Ethnopharmacological communication

Reproductive toxicity of *Campomanesia xanthocarpa* (Berg.) in female Wistar rats

Sarah Alves Auhareka, Maria do Carmo Vieirab, Claudia Andrea Lima Cardosoc, Rodrigo Juliano Olivierad, Andréa Luiza Cunha-Lauraa, n a
b c d

Centre of Biological Sciences and Health, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil
Department of Agricultural Science, Federal University of Grande Dourados, Dourados, MS, Brazil
Department of Chemistry, State University of Mato Grosso do Sul, Dourados, MS, Brazil
Faculty of Medicine, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil

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**Abstract**

**Ethnopharmacological relevance:** There is no evidence in the literature that substantiates the safety of *Campomanesia xanthocarpa* (Berg.) use during pregnancy.

**Materials and methods:** Thirty three female rats were randomly assigned to three groups. One group of animals received the *Campomanesia xanthocarpa* extract via gavage at a dose of 26.3 mg/kg/day from 6 to 15 days of pregnancy (organogenic period, T1) and another group received the same extract throughout the gestational period (from the 1st to the 20th day of pregnancy, T2). Control groups received distilled water. Euthanasia was done on 20th day, when the liver, kidney, spleen ovaries, fetuses and their respective placentas were removed. Implantations, reabsorptions, live and dead fetuses were recorded. Results and conclusions: *Campomanesia xanthocarpa*, in these experimental conditions, did not disturb the reproductive function of female rats and did not interrupt the progress of the embryofetal development. Moreover, our results provide further evidence that the *Campomanesia xanthocarpa* treatment reduces reabsorption sites, increases placenta weight and the number of live fetuses and may therefore have therapeutic applications. © 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

*Campomanesia xanthocarpa* Berg. (Myrtaceae), popularly known as “guavirova” is present in the Southern region of Brazil and is likewise found in Argentina, Paraguay, and Uruguay (Lorenzi, 1992). *Campomanesia xanthocarpa* leaves and stem bark are traditionally employed as a folk medicine to regulate the intestinal flux, for the treatment of cystitis, urethritis, dysentery and as an anti-inflammatory agent (D’ávila, 1990; Barroso, 1991; Alice et al., 1995). A recent study demonstrated that *Campomanesia xanthocarpa* extract has antiplatelet, antithrombotic, and fibrinolytic activities in mice and it may be effective in preventing thrombus formation through several pathways (Klafke et al., 2012). Additionally, Klafke et al. (2010) showed that *Campomanesia xanthocarpa* has reduced the blood cholesterol levels in hypercholesterolemic patients and may therefore have therapeutic applications (Klafke et al., 2010).

Based on the knowledge that toxicity often occurs during pregnancy and may have different effects on embryo development depending on the conceptus phase and the maternal conditions (Wilson 1977; Mason and Kang, 1989; Oliveira et al., 2009) and that *Campomanesia xanthocarpa* is widely used in folk medicine (Alice et al., 1995; Dickel et al., 2007), the aim of the present investigation was to evaluate the effect of *Campomanesia xanthocarpa* on pregnant rats exposed in two windows of treatment: throughout the gestational period (from the 1st to the 20th day of pregnancy) or during the organogenic period (from 6th to 15th day of pregnancy). The *Campomanesia xanthocarpa* single dose used in this experiment was based on the average consumption by the population since this plant is indiscriminately used during gestation and lactation, particularly by women of lower socioeconomic status.

2. Materials and methods

2.1. Preparation of extract

The leaves of *Campomanesia xanthocarpa* were collected in Mato Grosso do Sul State, Brazil in October 2006. A voucher...
specimen was identified by Maria do Carmo Vieira and deposited (No. 6123) in the herbarium of the Federal University of Grande Dourados (UFGD). The fresh leaves (200 g) were extracted in water at 95–100 ⁰C (10 L) by 10 min and left to macerate for 24 h in room temperature. After this period, the extract was filtered and concentrated under vacuum and lyophilizer. The yield of the crude extract was 16.7%. During the treatment, the extract was dissolved in distilled water.

2.2. Animals

The females were mated with males and the gestational day 0 (GD0) was determined if there were spermatozoa in the vagina. These animals were housed in a standard animal facility under controlled temperature (22 ⁰C) and photoperiod (12 h light, 12 h dark) with access to water and rodent food ad libitum. All procedures and protocols followed approved guidelines for the ethical treatment of animals, according to the Ethics Committee in Animal Experimentation from the Federal University of Mato Grosso do Sul (Protocol # 107/2006).

2.3. Experimental procedure

The mated females were randomly assigned to two experimental groups and exposed to *Campomanesia xanthocarpa* during organogenetic period (from 6th to 15th day of pregnancy, T1) or throughout the gestational period (from the 1st to the 20th day of pregnancy, T2). The dose used in this experiment corresponded to that used as a folk medicine (D’Avila, 1990; Alice et al., 1995). The females of the *Campomanesia xanthocarpa* exposed group received 26.3 mg/kg/day of the extract suspended in 0.5 mL in distilled water. The sign of pregnancy (GD0) was determined if there were spermatozoa in the vagina. The females were weighed on days 1, 6, 15 and 20 of pregnancy. These animals were housed in a standard animal facility under controlled temperature (22 ⁰C) and photoperiod (12 h light, 12 h dark) with access to water and rodent food ad libitum. All procedures and protocols followed approved guidelines for the ethical treatment of animals, according to the Ethics Committee in Animal Experimentation from the Federal University of Mato Grosso do Sul (Protocol # 107/2006).

2.4. Statistical analysis

Values are expressed as mean ± SEM and data were analyzed using the one-way ANOVA followed by the Tukey post-test using Graph-Pad Prism (version 5; Graph-Pad Software Inc., San Diego, CA, USA). The significance level was set at p < 0.05.

3. Results and discussion

In none of the experimental groups, maternal deaths, locomotor alterations, diarrhea or piloerection, that are clinical signals of maternal toxicity, were observed. The maternal body weight in both experimental groups investigated (T1 and T2) was similar. The *Campomanesia xanthocarpa* treatment did not affect the absolute weight of the spleen, liver and kidney (Table 1). Reduction or increases in the weight of these organs suggest toxicity (Queiroz et al., 2012). In addition, this study confirms earlier findings that *Campomanesia xanthocarpa* did not produce toxic symptoms in mice in doses up to 5 g/kg (Markman et al., 2004). Although *Campomanesia xanthocarpa* is used in folk medicine for weight loss, the present investigation did not confirm it in pregnant rats.

Table 1 summarizes the maternal and fetal parameters. A significant increase was observed in the placental weights of the *Campomanesia xanthocarpa* exposed animals (T1 and T2), in comparison with control. Moreover, it should be emphasized that placental weight has a significant role in fetal growth in terms of weight, body length, and cord length (Lo et al., 2002). Corroborating these findings, our results showed an increased fetal weight in both groups treated with *Campomanesia xanthocarpa*. The number of live fetuses were also significantly increased in the *Campomanesia xanthocarpa* exposed animals. Examination of live fetuses for external and visceral anomalies did not show a significant effect of the treatment.

The reproductive indices of implantation, reabsorption and pre- and postimplantation losses are shown in Table 2. The implantation index, considered an indicator of success of the blastocyst implantation in the endometrium (Tyl and Marr, 2006), was similar between the control and treated groups. However, the number of reabsorptions which are indicative of abnormal postimplantation development (Kalter, 1980) were decreased in *Campomanesia xanthocarpa* exposed animals. The effect of the *Campomanesia xanthocarpa* treatment on the period before embryo implantation was evaluated by determining the rate of preimplantation loss. This rate is a parameter used to establish a correlation between the number of released ova, which after fertilization, manage to implant in the uterus (Almeida and Lemonica, 2000). In comparison with control, the preimplantation loss in T2 group presented tendency to be lower, although it was not statistically significant. The treatment during the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=11)</th>
<th>T1 (n=11)</th>
<th>T2 (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>318 ± 22a</td>
<td>361 ± 7a</td>
<td>361 ± 7a</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>13 ± 1a</td>
<td>16 ± 0.3a</td>
<td>15 ± 0.4a</td>
</tr>
<tr>
<td>Spleen weight (g)</td>
<td>0.6 ± 0.05a</td>
<td>0.8 ± 0.01a</td>
<td>0.7 ± 0.04a</td>
</tr>
<tr>
<td>Kidney weight* (g)</td>
<td>0.9 ± 0.06a</td>
<td>1 ± 0.02a</td>
<td>1 ± 0.04a</td>
</tr>
<tr>
<td>Ovary weight** (mg)</td>
<td>94 ± 0.005a</td>
<td>105 ± 0.003a</td>
<td>105 ± 0.004a</td>
</tr>
<tr>
<td>Placenta weight</td>
<td>5.3 ± 0.8a</td>
<td>8.4 ± 0.4a</td>
<td>8.3 ± 0.4a</td>
</tr>
<tr>
<td>Uterine weight (g)</td>
<td>4.2 ± 0.4a</td>
<td>4.9 ± 0.3a</td>
<td>4.9 ± 0.2a</td>
</tr>
<tr>
<td>Corpora lutea (no.)</td>
<td>13 ± 0.5b</td>
<td>15 ± 0.5b</td>
<td>15 ± 0.5b</td>
</tr>
<tr>
<td>Implantation sites</td>
<td>11 ± 0.9b</td>
<td>14 ± 0.8b</td>
<td>14 ± 0.4a</td>
</tr>
<tr>
<td>Reabsorptions</td>
<td>1.1 ± 0.3b</td>
<td>0.2 ± 0.1b</td>
<td>0.1 ± 0.09b</td>
</tr>
<tr>
<td>Live fetuses (no.)</td>
<td>10 ± 1a</td>
<td>13 ± 0.8a</td>
<td>13 ± 0.6a</td>
</tr>
<tr>
<td>Dead fetuses (no.)</td>
<td>2.7 ± 0.5a</td>
<td>2 ± 0.6a</td>
<td>1.5 ± 0.5a</td>
</tr>
<tr>
<td>Fetuses size (cm)</td>
<td>3.7 ± 0.02a</td>
<td>4 ± 0.2a</td>
<td>4 ± 0.2a</td>
</tr>
<tr>
<td>Fetuses weight (g)</td>
<td>3 ± 0.06a</td>
<td>3 ± 0.03b</td>
<td>4 ± 0.02c</td>
</tr>
</tbody>
</table>

Results expressed in mean ± standard error. Different letters in the same line indicate statistical difference (P < 0.05).

* Left and right kidney average weights.
** Left and right ovary average weights.

<table>
<thead>
<tr>
<th>Indices (%)</th>
<th>Control (n=11)</th>
<th>T1 (n=11)</th>
<th>T2 (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation</td>
<td>88 ± 4a</td>
<td>91 ± 4a</td>
<td>98 ± 2a</td>
</tr>
<tr>
<td>Reabsorption</td>
<td>12 ± 4a</td>
<td>2 ± 1a</td>
<td>0.65 ± 0.65a</td>
</tr>
<tr>
<td>Preimplantation loss</td>
<td>12 ± 4a</td>
<td>9 ± 4a</td>
<td>2 ± 1.6a</td>
</tr>
<tr>
<td>Postimplantation loss</td>
<td>12 ± 3a</td>
<td>6 ± 1a</td>
<td>8 ± 3a</td>
</tr>
</tbody>
</table>

Results expressed in mean ± standard error. Different letters in the same line indicate statistical difference (P < 0.05).
organogenic period was intended to evaluate possible embryotoxic or embryolethal effects. The rate of postimplantation loss was not significant in both treated groups, which indicates that this window of treatment did not cause embryolethality.

4. Conclusion

As far as we know, our study is the first report in the literature which evaluates the effects of *Campomanesia xanthocarpa* in female rats during pregnancy. We can assume that *Campomanesia xanthocarpa*, in these experimental conditions, did not disturb the reproductive function of female rats and did not interrupt the progress of the embryofetal development. Moreover, our results provide further evidence that the *Campomanesia xanthocarpa* treatment reduces reabsorption sites, increases placenta weight and the number of live fetuses and may therefore have therapeutic applications.

Acknowledgments

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References


