Reproductive toxicity of *Echinodorus grandiflorus* in pregnant rats

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**ABSTRACT** — To evaluate the possible toxicity of the aqueous extract of *Echinodorus grandiflorus* in pregnant rats, animals were distributed in groups treated with 250, 500 and 1,000 mg/kg/day, by gavage, and a control group received saline solution. The treatment was carried out for 15 consecutive days, remaining during mating and until the 14th day of gestation. On the 15th day, pregnant animals were euthanized by exsanguination under anesthesia. A blood sample was destined to the hematological and biochemical analysis. The ovaries, liver, kidneys, spleen, and adrenal glands were removed and weighed. Liver, kidneys and spleen were processed for histopathological analysis. The number mated, cohabitated and pregnant rats were counted as well as the corpora lutea, implants, resorptions, and live and dead fetuses. Fetus body weight and placenta were measured. Treatment with 1,000 mg of extract caused anemia, leukocytosis, and an increase in AST and in cholesterol. The liver of animals treated with the two higher doses exhibited discrete inflammatory reaction, located mainly at the stroma which supports the portal space; in the kidneys of animals of T-500 and T-1000 groups there was an expressive decrease in the capsular space, and focal areas of vasodilatation and congestion, as well as a discrete hyalinization, and in the spleen of T-1000 group the red pulp presented excessive pigmentation suggestive of hemosiderin. There were no alterations in reproductive parameters, in fetus external morphology or in placenta weight. In conclusion, the extract causes maternal toxicity, though it does not alter the reproductive performance.

**Key words:** *Echinodorus grandiflorus*, Rats, Toxicity, Histopathology

**INTRODUCTION**

*Echinodorus grandiflorus* (Chamisso & Schlechtendal) Micheli (Alismataceae) is a herbaceous, semi-aquatic and perennial plant, with floating or emergent leaves, popularly known in Brazil as chapéu-de-couro, used to treat various diseases. In the leaves fatty acids, diterpenoids, phenolic acids, flavonols, alkaloids, derived from tartaric acid (see review in Brugiolo et al., 2009), saponins and tannins (Duarte et al., 2002) were identified.

The leaf tea is used by the population mainly as diuretic, prevention of arteriosclerosis, antirheumatic, depurative, anti-inflammatory and analgesic (see review in Brugiolo et al., 2009). The effects demonstrated for the
various extracts of *E. grandiflorus* in models *in vitro* and *in vivo* include anti-inflammatory and analgesic (Cardoso et al., 2003; Dutra et al., 2006); diuretic (Cardoso et al., 2003); antihypertensive (Lessa et al., 2008; Ribeiro et al., 1986); vasodilator (Tibiriçá et al., 2007); cholesterol reduction (Cardoso et al., 2005); antimicrobial (Souza et al., 2004) and immunosuppressive (Pinto et al., 2007) activities.

The increased use of natural products by the population brings about the indiscriminate use of medicinal plants that are used with the justification that what is natural does not harm health and, therefore, taken as free of side effects (Calixto, 2000), putting pregnant women and infants at greater risk once this group is culturally more likely to use these plants (Secretaria de Estado de Saúde, 2002). However, the risk of indiscriminate use of these plants is high, since the effects of most of them were devoid of any scientific basis and its use as a drug based only on popular tradition (Lopes et al., 2000).

In literature there are few studies on the toxicological potential of chapéu-de-couro, with preliminary toxicological investigations performed only for *E. macrophyllus*, a species of the same genus (Lopes et al., 2000), and another to evaluate the cytotoxicity and genotoxicity of the extract (Silva et al., 2010), but until the moment no research in the literature was found to evaluate reproductive toxicity of *Echinodorus*.

Many agents, among them the drugs, may affect the synthesis of hormones, gametogenesis, inhibit the processes of fertilization, cleavage, tubal transit and implantation of the blastocyst, that could result in changes in reproductive physiology (Hood, 2006). In pregnant females, exposure to extracts of plant can result in changes in embryo-fetal development (Calliari-Martin et al., 2001).

Considering the fact that chapéu-de-couro is widely used by the population, is part of the composition of soft drinks of great popular acceptance and that the toxicity of the plant has not yet been properly studied, the aim of this study was to examine the reproductive toxicity of the aqueous extract of *E. grandiflorus* in pregnant rats.

**MATERIALS AND METHODS**

**Plant material, extract preparation and chemical characterization**

The leaves of *E. grandiflorus* were collected in June 2006 from plantings under conditions of intermittent flooding, in the city of Juiz de Fora, state of Minas Gerais, Brazil (21° 45' 20'' S, 43° 20' 40'' W), by the botanist Dr. Daniel Sales Pimenta, who has identified and filed exsiccates in the Herbarium of Universidade Federal de Juiz de Fora (UFJF), number 49,707. To obtain the aqueous extract of *E. grandiflorus* (AEEg), the dried leaves (1,800 g) were extracted by aqueous infusion with distilled water (5,000 ml), staying for 15 hr in static maceration, and then filtered, stored in a freezer at -20°C and lyophilized (Liofilizador Freeze Dryer 18, Labconco, Kansas City, MO, USA). The yield of lyophilized residue corresponded to 13.45%.

The chemical characterization of the extract was carried out in the Department of Biochemistry of UFJF according to the sequential protocol of Matos (1997). The alkaloids were identified with the utilization of the Hager’s, Mayer’s, and Dragendorff’s reagent. The triterpenes and the steroids were identified by the reaction of the sample with acetic anhydride and concentrated sulfuric acid. The saponins were identified by the presence of a persistent froth when water was added to the sample. The coumarins were studied by the dripping of a solution of KOH at 10% into the sample on filter paper, with reading of 365 nm. The phenolic compounds were tested using a solution of FeCl₃ at 2%. The tannins were tested using acid chloride at 10% and gelatin solution at 2.5%, resulting in a white precipitate. The anthraquinones were determined using NaOH 0.5M. The flavonoids were tested through the dripping of the solution of AlCl₃ at 5% into the sample, leading to the appearance of a yellow fluorescence under UV light at 365 nm.

**Animals**

Adult nulliparous Wistar rats, two month old, with the average weight of 120 g, were used. Animals were obtained from the colony of the Center of Reproductive Biology of UFJF, where they are kept in polypropylene cages, provided with selected pinewood shavings as bedding, receiving water and pellet-type food *ad libitum*. The cages are kept in closed ventilated shelves (Allesco, Houston, TX, USA), located in an environment with temperature (22°C ± 2°C) and relative air humidity (40-60%) controlled, and a 12 hr light:12 hr dark regulated photoperiod.

**Experimental design**

In order to carry out the evaluation of reproductive toxicity, it was used the Guideline from the International Conference on Harmonisation of Technical Requirement for Registration of Pharmaceuticals for Human Use - ICH Harmonised Tripartite Guideline S5 (R2): Detection of Toxicity to Reproduction for Medical Products and Toxicity for Male Fertility, combined fertility and early embryonic development (2005), with little adaptation as follow.

The rats were distributed, randomly, in four experi-
Reproductive toxicity of *Echinodorus grandiflorus* in pregnant rats.

mental groups T-250, T-500 and T-1000 - treated groups, which received the AEEg by gavage, once a day, at doses of 250, 500 and 1,000 mg/kg/day, respectively. The lyophilized extract was weighed and the dose equivalent to body weight of rats was dissolved in 0.5 ml of distilled water. The C - control group, received 0.5 ml of saline solution by gavage, once a day. The treatment doses in this study were 10, 20 and 40 times higher than the one recommended for humans, which is of 25 mg/kg/day (Lopes et al., 2000).

After 15 days of treatment, the rats were mated with males with proven fertility (2 female: 1 male), continuing treatment during mating until the 14th day post-insemination. The day on which spermatozoa were found in the vaginal smear was considered the day zero of pregnancy. The rats were mated to obtain 16 pregnant rats/group in order to evaluate at least 16 litters.

On the 15th day post-insemination the rats were euthanized by exsanguination through cardiac puncture, performed under anesthesia with Ketamina (90 mg/Kg, Syntec, Cotia, São Paulo, Brasil) and Xilazine (10mg/Kg, Calmiun, Agener União, São Paulo, São Paulo, Brasil), via intraperitoneal.

The blood collected was destined to the determination of hematimetry, hemoglobin, hematocrit, total leukocyte and hematimetric indices (mean globular volume - MGV, mean globular hemoglobin - MGH, and mean globular hemoglobin concentration - MGHcb), determined by an automated hematology analyzer (DA-500, Celm, Barueri, São Paulo, Brasil) and cells counter (CC530/550, Celm), plasma cholesterol concentrations, triglycerides, aspartate transaminase (AST), alanine transaminase (ALT), urea and creatinine (SB - 190, Celm).

After euthanasia, the animals were submitted to laparothoracotomy, internal organs were examined, being removed: the ovaries, liver, kidneys, adrenal glands and spleen which were weighed in a precision balance (FA - 2108N, Bioprecisa, Brazil). Liver, kidney and spleen were subjected to routine histological processing in order to observe nefrotoxicity, hepatotoxicity and toxicity of spleen. The paraffin-embedded material was submitted to microscopy (Microtome HN - 340E, Micron, Boise, ID, USA), stained with hematoxylin and eosin (HE) and slides were mounted with Entellan (Merck 1.07961, Rio de Janeiro, Rio de Janeiro, Brasil). The samples were observed at full extension with a optical microscope (Zeiss, Hallbergmoos, Germany) at 50, 100 and 400X magnifications, by pathologist.

The ovaries were released from the ovarian bursa and the corpora lutea were counted with the aid of a stereoscopic microscope (Stemi SV6, Zeiss, magnification 10X). The uterine cornua were removed and placed according to their anatomic position and longitudinally sectioned. The live (cardiac beat present) and dead fetuses, and early and late resorptions were counted. Subsequently, the fetuses were weighed and fixed in Bouin solution during 60 min, and were examined with stereoscopic microscope (Stemi SV6, Zeiss, magnification 10X) for external observation of craniofacial or limb abnormalities, and verification of the neural tube closure.

The dams were inspected during the whole experimental process for clinical signs of toxicity: alteration of body weight, hyper or hypo-activity, piloerection, stereotyped activity, chromodacriorhea, vaginal bleeding, increase in diuresis, convulsion, diarrhea and death (Christian, 2001; Smith and Luo, 2004).

Statistical analysis
Data were analyzed by one-way ANOVA, followed by Dunnett’s test, for continuous data which would not violate the homocedasticity and normality of the sample. The female mating index, female fertility index, index of implantation/group and index of resorption/group were submitted to a non parametric test Chi-square. The index of post implantation loss was submitted at Kruskall-Wallis non parametric analysis. The significance test level was α = 0.05.

The experimental protocol was approved by the Ethics Committee on Animal Experimentation of UFJF, Minas Gerais, Brazil (Certificate 01/2007-CEEA).

RESULTS
The phytochemical characterization of the AEEg showed the presence of flavonoids, phenolic acids, triterpenes, saponins and tannins.

One rat from T-250 group and two from T-1000 group showed atrophy of one uterine cornua and no visual identification of implants, being eliminated of the experiment.

During the whole experiment, clinical signs of toxicity, such as presence of piloerection, deambulation alteration, diarrhea or death, weren’t observed in animals.

The body weight gain during 15 days of gestation was similar in pregnant rats of all the experimental groups (Fig. 1). The final body weight was similar in all the treatment groups when compared to the control group (Table 1). However, an increase in the liver relative weight was observed at 3 doses of treatment, an increase in the kidneys relative weight was observed at the doses of 500 and 1,000 mg/kg/day, and an increase in the spleen relative weight was observed at the dose of 1,000 mg/kg/day (Table 1).
A decrease in hematimetry, hemoglobin, hematocrit, and MGV, was observed in pregnant animals of T-250 group, a decrease in hematimetry and MGV was observed in animals of T-500 group, and a decrease in hematocrit, hemoglobin, MGV and an increase in total leukocyte count was observed in T-1000 group when compared to the control group (Table 2).

In relation to the biochemical data, an absolute increased in the dose-dependent way of the plasma cholesterol concentration was observed, though it had only been

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**Fig. 1.** Weight gain of pregnant Wistar rats which received saline solution (control) or AEEg administered by gavage at doses of 250 (T-250), 500 (T-500) and 1,000 (T-1000) mg/kg/day from the 1st to the 14th day of gestation.

**Table 1.** Final body weight, and absolute and relative weight of organs of pregnant Wistar rats which received saline solution (control) or AEEg administered by gavage at doses of 250 (T-250), 500 (T-500) and 1,000 (T-1000) mg/kg/day from the 1st to the 14th day post-insemination

<table>
<thead>
<tr>
<th>Organ Weight</th>
<th>Control (n = 16)</th>
<th>T-250 (n = 15)</th>
<th>T-500 (n = 16)</th>
<th>T-1000 (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight (g)</td>
<td>193.58 ± 13.21</td>
<td>193.55 ± 11.43</td>
<td>196.25 ± 16.10</td>
<td>197.20 ± 9.55</td>
</tr>
<tr>
<td>Liver absolute (g)</td>
<td>8.19 ± 0.89</td>
<td>9.22 ± 0.53*</td>
<td>8.98 ± 0.88*</td>
<td>9.68 ± 0.85*</td>
</tr>
<tr>
<td>Liver relative</td>
<td>4.22 ± 0.29</td>
<td>4.73 ± 0.20*</td>
<td>4.51 ± 0.17*</td>
<td>4.91 ± 0.43*</td>
</tr>
<tr>
<td>Kidneys absolute (g)</td>
<td>1.39 ± 0.13</td>
<td>1.44 ± 0.07</td>
<td>1.49 ± 0.12*</td>
<td>1.50 ± 0.12*</td>
</tr>
<tr>
<td>Kidneys relative</td>
<td>0.71 ± 0.06</td>
<td>0.74 ± 0.03</td>
<td>0.76 ± 0.05*</td>
<td>0.75 ± 0.02*</td>
</tr>
<tr>
<td>Spleen absolute (mg)</td>
<td>514.06 ± 71.90</td>
<td>552.53 ± 51.04</td>
<td>549.81 ± 68.68</td>
<td>649.43 ± 125.14*</td>
</tr>
<tr>
<td>Spleen relative</td>
<td>0.26 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.28 ± 0.03</td>
<td>0.32 ± 0.05*</td>
</tr>
<tr>
<td>Ovaries (mg)</td>
<td>68.06 ± 12.91</td>
<td>75.46 ± 13.41</td>
<td>74.94 ± 7.28</td>
<td>75.28 ± 8.09</td>
</tr>
<tr>
<td>Adrenal glands (mg)</td>
<td>68.62 ± 8.48</td>
<td>73.06 ± 7.30</td>
<td>69.56 ± 8.33</td>
<td>71.71 ± 13.40</td>
</tr>
</tbody>
</table>

Relative weight: absolute organ weight/final body weight X 100.
Results expressed as average ± standard-deviation (number of case studies).
*p < 0.05 in relation to the control group.
Table 2. Hematological and biochemical analysis of pregnant Wistar rats which received saline solution (control) or AEEg administered by gavage at doses of 250 (T-250), 500 (T-500) and 1,000 (T-1000) mg/kg/day from the 1st to the 14th day of pregnancy.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 16)</th>
<th>T-250 (n = 15)</th>
<th>T-500 (n = 16)</th>
<th>T-1000 (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematimetry (millions/mm³)</td>
<td>6.120 ± 0.388</td>
<td>5.875 ± 0.736*</td>
<td>6.105 ± 0.539*</td>
<td>5.869 ± 0.865</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.70 ± 4.50</td>
<td>35.46 ± 4.85*</td>
<td>39.42 ± 2.61</td>
<td>37.07 ± 4.87*</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.89 ± 0.82</td>
<td>11.76 ± 0.86*</td>
<td>12.14 ± 0.91</td>
<td>11.90 ± 0.59*</td>
</tr>
<tr>
<td>MGV (µ/m³)</td>
<td>69.99 ± 6.32</td>
<td>57.82 ± 5.49*</td>
<td>64.26 ± 5.83*</td>
<td>65.00 ± 4.54*</td>
</tr>
<tr>
<td>MGH (pg)</td>
<td>21.12 ± 1.58</td>
<td>20.02 ± 1.84</td>
<td>20.48 ± 1.87</td>
<td>20.5 ± 2.37</td>
</tr>
<tr>
<td>MGHC (g/dl)</td>
<td>30.43 ± 2.06</td>
<td>30.12 ± 6.35</td>
<td>31.69 ± 1.50</td>
<td>30.14 ± 2.76</td>
</tr>
<tr>
<td>Leukometry (cel/mm³)</td>
<td>5363 ± 1186</td>
<td>6106 ± 893</td>
<td>6287 ± 1985</td>
<td>8122 ± 2213*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>57.95 ± 10.90</td>
<td>61.13 ± 8.45</td>
<td>64.55 ± 12.04</td>
<td>69.15 ± 15.17*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>72.26 ± 25.52</td>
<td>85.86 ± 20.44</td>
<td>71.10 ± 33.22</td>
<td>82.18 ± 23.24</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>56.93 ± 11.67</td>
<td>52.33 ± 7.93</td>
<td>59.21 ± 12.50</td>
<td>56.92 ± 8.98</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.59 ± 0.10</td>
<td>0.70 ± 0.08</td>
<td>0.66 ± 0.12</td>
<td>0.62 ± 0.22</td>
</tr>
<tr>
<td>AST (u/l)</td>
<td>36.25 ± 11.72</td>
<td>54.66 ± 13.31*</td>
<td>43.20 ± 20.33*</td>
<td>64.50 ± 14.25*</td>
</tr>
<tr>
<td>ALT (u/l)</td>
<td>56.62 ± 18.75</td>
<td>47.00 ± 12.69</td>
<td>49.53 ± 14.08</td>
<td>62.64 ± 18.91</td>
</tr>
</tbody>
</table>

MGV (mean globular volume) = hematocrit x 10/hematimetry.
MGH (mean globular hemoglobin) = hemoglobin x 10/hematocrit.
MGHC (mean globular hemoglobin concentration) = Hb x 100/hematocrit.
AST: Aspartate transaminase
ALT: Alanine transaminase
Results expressed as average ± standard-deviation (number of case studies).
* p < 0.05 in relation to the control group.

significant in the T-1000 group (Table 2). An increased of the AST was observed at the 3 doses tested when compared to the control group (Table 2). The concentration of triglycerides, urea, creatinine, and ALT did not show any alterations when compared to the control group (Table 2).

The histopathological analysis of liver, kidneys and spleen of animals from T-250 group did not show any difference when compared to the control group (Figs. 2a, b and c).

In animals from T-500 group, the sections of liver presented large dilated terminal venules showing great congestion, associated with discrete inflammatory reaction, located mainly at the stroma which supports the portal space. The cellular infiltrate, surrounding at least 50% of the vessel wall, was predominantly composed of cells with morphology suggestive of lymphocyte cells (Fig. 3a). In all the sections of kidneys of animals of the same group, there was an expressive decrease in the capsular space, and focal areas of vasodilatation and congestion, as well as a discrete hyalinization (Figs. 3b and c).

The analysis of the spleen, liver and kidneys sections from the T-1000 group showed that, in all the sections, the white pulp in the spleen did not show evidence of antigenic stimulus, whereas the red pulp presented excessive chestnut pigmentation, suggestive of deposition hemosiderin in the whole compartment (Fig. 4a). All the liver sections showed congested terminal portal venules, and also vasodilatation in the terminal hepatic venules, in the sinusoids and in the space of Disse. Inflammatory alterations showed to be associated to the discrete mononuclear inflammatory infiltrate in most perivascular tissue (Fig. 4b). In all the kidneys section, it was registered vasodilatation of intertubular capillaries and intracytoplasmic hyalinization in cells of the proximal tubules (Fig. 4c).
Fig. 2.  a: Histological section of spleen of the control group presenting white pulp (white X), central arteriole (white asterisk), red pulp (black asterisk), and capillary sinuses (white arrows). HE stain. Magnification 400X. b: Histological section of liver of the control group presenting the elements of the portal triad (black arrows) and centrilobular venule (black X). HE stain. Magnification 250X. c: Histological section of kidney of the control group, cortical region, showing vascular pole of the corpuscle (V), outer layer of the capsule of Bowman (black arrow), inner layer of the capsule of Bowman (white arrow), capsular space (black asterisk), proximal tubule (black X), distal tubule (white X) and peritubular microvascularization (white asterisk). HE stain. Original magnification 400X.
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Fig. 3. a: Histological section of liver from T-500 group presenting congested terminal portal venules (black asterisk) and discrete mononuclear inflammatory infiltrate in the stroma which supports the portal space (arrows). The mononuclear inflammatory infiltrate was predominantly composed of cells with morphology suggestive of lymphocyte cells. HE stain. Magnification 250X. b: Histological section of kidney from T-500 group, cortical region, presenting a decrease in the capsular space (black asterisk). The light of the proximal tubules might disappear due to the routine histological procedure, even when the light of the distal tubule is kept (black X). HE stain. Magnification 400X. c: In the sections of cortical area of the kidneys of animals of the same group, there was an expressive decrease in the capsular space of the glomerulous, and focal areas of vasodilatation and congestion in peritubular vessels (black asterisk) and a discrete hyalinization in all most epithelial cells of the cortical tubules (white asterisk). HE stain. Magnification 400X.
Fig. 4. a: Histological section of spleen from T-1000 group presenting a red pulp with massive pigmentation suggestive of hemosiderin. HE stain. Magnification 250X. In detail: intracytoplasmic deposit. HE stain. Magnification 400X. b: Histological section of liver from T-1000 group, with terminal venules showing great congestion (white asterisk), mononuclear inflammatory infiltrate in the stroma which supports the portal space (arrows). HE stain. Magnification 250X. In detail: dilatation of the terminal hepatic venules (black asterisk) and hepatocytes in various death stages surrounding the dilated sinusoids (arrows). HE stain. Magnification 400X. c: Histological section of kidney from T-1000 group, cortical region, showing vasodilatation of intertubular capillaries (black asterisks) and glomerular vasodilatation (white asterisks), and hyalinization in the proximal tubules (white X). The distal tubules are within its normal conditions (black X). HE stain. Magnification 400X.
These observations suggest that the T-1000 group exhibit a subclinical signs of toxicity, expressed by the presence of the vascular reactions and leucocyte infiltration.

In relation to the reproductive performance, there were no differences in mating and fertility indices, in the total of corpora lutea and average per dam, total of implants and average per dam, average of fetuses per dam, number of resorptions, placental weight and litter weight, in the animals of the treatment groups when compared to the control group (Table 3). Likewise, the index of post-implantation loss, the implantation index per group and the resorption index per group were similar in all experimental groups (Table 3). No external malformation was observed in the fetuses.

**DISCUSSION**

The relationship of maternal toxicity and embryo/fetal toxicity is a matter of discussion (Khera, 1984, 1985; Chernoff et al., 1989; Chahoud et al., 1999), but the maternal metabolism, maternal stress, and some maternal toxicity may affect the conceptus development (Burdan et al., 2009). The end point of maternal toxicity includes clinical observation, body and organs weight and if necessary histopathological analysis of the organs.

Treated animals showed no clinical signs of toxicity, there were no body weight loss, but an increase in the relative liver weight at the three doses, kidneys at doses of 500 and 1,000 mg/kg/day, and spleen at the dose of 1,000 mg/kg/day were observed when compared to the control group, what could be an indicative of toxicity.

In the literature weren’t reported studies about the

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**Table 3.** Reproductive performance of pregnant Wistar rats which received saline solution (control) or AEEg administered by gavage at doses of 250 (T-250), 500 (T-500) and 1,000 (T-1000) mg/kg/day from the 1st to the 14th day post-insemination

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 16)</th>
<th>T-250 (n = 15)</th>
<th>T-500 (n = 16)</th>
<th>T-1000 (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mating index (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fertility index (%)</td>
<td>80</td>
<td>72</td>
<td>72</td>
<td>80</td>
</tr>
<tr>
<td>Number of corpora lutea</td>
<td>200</td>
<td>163</td>
<td>212</td>
<td>170</td>
</tr>
<tr>
<td>Average of corpora lutea/dam</td>
<td>12.5 ± 1.36</td>
<td>10.85 ± 1.29</td>
<td>13.25 ± 1.06</td>
<td>12.14 ± 1.40</td>
</tr>
<tr>
<td>Number of implants</td>
<td>184</td>
<td>159</td>
<td>180</td>
<td>154</td>
</tr>
<tr>
<td>Average of implants/dam</td>
<td>11.50 ± 1.67</td>
<td>10.73 ± 1.22</td>
<td>11.18 ± 2.80</td>
<td>11.00 ± 1.66</td>
</tr>
<tr>
<td>Number of fetuses</td>
<td>176</td>
<td>152</td>
<td>170</td>
<td>144</td>
</tr>
<tr>
<td>Average of fetuses/dam</td>
<td>11.0 ± 1.71</td>
<td>10.06 ± 1.57</td>
<td>10.62 ± 2.68</td>
<td>10.07 ± 1.68</td>
</tr>
<tr>
<td>Number of resorptions</td>
<td>8</td>
<td>7</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>1.55 ± 0.23</td>
<td>1.58 ± 0.28</td>
<td>1.52 ± 0.37</td>
<td>1.49 ± 0.25</td>
</tr>
<tr>
<td>Average litter weight (g)</td>
<td>1.85 ± 0.28</td>
<td>1.81 ± 0.41</td>
<td>1.82 ± 0.48</td>
<td>1.72 ± 0.31</td>
</tr>
<tr>
<td>Index of post-implantation loss</td>
<td>11.47 ± 4.89</td>
<td>13.58 ± 7.77</td>
<td>8.50 ± 1.34</td>
<td>10.86 ± 2.96</td>
</tr>
<tr>
<td>Index of implantation/group (%)</td>
<td>92.00 (184/200)</td>
<td>97.54 (159/163)</td>
<td>85.00 (180/212)</td>
<td>90.58 (154/170)</td>
</tr>
<tr>
<td>Index of resorption/group (%)</td>
<td>4.34 (8/184)</td>
<td>4.40 (7/159)</td>
<td>5.55 (10/180)</td>
<td>6.49 (10/154)</td>
</tr>
</tbody>
</table>

*: Results expressed as average ± standard-deviation (number of case studies).
Mating index = number females mated/number of cohabitated females X 100.
Fertility index = number of pregnant females/number of cohabitated females X 100.
Index of post-implantation loss: number of implants - number of living fetuses X 100/number of implants.
Index of implantation/group = number de implants/number of corpora lutea X 100.
Index of resorption/group = number of resorptions/number of implants X 100.
toxicity of *E. grandiflorus* in pregnant rats. Lopes et al. (2000) evaluated the toxicity of the *E. macrophyllus* in Swiss males mice and didn’t observed changes in liver weight, but a modest increase in the transaminase, what could be evidence of hepatotoxicity. In this work, animals of T-1000 group, showed histopathological changes suggestive of chronic inflammation in the liver, kidneys and spleen. The hepatic lesions observed are compatible with the finding laboratory data, like the increase in AST and in cholesterol.

Lopes et al. (2000) observed DNA damage in kidneys cells of Swiss mice treated with *E. macrophyllus*, and suggest that this result occurs by the presence of substances with potential genotoxic against the cells of this organ. However, Silva et al. (2010), observed low genotoxic activity through the Comet test. The kidneys are particularly vulnerable to toxic agents, due to high rate of perfusion and its capacity to concentrate a variety of substances in the tubular lumen (Finco, 1997). This vulnerability may increase during pregnancy because of the physiological enhance of the glomerular filtration rate. In the present study an increase in the relative kidneys weight of the animals of T-500 and T-1000 groups was observed and this may be caused by vasodilatation and obstruction of glomerular capillaries, and hyalinization in the proximal tubules, found in histopathological examinations. The renal lesion, however, not appear to induce changes in fetal development, as demonstrate by Kavlock et al. (1993).

The spleen participates in red blood cell storage and destruction in the red pulp, besides it is a source of an antigenic stimulus rapidly responsive and a source of immunoglobulin production in its white pulp. Due to its functional diversity, the spleen may be affected by several conditions which compromise blood metabolism or metabolism of lymphatic system, even as liver illness and infectious processes (Failace, 1992). Animals treated with 1,000 mg/kg/day of the AEEg had an increase in the relative spleen weight and presence of pigmentation suggestive of the hemosiderin in the whole red pulp in the spleen histological sections and this may be due to hematicy anemia or an acute episode of loss of hemoglobin in the urine (Failace, 1992).

Saponins are phenolic compounds present in the AEEg, which are able to interfere with the complexity of phospholipids of cell membranes, altering their permeability or causing their destruction (Schenkel et al., 2000). Therefore, it is possible that saponins may have caused the rupture of red blood cells with hemoglobin degradation and subsequent release of iron ion more intensely in group T-1000. The presence of hemosiderin in animal spleens of this group is compatible with the hematological observation of reticulocytosis and anemia, as well as hepatomegaly.

In this work, the anemia was observed in all treated groups, as Moreti et al. (2006), in an experiment carried out with male rats treated for 7 days with the infusion of *E. macrophyllus*. The anemia has many causes, such as reduction of erythropoiesis and the low concentration of hemoglobin. The hemoglobin is controlled by the hormone erythropoietin, produced in the kidneys as a response to the total oxygen needed in the organism (Miranda et al., 2009). The renal injuries may contribute to the reduction of erythropoiesis (Hutchinson and Jones, 1997; Miranda et al., 2009). The renal changes observed, such as glomerulonephritis associated with vasodilatation of intertubular capillaries and a more intense hyalinization in the proximal tubules, might be associated with toxicity and renal injury, leading to an increase in creatinine and anemia for insufficient erythropoietin. However, this hypothesis would be appropriate to groups where there were renal lesions, but does not explain the anemia observed in the group treated with lower dose.

The reproductive function of females treated with extract during 15 days, that is, three consecutive estrous cycles (Marcondes et al., 2002), evaluated by the mating index and fertility index, indicate that AEEg seem not to alter the physiological mechanisms involved in folliculogenesis, oogenesis, in the sexual behavior of females and fertilization.

Treated animals had reproductive performance similar to the control group. The index of implantation, which is an indicator of blastocyst implantation success in the endometrium, and the index of resorption, which indicates disturbances in embryonic development (Almeida and Lemonica, 2000), did not present significant differences among the experimental groups.

The maternal anemia could cause hypoxia and intrauterine growth retardation (Ergaz et al., 2005; Gortner et al., 2005), but the weight of the animals was similar in all experimental groups. Moreover, in all groups, the external morphology of fetuses did not present any alteration and the weight of placenta was similar between groups, suggesting that AEEg induced no alteration in the reproductive performance and no gross abnormalities.

Taken together, the data of the present work suggest that aqueous extract of *E. grandiflorus*, at higher doses may be toxic to the maternal liver, kidneys and spleen, and at all dose induced to anemia, though it did not lead to detectable clinical signs of toxicity. The reproductive performance was not altered and no external malformation was observed in the fetuses.
Reproductive toxicity of *Echinodorus grandiflorus* in pregnant rats.

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