine (*Solanum melongena*) on serum and hepatic cholesterol and triglycerides in rats. *Braz Arch Biol Technol* **42**:339-342.
- Silva CR, Greggi Antunes LM, Bianchi MdL (2001) Antioxidant action of bixin against cisplatin-induced chromosome aberrations and lipid peroxidation in rats. *Pharmacological Research* **43**:561-566.
- Turbino-Ribeiro SM, Silva ME, Chianca DA, Jr., de Paula H, Cardoso LM, Colombari E, Pedrosa ML (2003) Iron overload in hypercholesterolemic rats affects iron homeostasis and serum lipids but not blood pressure. *J Nutr* **133**:15-20.

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