Evaluation of the hepatotoxicity of Abarema cochliacarpos extracts in mice Mus musculus

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Abstract: Abarema cochliacarpos (Gomes) Barneby & J.W. Grimes, Fabaceae, is a native species of Brazil popularly known as “barbatimão”, frequently found along the north coast of the state of Bahia. Local communities make an infusion from its stem bark, which is used to treat several diseases. This study aimed to evaluate the hepatotoxicity of A. cochliacarpos extracts in mice Mus musculus. The bark infusion and hydroalcoholic extract were administered nasogastrically into two groups of eight animals (four male and four female each). After 45 days all mice were killed and the livers were collected for further histological analysis. Hepatic steatosis, congestion of the hepatic vessels and presence of macrophages and lymphocytes infiltrates in the liver, were observed in both group of animals, additionally animals that received the stem bark infusion presented an accumulation of pigments. None of the animals belonging to the negative control group showed any of the symptoms described above. In conclusion, the hydroalcoholic extract and infusion of A. cochliacarpos stem bark were proven to cause intoxication in mice. The hepatotoxicity of the infusion was more aggressive in females. Further studies are necessary to isolate compounds responsible for the toxic characteristics of A. cochliacarpos.

Introduction

According to the World Health Organization, 80% of the world’s population use medicinal plants to treat health problems. In Brazil, the knowledge left behind by the Indigenous and African communities about medicinal plants is a powerful alternative for the treatment of several diseases. (Almeida, 2000; Albuquerque et al., 2007). Despite the pharmacological properties of these plants, its indiscriminate use can lead to serious asymptomatic effects, such as carcinogenesis, hepatotoxicity and nephrotoxicity, as it has been described for extracts obtained from Symphytum officinale L., Boraginaceae (Ramos, 2005). Abarema cochliacarpos (Gomes) Barneby & J.W. Grimes, popularly known as "barbatimão", "babatemão" and "barbatião", belongs to the Fabaceae family and it is a medium size tree growing up to 8 m high (Santos, 2004; Silva et al., 2011). Silva and collaborators (2009) studying the chemical composition of the A. cochliacarpos reported the presence of saponins, catechins, tannins, phenols and anthraquinones in the extracts. Recently, Silva et al. (2011) evaluated the anti-inflammatory activity of A. cochliacarpos methanolic and butanolic extracts on Wistar mice and showed that the treatment with A. cochliacarpos extracts significantly decreased the symptoms of the disease on the intestinal mucosa. However, Melo and co-workers (2008) showed that the administration of either saponin or tannin fractions obtained from Mascagnia rigida Griseb., Malpighiaceae, caused an increase of the CK-MB enzyme, highlighting that M. rigida has the ability to cause damage to myocardial fibers.

According to Cotran et al. (2000), the liver can be stricken by a variety of metabolic, toxic, microbial, circulatory and neoplastic illnesses. A liver injury may be visualized through the accumulation of fat droplets within hepatocytes (steatosis), badly stained and mummified hepatocytes, necrosis, parenchyma with clumps of inflammatory cells (inflammation), yellowish skin caused by bilirubin retention (jaundice), or parenchymal damage caused by the destruction of hepatocytes (liver failure and cirrhosis).
The increase in liver fat, even without any alcohol consumption, can cause cirrhosis, and in some cases even liver cancer. The steatohepatitis affects children, but can also affect adults between the ages of 20 and 60 years old. In general, women are more affected by this disease (Campos, 2007).

The low output caused by veno-occlusive diseases and other liver damages can lead to portal hypertension followed by venous congestion and dilation on the microcirculation, thereby altering the transport of nutrients and hepatotrophic factors. Liver sinusoidal lesions can also lead to fibrosis and cirrhosis, causing the death of the organ (Aguiar et al., 2011).

Liver cells secrete phospholipids which accounts for up to 2% of the bile weight (e.g. lecithin) through the membrane-associated protein transporter MDR3 (Multidrug Resistance Protein 3). Cholesterol is also secreted and accounts for up to 4% of the bile weight. Cholesterol binds to two specific transporters (ABCG5/ G8), and is secreted along with the bile salts (Ikonen, 2006).

The inflammation is an immune system response against the infection that arises over the course of the liver injury. It is characterized by the infiltration or clusters of white blood cells scattered throughout the lobes of the liver parenchyma. Regarding liver poisoning, in a survey about drug poisoning among the elderly, Bernardes et al. (2005) showed that aging process is associated with pharmacokinetic and pharmacodynamic changes, such as body composition, basal metabolism, hepatic blood flow and glomerular filtration rate. Therefore, drugs prescriptions for the elderly should take into account aspects such as decreased muscle mass, which reduces the distribution of the drug, as well as a narrow therapeutic window, which makes therapeutic doses much closer to toxic doses.

Since the medicinal properties of A. cochliacarpos are well known and described by local communities, this study aimed to evaluate the hepatotoxicity of its extracts on mice Mus musculus.

Materials and Methods

Plant material

Drying, selection and storage conditions were performed according to the rules described in Campos (1991) and the Resolution-RDC No. 17 of 24th February 2000. Abarema cochliacarpos (Gomes) Barneby & J.W. Grimes, Fabaceae, stem bark was collected in the ecological park of Instituto de Desenvolvimento Sustentável do Litoral Norte da Bahia, located in Vila de Sauípe, coordinates 12° 33’16” South latitude and 037° 55’27” West longitude (Megallan, GPS 315), Mata de São João, Bahia, Brazil. The voucher specimen (51540) was identified by Dra. Hortensia Pousada Bautista and is stored at Radambrasil (HRB) herbarium, Salvador, Bahia. The plant material was collected in the morning and transported on trays lined with paper towels, as described by Campos (1991). The material was dried in an oven at 40-45 °C for twenty days. Dry weight was determined using an analytical balance (Mettler Toledo AG-285), until no variation in the weight was observed.

Preparation of the infusion

Dried stem bark (20 g) was used to prepare the infusion by adding 125 mL of boiling distillated water during 15 min. Dilutions of this infusion (12.5, 25 and 50% v/v) were prepared with distilled water.

Preparation of the crude extract

The dried and powdered stem bark (68 g) were extracted exhaustively (three consecutive times) by maceration, using ethanol 70% (200 mL), and leaving at room temperature, in the dark, for two days. The combined extract was filtered using filter paper (Qualy, 14 μm) and a vacuum pump (Marconi, MA 057/1). It was then concentrated under reduced pressure at 40 °C (Heidolph, Taiff 2100). Finally, the crude extract was lyophilized (Labconco Mark) to remove the solvent completely.

Animals

The number of swiss mice used in this work was established in accordance with the methodology described by Oliveira & Andrade (2001). Twenty adult swiss mice, supplied by the Laboratory of Environmental Studies at the Catholic University of Salvador, were divided into two groups of eight animals (four males and four females) plus a negative control group of four animals (two males and two females). Animals used in this experiment were 2.5 month old, weighing approximately 30 g each and were fed with rodent chow. Negative control animals were chosen randomly. Experiments using animals were approved of by the Ethics Committee on the Use of Animals in the School of Veterinary Medicine, Federal University of Bahia, protocol 05/2011.

Hepatotoxicity assay

Mice were deprived of food and water for 3 h prior to the administration of the infusion and crude extract dilutions. They were anesthetized with ether and a 5 cm of a nasogastric tube was introduced through their mouths into their stomachs. Within the first group of animals, four groups of two animals each (one male and one female) were assigned to receive different dilutions of the infusion. An aliquot of 1 mL of the bark infusion...
and its dilutions (12.5, 25 and 50% v/v) were administered by using nasogastric tubes and disposable syringes. The administration of the infusion was performed via oral route as this is the traditional way of taking the infusion by local population where the plant was collected. Similarly, the second group of animals was subdivided in four groups of two animals each (one male and one female). Different concentrations (125, 250, 500 and 1000 mg/mL) of the crude extract were prepared in water and 1 mL of each solution was administered using nasogastric tubes and disposable syringes. In total, four animals were used as negative control.

Histological studies

Histology slides were prepared according to the method described by Young & Heath (2001). Briefly, we used tissue samples from the liver of each animal and fixed in 37% formaldehyde, then submerged into paraffin. Tissue slices (5 μm) were obtained and stained with hematoxylin-eosin in accordance with the standard procedures for histological evaluation described by Timm (2005). All tissue slices were examined in an Olympus Color 3 (BX 51) microscope. Image-Pro-Plus® software was used to characterize histopathological changes. Besides the histological studies, the toxicity of A. cochliacarpos infusion and crude extract were evaluated by observing some characteristics in the animals’ behavior, such as breathing, feeding, abdominal aspect and locomotion.

Results and Discussion

Abarema cochliacarpos (Gomes) Barneby & J.W. Grimes, Fabaceae, infusion has been used by many communities in the treatment of several gastrointestinal disorders. However, indiscriminate use of plants in popular medicine can lead to serious asymptomatic effects (Ramos, 2005). After the administration of A. cochliacarpos bark infusion and crude extract in Swiss mice, changes in locomotion, breathing, feeding and abdominal aspect were observed. Steatosis was observed in the group of animals treated with the bark infusion and with the crude extract of A. cochliacarpos, evidenced by the accumulation of fat droplets within the hepatocytes (Figure 1). Steatosis, also known as fatty change, fatty degeneration or adipose degeneration, is characterized by the presence of fat droplets on the interior of the liver hepatocytes. This process is often associated with accumulations of lymphocytes and macrophages (inflammatory cell infiltration). Liver secretes bile on fats through ABCG5/G8 carriers, and this is the main form of excretion of cholesterol in the human body (Ikonen, 2006).

Figure 1. Effect of bark infusion and crude extract of Abarema cochliacarpos on liver of Swiss mice (steatosis). Histological appearance of animals treated with crude extract (A, male; C, female) and bark infusion (B, female; D, male) show accumulation of fat droplets (steatosis). Stained with hematoxylin and eosin. Original magnification: 100x.
These results are in agreement with Brito et al. (2001) which reported the effect of *Stryphnodendron obovatum* (family Fabaceae, subfamily Mimosoideae), also known as barbatimão, in young cattle. It was observed anatomical and histological changes associated with intoxication, like epithelial detachment and congestion, ulcers and focal hemorrhages throughout the digestive tract. According to Ramos (2005), indiscriminate use of *Symphytum officinale* L. extract and infusion can lead to several liver diseases. Rebecca and co-workers (2003) reported that *Stryphnodendron aqueous* extract hampers hepatic metabolism in three different ways: during oxidative phosphorylation, inhibition of mitochondrial electron transport and ATP synthesis inhibition. Both group of animals presented congestion of the hepatic vessels (Figure 2) and presence of macrophages and lymphocytes infiltrates (Figure 3C, D and E). However, presence of pigments in liver parenchyma was just observed in animals treated with the crude extract (Figure 3A and B). None of the animals in the negative control group showed hepatic steatosis, accumulation of pigments, congestion of the hepatic vessels, presence of macrophages and lymphocytes infiltrates.

According to Silva and collaborators (2010) *A. cochliacarpos* extracts are effective as healing, anti-ulcerogenic and analgesic. Silva (2004) described that extracts and infusions prepared with *A. cochliacarpos* stem bark induced hepatitis, steatosis and hepatic vessels congestion in agreement with results found in this study. Audi and colleagues (1999) reported the use of *S. adstringens* extracts in the treatment of induced gastric lesions in mice.

Phytochemical investigation on *A. cochliacarpos* has shown presence of saponins, catechins, tannins, phenols and anthraquinones, which may be responsible for the toxic and pharmacological properties of this plant (Santos et al., 2006; Silva et al., 2009).

**Figure 2.** Effect of bark infusion and crude extract of *Abarema cochliacarpos* on liver of Swiss mice (congestion of the hepatic vessels). Histological appearance of animals treated with crude extract (A, female; C and E, male) and bark infusion (B, female; D, male) show congestion of the hepatic vessels. Stained with hematoxylin and eosin. Original magnification: 100x.
Evaluation of the hepatotoxicity of *Abarema cochliacarpos* extracts in mice
*Mus musculus*
Roseli F. Oliveira et al.

**Conclusion**

Both *A. cochliacarpos* bark infusion and crude extract caused intoxication in mice, where histological studies showed the presence of steatosis, accumulation of pigments, congestion of the hepatic vessels and presence of macrophages and lymphocytes infiltrates in the liver. It was observed that intoxication was more aggressive among females. Even though several studies have been published regarding *A. cochliacarpos* activity in the treatment of different diseases, this report describes its hepatotoxicity and these results are especially important because many poor communities use *A. cochliacarpos* infusion to provide treatments for their illnesses.

Further phytochemical analysis of the bark infusion and crude extract is necessary to separate and identify chemical compounds responsible for the hepatotoxicity of *A. cochliacarpos* in mice *Mus musculus*.

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**Authors’ contributions**

RFO contributed in collecting plant sample and identification, confection of herbarium, running the laboratory work, analysis of the data and drafted the paper. CSO, PRCS and HAO contributed to biological studies. HPB contributed in plant identification and herbarium confection. PRR contributed to critical reading and draft of the manuscript. LGF and RCT designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

**Figure 3.** Effect of bark infusion and crude extract of *Abarema cochliacarpos* on liver of Swiss mice (pigments, macrophages and lymphocytes infiltrates). Histological appearance of animals treated with crude extract show presence of pigments in the liver parenchyma (A, male; B, female) and presence of macrophages and lymphocytes infiltrates (C, male; E, female). Animals treated with bark infusion (D, female) presence of macrophages and lymphocytes infiltrates. Stained with hematoxylin and eosin. Original magnification: 100X.
Reference text in the document is as follows:

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