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# Evaluation of the subchronic toxicity of oral treatment with *Chenopodium ambrosioides* in mice

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ABSTRACT

*Aim of the study:* The leaves of *Chenopodium ambrosioides* L. (Chenopodiaceae) have been used by native people to treat many diseases. Recently, we showed that the treatment with small dose (5 mg/kg) of hydroalcoholic extract (HE) from *Chenopodium ambrosioides*' leaves has immunestimulatory effects. The aim of this study was to investigate the subchronic toxicity of the oral treatment with this HE in preclinical assays.

*Material and methods:* Swiss mice were divided into 4 groups ($n = 10$/group). They received the HE daily at the doses of 5, 50 and 500 mg/kg by gavage during 15 days. The control group received only water. They were observed each hour for 24 h and each day for 15 days, when the blood was collected. The serum was used to perform the biochemical analysis. The mice were then killed and the vital and lymphoid organs were collected and evaluated.

*Results:* There was neither death nor alterations in the body weight in the HE-treated groups, but there were alterations in the weight of some organs. There was an increase in the lymph node cells number in the highest two doses. The number of cells in the bone marrow was high in the HE-treated groups, but the number of peritoneal cells was smaller in the HE-treated groups when compared to the control. There was no alteration in the AST, but there was a reduction in the albumin levels in the HE500 group and in the triglycerides and VLDL in the highest doses.

*Conclusion:* The subchronic treatment with HE induced punctual alterations in the groups treated with the highest doses. However, the HE treatment was not lethal and did not induce toxic alterations using the therapeutic dose, suggesting that it is safe to use this product in the adequate dose.



## 1. Introduction

Plants, vegetables and herbs used as food and in the folk treatment have been accepted currently as one of the main source of drug discovery and development, but only a few of them have been scientifically investigated, especially about their toxic aspects. One example is *Chenopodium ambrosioides* L. (Chenopodiaceae) which have been used for centuries by native people as anti-helmintics, as anti-inflammatory, as anti-tumoral, and as healer, including the healing of skin ulceration caused by the *Leishmania* species (Klicks, 1985; Franca et al., 1996). It is an herbaceous shrub known in Brazil as 'mastruz' or 'erva-de-Santa-Maria' and as American wormseed, goosefoot, 'epazote' and 'paico' in other countries of America.

Some of these activities have been scientifically confirmed using both the essential oil and the extracts from *C. ambrosioides* leaves such as the anti-helminthic effect against *Ancylostoma duodenale*, *Trichuris trichiura* and *Ascaris lumbricoides* (Giove, 1996; Macdonald et al., 2004), and also the anti-*Trypanosoma cruzi* (Kiuchi et al., 2002), anti-*Plasmodium falciparum* (Pollack et al., 1990) and anti-*Leishmania amazonensis* (Monzote et al., 2006; Patrício et al., 2008) activities. The anti-inflammatory and analgesic effect of *C. ambrosioides* was previously showed (Ibironke and Ajiboye, 2007), as well the immunestimulatory effect using lymphocytes (Rossi-Bergamann et al., 1997) and macrophages (Cruz et al., 2007), the anti-tumoral effect (Nascimento et al., 2006a), and the healer activity in skin lesions and bone fractures (Pinheiro Neto et al., 2005).

Despite the spreading use of *C. ambrosioides* in Latin America, especially in Brazil, and the promissory biological effects associated with this specie, it is empirically described by native people that the use of *C. ambrosioides* is toxic, and that this toxicity is abolished by mixing the leaves with milk before the ingestion.

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In fact, some cases of intoxication with the essential oil has been shown in human beings and rats specially when it was used in large amounts, inducing toxic effects in the kidneys, liver and gut (De Pascual et al., 1980; Chevallier, 1996; Ruffa et al., 2002). Likely these toxic effects are related to the presence of terpenoids that besides have diverse pharmacological properties, also have toxic aspects (Di Carlo et al., 1999; Kiuchi et al., 2002; Liu, 2004). The ascaridol, for example, a monoterpene found in the essential oil could explain these effects, together with the terpenic peroxide which corresponds to about 70% of oil components (De Pascual et al., 1980; Sagrero-Nieves and Bartley, 1995).

However the essential oil is not the material used by native people. Decoctions or infusions using the leaves are the most commons ways of use. Considering that there is only one study in rats suggesting a toxic effect chronic treatment with *C. ambrosioides* (Amole and Izegbu, 2005) and that there are no pre-clinical assays using mice treated subchronically by oral route with the extract from *C. ambrosioides* leaves; considering the potential biological activities showed to this extract; and also considering the presence of terpenoids, which are potentially toxic, it is essential to evaluate the toxicity of high doses of this product in subchronic oral treatment to really assure the safety of the use and dissemination of *C. ambrosioides* as a natural therapeutic possibility.

## 2. Materials and methods

### 2.1. Animals

Male and female Swiss mice of about 8–12 weeks old, 25 g weight were used, supplied by the Central Animal Facilities of Universidade Federal do Maranhão (UFMA) and kept in the Experiments Sector of the Animal Facilities of the Laboratory of Immunophysiology under standardized environmental conditions, fed with balanced diet and water *ad libitum*. The use of animals was approved by UFMA's Ethics Committee on the use of animals and is in conformity with the Brazilian College of Animal Testing (Protocol CEP/UFMA no. 012975/2008-43).

### 2.2. Plant material

Leaves of *Chenopodium ambrosioides* L. (Chenopodiaceae) were collected and identified at the Ático Seabra Herbarium of the Universidade Federal do Maranhão (São Luís, MA, Brazil) (voucher specimen N° 0998). The leaves were dried at 30 °C and powdered. Dry powdered leaves (200 g) were extracted with 1 L of ethanol (70%) and mixed each 8 h during 24 h. After this period the hydroalcoholic extract was filtered using a cotton funnel and the same procedure was repeated four times. After this process the hydroalcoholic extract (HE) was concentrated under low pressure. The yield obtained was 10.4% (w/w). Finally, the extract was dried and the remainder was later lyophilized.

### 2.3. Evaluation of subchronic toxicity of HE

The animals were weighed, divided into 4 groups ($n = 10$/group) and treated for 15 days by gavage with water (control) or HE at the doses of 5 (HE5), 50 (HE50) or 500 (HE500) mg of dried plant material/kg of body weight. These doses were chosen according to the previously described by Patrício et al. (2008), which showed that the oral therapeutic dose of HE was 5 mg/kg.

The mortality and the toxicity signs were analyzed according to the methodology described by Malone (1977). For each group it was offered 150 g of food and 250 mL of filtered water. Each 3 days, the mice were observed until 15 days after the beginning of treatment. In that observation period the following parameters were measured: weight, food and water consumption and fatality recording.

### 2.4. Biochemical parameters evaluation

The animals were anesthetized and blood samples were collected from the retro-orbital plexus and centrifuged at 150 g, for 10 min for biochemical analysis. The serum was used for determining the concentration of total protein, albumin, urea, aspartate aminotransferase (AST), total cholesterol (TC) and triglycerides (TG), by means of automatic procedures - Architect - C8000 (Abbott®), and the Labtest kits - Brazil.

### 2.5. Organs necropsy

After blood collection, the animals were sacrificed by cervical dislocation for removal of the liver, stomach, intestine, heart, kidney, spleen, lymph node and bone marrow. All organs were weighted and submitted to macroscopic analysis. Histopathological analysis was performed only in the liver, fixed with formalin and placed in paraffin. 5 μm diameter sections were obtained and stained with hematoxylin and eosin. Glass slides were analyzed and transversal sections were photographed with a photomicroscope Olympus PM-104 K3, at 400× magnification.

### 2.6. Peritoneal cell harvesting

The peritoneal cells were aseptically collected by washing the peritoneal cavity with 5 mL sterile ice-cold PBS devoid of calcium and magnesium ions. For total cell determination, nine volumes of peritoneal cells were added to 1 volume of 0.05% crystal violet dissolved in 30% acetic acid and the cells were counted using a bright-line hemocytometer (Sigma, St. Louis, MO, USA).

### 2.7. Spleen, lymph node and bone marrow's cells counting

After the collection of the peritoneal fluid, the spleen, the lymph node, and the femur were collected. The lymph node and spleen were processed in 1 mL or 5 mL of PBS, respectively. The femur was perfused with 1 mL of PBS to obtain the bone marrow's cells. For total cell determination, nine volumes of peritoneal cells were added to 1 volume of 0.05% crystal violet dissolved in 30% acetic acid and the cells were counted using a bright-line hemocytometer (Sigma, St. Louis, MO, USA).

### 2.8. Statistical analysis

Results were analyzed aided by the Graph Pad Prism Software, version 5.0, by one-way analysis of variance (ANOVA) followed by the Tukey test, using $p < 0.05$ as the level of significance. Data were expressed with mean ± standard error of mean (S.E.M.) of 10 animals per group and 5 animals per sex.

## 3. Results

### 3.1. Effect of oral treatment on the organs weight and the cellularity

The HE induced no alterations in the body weight. There was also no alteration of food and water consumption. There was no macro and microscopic alteration in the analyzed organs. There were alterations only in the weight of a few organs but this was not observed with all doses (Table 1).

There was an increase in the lymph node cells number in HE50 and HE500. The number of cells in the bone marrow was higher in the HE-treated groups. On the other hand, the number of peritoneal