SHORT COMMUNICATION

Effects of Bixa orellana L. Seeds on Hyperlipidemia

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Bixa orellana L., urucum, or urucu, a native tropical tree of Central and South American rain forests is used to treat various diseases in popular medicine. In Ceará, Northeast of Brazil, the seeds of urucum have been used for the treatment of high lipid blood levels. The present study investigated the effects of the aqueous extract from Bixa orellana seeds (AEBO) in mice with hyperlipidemia induced by tyloxapol, fructose and ethanol. In hyperlipidemia induced by Triton WR1339, 400 and 800 mg/kg AEBO reduced triglycerides (TG) serum levels at 24h and 48h. In the study of hypertriglyceridemia induced by fructose, AEBO in doses of 400 mg/kg and 800 mg/kg reduced TG levels by 48.2% and 48.7%, respectively. Finally, the ethanol experimental model with 400 mg/kg AEBO promoted a reduction of 33.6% of TG levels, while the 800 mg/kg concentration reduced hypertriglyceridemia in 62.2%. In conclusion, the aqueous extract of the seeds of Bixa orellana was capable of reversing the hypertriglyceridemia induced by Triton, fructose and ethanol, demonstrating a hypolipidemic effect. However, further studies are necessary to discover the precise mechanism of action. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: Bixa orellana; hypolipemic; fructose; Triton; ethanol.

INTRODUCTION

The Brazilian flora has been used as a therapeutic source for the treatment of different conditions, such as inflammation, diarrhea, hepatic disorders, diabetes mellitus and alterations in lipid metabolism (Silva, 2000). Some studies on plants have been carried out with the objective of discovering new therapeutic agents capable of reducing serum level lipids. For example, a study of the action of an isolated protein from Amaranthus cruentus seeds in the lipid metabolism can be cited (Escudero et al., 2006), as well as the hypolipidemic activity of Cassia tora seeds (Patil et al., 2004), the demonstration of the potential of Mormodica charantia as a reducing agent of serum triglyceride levels (Senanayake et al., 2004) and the investigation of the effect of a diterpene isolated from Croton cajucara Benth in triglyceride reduction (Silva et al., 2001a, 2001b).

Bixa orellana L. (annatto), ‘urucum’ aka ‘urucu’, family Bixaceae, a tropical tree native to the Central and South American rain forests is used in popular medicine to treat various diseases (Paumgarten et al., 2002). The indigenous Amazon people have used urucum for body painting for centuries and it is believed that the original Aztec chocolate beverage contained annatto seeds in addition to cocoa (De-Oliveira et al., 2003). In Latin America, urucu has been used as a color additive in drugs, cosmetics and also in food products (Hallagan et al., 1995; De-Oliveira, 2003). Fleischer et al. (2003) demonstrated the antimicrobial activity of the leaves and seeds of Bixa orellana. It described the hypoglycemic activity of Bixa orellana extract (Russell et al., 2008). In the West Indies (Jamaica), a folk remedy (bush tea) made from Bixa orellana is used to treat diabetes mellitus (Morrison et al., 1991). In Brazil, the seeds, leaves and roots of Bixa orellana have also been used popularly as an aphrodisiac medicine, as well as a remedy to treat fevers, inflammatory conditions and parasitic diseases (Di Stasi et al., 1989; Paumgarten et al., 2002). Previous pharmacological studies have revealed that Bixa orellana extracts possess antiprotozoal, anthelmintic and platelet antiaggregant activity (Villar et al., 1997; Barrio et al., 2004). In Ceará, Northeast of Brazil, the seeds of urucum have been used for the treatment of high lipid blood levels. The present study investigated the effects of the aqueous extract from Bixa orellana seeds in hyperlipidemia induced by tyloxapol (Triton WR-1339), fructose and ethanol.

MATERIALS AND METHODS

Materials. Total cholesterol (TC) and triglyceride (TG) diagnostic kits were provided by Labtest Diagnosis SA* (Minas Gerais, Brazil). Triton WR-1339 was purchased from Sigma (St Louis, MO, USA). Gemfibrozil was purchased from EMS S/A (Sigma Pharma). The other chemicals used in this study were all reagent grade.

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**Plant extract.** The Bixa orellana seeds were collected in Fortaleza, State of Ceará, Northeastern Brazil. A voucher specimen (no. 040411) was deposited in the Prisco Bezerra Herbarium of the Department of Biology, Federal University of Ceará. The seeds were ground (500 μm) and then 30 g of the resulting powder was mixed with 140 mL of hot distilled water for 20 min. Afterwards, the mixture of water and powder was filtered, from which 10 mL was transferred and dried in a Petri dish at a temperature of 105 °C. At last a concentration of the extract per mL was obtained by gravimetric techniques.

**Animals.** Male Swiss mice (30–40 g) from the Central Animal House of Federal University of Ceará (Fortaleza, Brazil) were maintained under standard conditions (temperature of 22 ± 1 °C, relative humidity 60 ± 5%, 12 h light/dark cycle) with standard diet from Biobase (temperature of 22 °C). Animal care followed the guidelines of the Brazilian National Council of Animal Experimentation (Brazil). Mice were divided into three groups of six animals: control group (fructose) and test groups (AEBO 400 mg/kg or 800 mg/kg). All groups except saline received 0.1 mL of 26% ethanol, after 30 min of treatment with saline or AEBO for 4 days. The 10% ethanol was added to the drinking water of groups ethanol and AEBO (400 or 800 mg/kg) overnight, whereas the saline group received only water; afterwards, water was given for 6 h. Blood samples were collected from the orbital plexus of the animals, before starting and at the end of the experiment in order to determine total cholesterol (TC) and triglycerides (TG), using diagnostic kits (Labtest®, Brazil).

**Hyperlipidemia induced by Triton WR-1339.** Hypertriglyceridemia and hypercholesterolemia were induced in four groups of mice by a single intraperitoneal injection of Triton WR1339 at a dose of 400 mg/kg (Hall et al., 2000). Mice were divided into five groups of six animals, each group being treated three times (1 h before, 22 h and 46 h after Triton administration) with the aqueous extract of Bixa orellana seeds (AEBO 400 or 800 mg/kg); gemfibrozil (100 mg/kg; positive control), a conventional hypolipidemic drug, or vehicle (saline solution), the latter in normal and hyperlipidemic controls. At 24 h and 48 h after Triton injection, blood samples were collected from the animal orbital plexus in tubes with anticoagulant (heparin), and these tubes were submitted to subsequent centrifugation at 3500 rpm for 15 min. The resulting plasma was used to determine the levels of total cholesterol and triglycerides. Lipids were measured by means of colorimetric methods in a semi-automatic analyser (Labquest®) using diagnostic kits (Labtest®, Brazil).

**Hypertriglyceridemia induced by fructose.** Acute administration of fructose leads to a sharp increase in plasma triglyceride level in mice as described by Cignarella et al. (1996) and Xie et al. (2007), whose methods were somewhat modified for the purpose of our experiment. Mice were divided into three groups of six animals: control group (fructose) and test groups (AEBO 400 mg/kg or 800 mg/kg). Blood samples were collected from the orbital plexus of all groups before and after hypertriglyceridemia induced by fructose for TC and TG serum levels determination. For the induction of hypertriglyceridemia two different concentrations of fructose were utilized: 15 g/100 mL drinking water overnight and 100 g/100 mL by oral gavage (0.3 mL/animal) 7 h before the last collection of the blood samples. Test groups received AEBO four times at a dose of 400 mg/kg or 800 mg/kg; at the same time the control group was treated with saline.

**Hypertriglyceridemia induced by ethanol.** Hypertriglyceridemia was induced in four groups of six mice by administration of 10% and 26% ethanol (Silva et al., 2001a, 2001b): saline, ethanol and AEBO (400 or 800 mg/kg). All groups except saline received 0.1 mL of 26% ethanol, after 30 min of treatment with saline or AEBO for 4 days. The 10% ethanol was added to the drinking water of groups ethanol and AEBO (400 or 800 mg/kg) overnight, whereas the saline group received only water; afterwards, water was given for 6 h. Blood samples were collected from the orbital plexus of the animals, before starting and at the end of the experiment in order to determine total cholesterol (TC) and triglycerides (TG), using diagnostic kits (Labtest®, Brazil).

**Statistical analysis.** All values were expressed as mean ± SEM in order to determine differences among the groups, data were compared by one-way analysis of variance (ANOVA) followed by Newman-Keuls (p < 0.05) using the GraphPad program (Prism 3.0). Values of *p < 0.05 were considered to be statistically significant.

**RESULTS AND DISCUSSION**

The intraperitoneal administration Triton WR1339 (400 mg/kg), a nonionic detergent, elevated the triglycerides and total cholesterol levels. The AEBO treatment (400 mg/kg) caused a reduction of TG serum levels in 47.5% (24 h) and 50.7% (48 h), while 800 mg/kg dose promoted a reduction of 56.4% and 44.6% at 24 h and 48 h after Triton administration. Gemfibrozil reduced triglyceride in 51.8% (24 h) and 69.8% (48 h) (Fig. 1a). Triton administration elevated TC levels at 24 h (420.5%) and at 48 h (180.6%) after the treatment. AEBO was not able to reduce TC levels as shown in Table 1. The Triton administration caused dyslipidemia through blockade of the removal of triglyceride in the plasma and hypercholesterolemia through mechanisms that include stimulation of the synthesis of hepatic cholesterol and increased cholesterol efflux from tissues to the circulation. It is reported that Triton forms a layer on lipoprotein surfaces, especially VLDL, protecting them from enzymatic action; thus there seems to be a protection against hydrolysis provoked by lipoprotein lipase, with hypertriglyceridemia as a result (Hall et al., 2000).

A carbohydrate rich diet causes some metabolic effects such as hyperinsulinemia, hypertension and hypertriglyceridemia, both in experimental animals and humans. To explain the hypertriglyceridemic phenomenon, studies suggest that an elevated hepatic production of very low density lipoprotein (VLDL-TG) occurs, as well as an increase of fatty acid synthesis through lipogenesis (Park et al., 1992). Other authors showed that besides...
Lipid metabolism as lipoprotein lipase and hepatic triglyceride lipase, enabling experimental models of induction of hypertriglyceridemia (Futnatsu et al., 2003). In the present study, the AEBO hypotriglyceridemic effect in mice could be due to the increase of activity of lipoprotein lipase and hepatic triglyceride lipase, because lipoprotein lipase is a very important enzyme for lipid catabolism promoting the absorption and metabolism of fatty acids.

Fructose, also known as levulose, found in fruits and vegetables, is used as a model of hypertriglyceridemia induction. The increase of triglyceride as a result of feeding with high levels of fructose is associated with a greater hepatic synthesis of glycerol and fatty acids, the increase being 1.4–18.9 times that of glucose (Barreiros et al., 2005). In the present study, the fructose administration produced an elevation in the serum levels of triglyceride (502.6 ± 38.15 mg/dL) equivalent to an increase of 135.6% when compared with the fructose group before the start of the treatment (213.3 ± 13.16 mg/dL). Animals treated with 400 mg/kg and 800 mg/kg AEBO had a significantly reduced hypertriglyceridemia induced by fructose by 48.2% and 48.7% respectively (Fig. 1b). The values of TC were not affected during the animal treatment (AEBO 400 mg/kg before treatment = 127.50 ± 4.11 mg/dL; AEBO 400 mg/kg after treatment = 136.50 ± 5.44 mg/dL), (AEBO 800 mg/kg before treatment = 137.20 ± 7.58 mg/dL; AEBO 400 mg/kg after treatment = 139.30 ± 3.74 mg/dL). The hypotriglyceridemic effect of the extract could be due to the fact that it might contribute to an increase in the enzymes taking part in lipid metabolism. There could be a possible similarity in the mechanism of action of the extract in hyperlipidemia induced by the Triton and fructose models, since the lipoprotein lipase enzyme and the metabolism of VLDL-TG are both involved in the models used.

In experimental models of hypertriglyceridemia induced by ethanol, the AEBO treatment reduced serum triglyceride levels by 33.6% (400 mg/kg) and 62.2% (800 mg/kg) (Fig. 1c). The TC values were not affected during the animal treatment (AEBO 400 mg/kg before treatment = 122.00 ± 7.03 mg/dL; AEBO 400 mg/kg after treatment = 107.80 ± 4.54 mg/dL), (AEBO 800 mg/kg before treatment = 107.80 ± 4.00 mg/dL; AEBO 400 mg/kg after treatment = 122.30 ± 6.65 mg/dL).

The results found may be due to the fact that Bixa orellana contains carotenoids, substances possessing antioxidant properties, suggesting that the extract exerts a protective liver effect.

In conclusion, an aqueous extract of the seeds of Bixa orellana was capable of reversing the hypertriglyceridemia induced by Triton, fructose and ethanol, demonstrating a hypolipidemic effect. However, further studies are necessary to discover the precise mechanism of action.

Acknowledgements

The authors wish to express their gratitude to Labtest® diagnostics kits and Funca/CAPES, Brazil, for financial support.

Conflict of Interest

The authors declare no conflict of interest.

Table 1. Effects of aqueous extract of Bixa orellana seeds on total cholesterol levels in the Triton-WR1339-induced hypercholesterolemia

<table>
<thead>
<tr>
<th>Group</th>
<th>24 h (mg/dL)</th>
<th>48 h (mg/dL)</th>
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<tbody>
<tr>
<td>Saline</td>
<td>110.00 ± 4.80</td>
<td>113.90 ± 3.29</td>
</tr>
<tr>
<td>Triton (400 mg/kg)</td>
<td>572.50 ± 28.45a</td>
<td>319.60 ± 53.27a</td>
</tr>
<tr>
<td>Gemfibrozil (100 mg/kg)</td>
<td>416.30 ± 29.22b</td>
<td>149.50 ± 8.06b</td>
</tr>
<tr>
<td>AEBO (400 mg/kg)</td>
<td>531.00 ± 29.32b</td>
<td>281.60 ± 19.53</td>
</tr>
<tr>
<td>AEBO (800 mg/kg)</td>
<td>511.30 ± 42.36b</td>
<td>376.40 ± 69.24b</td>
</tr>
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Saline, normal control; Triton, hyperlipidemic control; AEBO, aqueous extract of Bixa orellana seeds. *p < 0.05 compared with normal control. **p < 0.05 compared with hyperlipidemic control.


