Full Length Research Paper

Antilipemic activity of ethanolic and hexane extracts from seeds of *Bixa orellana* Linn. in hyperlipidemic rats

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The seeds of *Bixa orellana* are used in popular medicine as a hypolipidemic drug. This study evaluated the antilipidemic activity of ethanolic and hexane extracts from seeds of *B. orellana* in dyslipidemic rats. Dyslipidemia was induced by an intraperitoneal injection of Triton WR1339 in male Wistar rats. The animals were treated with the ethanolic and hexane extracts of *Bixa orellana*. Plasma levels of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by enzymatic methods. The ethanolic and hexane extracts from seeds of *B. orellana* reduced TG, TC and LDL-C and increased HDL-C in dyslipidemic rats. The treatments did not promote hepatic toxicity. The data showed that treatment with *B. orellana* extracts decreased plasma lipids in dyslipidemic rats.

Key words: *Bixa orellana*, annatto, dyslipidemia.

INTRODUCTION

Atherogenic dyslipidemia is a lipoprotein metabolism disorder and the most common cause of cardiovascular disease (CVD) that contributes to the high rate of morbidity and mortality (Genest et al., 2009). Atherosclerosis is a progressive disease characterized by lipid accumulation and fibrous elements in arteries that are responsible for the onset of CVD. It can accelerate the development of CVD and progressively cause atherosclerotic lesions (Parsaee et al., 2006; Jeong et al., 2005). Concentrations of cholesterol, particularly low-density lipoprotein cholesterol (LDL-C), promote the accumulation of substances in the sub-endothelial extracellular space in arteries. LDL-C can be modified to form oxidized LDL-C, which is an atherogenic substance that is toxic to vascular cells (Regnstrom et al., 1992). In hypercholesterolemia, an increase in apolipoprotein-CIII inhibits the action of lipoprotein lipase (McConathy et al., 1992) with consequent increases in triglycerides. The increase in hepatic lipase concomitantly catabolizes and reduces high-density lipoprotein cholesterol (HDL) levels (Vega and Grundy, 1996). The therapeutic use of statins has been shown to reduce the morbidity and mortality associated with coronary events and other cardiovascular diseases by 42% in patients with established CVD (Nicholls et al., 2011). These substances inhibit the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which regulates cholesterol synthesis.

*Bixa orellana*, also known as achiote or annatto, is a plant belonging to the Bixaceae family. Their seeds are used in folk medicine as a natural antilipemiant (Silva et al., 2010). The dye extracted from the pericarp of *B. orellana* seeds is widely used in the food industry to replace synthetic dyes because of its lower cost and lack of toxicity (Paumgartten et al., 2002). Phytochemical studies of extracts from different parts of *B. orellana*

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showed the presence of steroidal compounds, flavonoids (Shilpi et al., 2006), carotenoids (Jondiko and Pattenden, 1989) and isoprenoids such as geranylgeraniol, farnesylacetone, geranylgeranyl octadecanoate and tocotrienol (Scotter et al., 1994; Jondiko and Pattenden, 1989). The present study evaluated the antilipemic activity of the hydroalcoholic and hexane extracts from *B. orellana* in experimental dyslipidemia induced by Triton WR1339 in rats.

### MATERIALS AND METHODS

Pharmacological assays were performed from May 2009 to July 2011, in the Laboratory of Inflammation, State University of Maringá. The plant material (seeds) of *B. orellana* was collected in February 2009 in Uberlândia, Minas Gerais, Brazil, and identified by Professor Maria Aparecida Serti. After botanical identification, a voucher specimen (no. 20019113) was deposited in the Biology Institute, Federal University of Uberlândia, Uberlândia.

#### Extract preparation

The seeds (850 g) were used for the preparation of *B. orellana* hydroalcoholic and hexane extracts. Four hundred grams of the seeds were used for hexane extraction in a Soxhlet apparatus with 500 ml of n-hexane for 8 h. The hexane extract was concentrated under vacuum at 40 °C to obtain an oily extract (OE; 31.5 g). The remaining seeds (450 g) were soaked in a solution of ethanol: water (9:1, v/v) at room temperature for 7 days. This extract was filtered and concentrated under vacuum at 40 °C, and the lyophilized ethanolic extract (EE) yield was 91 g.

#### Identification of compounds

Experiments were performed to identify the compounds present in the plants using nuclear magnetic resonance (NMR), mass spectrometry, high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection. $^1$H NMR and $^{13}$C NMR were performed at 300 and 75 MHz, respectively, with a deuterated chloroform (CDCl$_3$) Bruker DRX-400 NMR spectrophotometer (Varian Gemini 300 model). The standard reference scale for $^1$H NMR was tetramethylsilane (TMS), and $^{13}$C NMR chemical shifts of CDCl$_3$ were analyzed at a constant temperature of 25 °C. The analysis of the hydroalcoholic and hexane extracts by mass spectrometry and HPLC were performed using a Micromass Quattro LC Triple Quadrupole mass spectrometer and a Shimadzu LC20AT HPLC apparatus with an LC-10AT solvent pump, SPD-M20A diode array detector (SIL-20A), and rhodamine injector with a 20 µl “loop” and “online” degasser. All of the separations were conducted on a C18 column (Spherisorb ODS-2, 150 mm x 4.6 mm, 3 mm particle size) at a flow rate of 1 ml/min eluted with methanol. The OE (1.0 mg) and EE (1.0 mg) were dissolved in 1 ml of CH$_3$CN/H$_2$O (65:35, v/v), filtered through a "Millex" filter and injected at a volume of 20 µl to the interior of the HPLC system. The peaks of the carotenoids and isoprenoid derivatives were identified by their chromatographic characteristics, and the UV-visible spectra provided by the detector were compared with diode array data from the literature.

#### Experimental animals

Male Wistar rats weighing 200 - 250 g, were provided by the Central Animal House of the State University of Maringá. All of the animals received commercial chow and water *ad libitum*. The animals were housed at 22 ± 2 °C under a 12 h/12 h light/dark cycle. Prior to the experiments, the animals were fasted overnight, with water provided *ad libitum*. The experimental protocols were approved by the Ethical Committee in Animal Experimentation of the State University of Maringá (CEAE/UEM 066/2010).

### RESULTS

#### Chemical identification

HPLC and mass spectrometry identified the major compounds in the EE and OE to be geranylgeraniol, carotenoids, bixin, norbixin and lycopene derivatives, which were confirmed by UV spectrometry in comparison with authentic standard purchased from Sigma-Aldrich and literature data (Davies, 1976; Coates et al., 1978; Scotter et al., 1994; Britton, 1995). A mixture of polysaccharides was also identified in the EE.

#### Effect of *B. orellana* treatment on experimental dyslipidemia

Total cholesterol, TG and LDL-C levels were significantly higher and HDL-C levels were lower in dyslipidemic animals (Table 1). Lovastatin treatment decreased TC
Table 1. Effect of different treatments on lipid profile in normal and dyslipidemic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cholesterol (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76.8 ± 10.8</td>
<td>87.8 ± 13.6</td>
<td>88.7 ± 14.83</td>
<td>48.4 ± 5.8</td>
</tr>
<tr>
<td>Triton</td>
<td>332.6 ± 5.8</td>
<td>2357.6 ± 10.3</td>
<td>808.2 ± 14.00</td>
<td>34.3 ± 4.6</td>
</tr>
<tr>
<td>Triton + Lovastatin</td>
<td>301.1 ± 8.6</td>
<td>2014.0 ± 12.8*</td>
<td>720.9 ± 12.18*</td>
<td>38.7 ± 9.9*</td>
</tr>
<tr>
<td>Triton + 100 mg EE</td>
<td>297.0 ± 8.0*</td>
<td>2338.0 ± 15.4</td>
<td>759.4 ± 16.8*</td>
<td>33.53 ± 8.6</td>
</tr>
<tr>
<td>Triton + 500 mg EE</td>
<td>279.4 ± 7.4**</td>
<td>2130.4 ± 12.8*</td>
<td>704.2 ± 3.8*</td>
<td>43.5 ± 11.3*</td>
</tr>
<tr>
<td>Triton + 750 mg EE</td>
<td>273.4 ± 12.2*</td>
<td>1831.2 ± 9.8*</td>
<td>632.1 ± 10.7**</td>
<td>76.6 ± 5.8**</td>
</tr>
<tr>
<td>Triton + 500 mg OE</td>
<td>271.6 ± 6.35**</td>
<td>777.0 ± 9.9*</td>
<td>460.8 ± 12.5*</td>
<td>69.4 ± 6.3**</td>
</tr>
</tbody>
</table>

The data are expressed as mean ± SEM; n = 8-10 rats per group. EE, Ethanolic extract; OE, oily extract. *p < 0.05, compared with dyslipidemic group (Triton); **p < 0.05, compared with lovastatin-treated group (ANOVA, Tukey's test).

Table 2. Effect of different treatments on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity in control and Triton-treated groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.2 ± 5.9</td>
<td>115.6 ± 5.2</td>
</tr>
<tr>
<td>Triton</td>
<td>76.3 ± 6.4*</td>
<td>195.6 ± 4.4*</td>
</tr>
<tr>
<td>Triton + lovastatin</td>
<td>66.1 ± 7.2*</td>
<td>153.9 ± 7.5*</td>
</tr>
<tr>
<td>Triton + 100 mg EE</td>
<td>63.9 ± 7.3*</td>
<td>156.2 ± 5.3*</td>
</tr>
<tr>
<td>Triton + 500 mg EE</td>
<td>67.1 ± 5.6*</td>
<td>198.8 ± 5.9*</td>
</tr>
<tr>
<td>Triton + 750 mg EE</td>
<td>100.1 ± 4.8*</td>
<td>277.6 ± 10.8*</td>
</tr>
<tr>
<td>Triton + 500 mg OE</td>
<td>53.9 ± 10.1</td>
<td>116.9 ± 10.0</td>
</tr>
</tbody>
</table>

The data are expressed as mean ± SEM. n = 8-10 rats per group. *p < 0.05, compared with control group (ANOVA followed by Tukey's test).

Hyperlipidemia comprises a state of increased concentrations of TG, TC and LDL-C and is an important risk factor for the development and progression of atherosclerosis and coronary heart disease (Genest et al., 2009). Epidemiological studies have shown that elevated HDL-C levels may have anti-atherogenic effects, including inhibition of LDL-C oxidation, providing a protective effect on vascular endothelial cells by preventing the cytotoxic effects of oxidized LDL-C (Assmann and Nofer, 2003; Harrison et al., 2003). Furthermore, clinical studies have found a significant reduction of LDL-C and elevation of HDL-C in hypercholesterolemic subjects after ingestion of natural products (Hasani-Ranjbarth et al., 2010). In the present study, we found that Triton effectively induced experimental dyslipidemia. In fact, this drug increased plasma lipid concentrations, thus characterizing experimental dyslipidemia. Triton is a hyperlipidemic agent (Frantz and Hinkelman, 1955) and nonionic detergent that increases the plasma concentrations of TC and TG by increasing the activity of HMG-CoA reductase (Goldfarb, 1978) and acting on cholesterol synthesis. This drug inhibits lipase by inhibiting the removal of plasma lipoproteins to extrahepatic tissues. Thus, an increase in TC and plasma TG is observed 72 h after Triton administration (Goldfarb, 1978; Catanozi et al., 2001).

DISCUSSION

*B. orellana* is used for natural coloring and flavoring, especially in the food industry. Despite the few empirical reports of its biological activity, the seeds of this plant have been used as an antilipemic drug in folk medicine in many countries including Brazil (Silva et al., 2010). This study evaluated the antilipemic activity of the hydroalcoholic and hexane extracts of *B. orellana* in dyslipidemic rats.
Our results also showed that the \textit{B. orellana} extracts were able to increase the plasma concentrations of HDL-C in dyslipidemic rats. The LDL-C/HDL-C ratio has a direct correlation with the incidence of CVDs and is considered important for therapeutic strategies, including dietary and pharmacological (e.g., antilipemic drugs), that prevent and treat dyslipidemia and atherosclerosis. Geranylgeraniol is an isoprenoid intermediate of cholesterol synthesis and was present in high concentrations in these \textit{B. orellana} extracts. In this study, the administration of the EE and OE in hyperlipidemic rats significantly reduced plasma concentrations of TG, TC and LDL-C. Therefore, this substance and others constituents in these extracts could participate in reducing the dyslipidemia induced by Triton.

Apocarotenoids and carotenoids found in \textit{B. orellana}, such as bixin and norbixin (Jondiko and Pattenden, 1989; Mercadante et al., 1996), may have antioxidant activity (Kiokias and Gordon, 2003) that is important for the prevention of atherosclerosis. Isoprenoid compounds are substrates in cholesterol biosynthesis. This class of compounds includes sterols, oxysterols, farnesol and geranylgeraniol and their derivatives. These isoprenoids exert antimicrobial (Inoue et al., 2005), anti-inflammatory (Silva et al., 2004) and antioxidant (Wassmann et al., 2001) effects.

Polysaccharides and protein (e.g., glycoprotein) complexes present in plant extracts (Oh et al., 2006) could also be involved in the hypolipidemic and anti-atherosclerotic effects of \textit{B. orellana}. Therefore, the association between these complexes and carotenoids and others constituents may play an important role in the biological activity of this plant. A proposed antioxidant effect in the dyslipidemia may involve LDL-C peroxidation inhibition, as observed in the ethanolic extract which contains high concentrations of polysaccharides (Amaral et al., 2001) and carotenoids (Kiokias and Gordon, 2003).

In fact, these substances can reduce lipoprotein peroxidation in hyperlipidemic rats and therefore prevent atherosclerosis, which has already been demonstrated (Parthasarathy et al., 1999). Carotenoids also may regulate adipogenesis through mechanisms that involve peroxisome proliferator-activated receptors (Takahashi et al., 2002). However, the mechanism by which \textit{B. orellana} exerts antilipemic activity is unclear, and further studies should be conducted. The hypotriglyceremic effect of natural products may be attributable to a decrease in fatty acid synthesis (Bopanna et al., 1997), enhanced LDL catabolism (Khanna et al., 2002), or acetyl-CoA carboxylase inhibition (Lemhadri et al., 2006) and by influencing lipoprotein metabolism primarily by increasing LDL-C receptors (Slater et al. 1980). Another possible mechanism for the reduction of TC may be related to the removal of cholesterol biosynthesis by reducing 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity, thus limiting the presence of this enzyme in the cholesterol biosynthesis pathway. Our data demonstrate that \textit{B. orellana} extract treatment reduced cholesterol levels in dyslipidemic rats, possibly through an effect on enzyme activity, similar to treatment with lovastatin, an HMG-CoA reductase inhibitor.

The effects of the EE (100, 500 and 750 mg/kg) and OE (500 mg/kg) on transaminase (ALT and AST) activity was also studied in hyperlipidemic rats. Transaminases are important enzymes for the study of liver toxicity. ALT is found predominantly in the liver, with fewer quantities in the kidneys, heart and skeletal muscles. As a result, ALT is a more specific indicator of liver inflammation than AST. AST may also be elevated in diseases that affect other organs, such as the heart and muscles. Our results indicate that only treatment with the OE of hyperlipidemic rats reduced the activity of these enzymes, suggesting that this extract which is rich in geranylgeraniol, effectively reduced the toxic effect of Triton on these enzymes. Furthermore, previous research demonstrated a similar protective effect of certain carotenoids in \textit{B. orellana}, such as bixin and norbixin (Paumgartten et al., 2002; Bautista et al., 2004). Our data showed the effect of \textit{B. orellana} extracts in reducing the experimental dyslipidemia in rats. This study contributes to the evaluation of anti-dyslipidemic activity of natural products.

**Conclusion**

We demonstrated that the ethanolic and hexane extracts obtained from \textit{B. orellana} have hypolipidemic effects in an experimental model of Triton-induced dyslipidemia. This effect could be attributable to the isolated or associated chemical constituents present in \textit{B. orellana} extracts. Further studies are needed to reveal the mechanisms involved in hypolipidemic effect in the extracts of this plant.

**ACKNOWLEDGEMENTS**

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