Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
21(4): 710-714, Jul./Aug. 2011

Evaluation of acute toxicity of babassu mesocarp in mice

Elizabeth S. B. Barroqueiro, Fernanda S. B. Barroqueiro, Mayara T. Pinheiro, Márcia C. G. Maciel, Priscila S. Barcellos, Lucilene A. Silva, Adelson S. Lopes, Flávia R. F. Nascimento, Rosane N. M. Guerra*

Laboratório de Imunofisiologia, Centro de Ciências Biológicas e da Saúde, Universidade Federal do Maranhão, Brazil.

Article

Received 29 Jul 2010
Accepted 4 Jan 2011
Available online 8 Jul 2011

Keywords:
babassu
blood biochemistry
mesocarp
oral acute toxicity
Orbignya phalerata

ISSN 0102-695X
doi: 10.1590/S0102-695X2011005000121

Abstract: The safety of babassu mesocarp (*Orbignya phalerata* Mart., Arecaceae), which exhibited anti-inflammatory and antithrombotic activities, was evaluated by determining the potential acute toxicity in mice. A lyophilized ethanol extract of babassu mesocarp (BME) was administered to C3H/HePas mice (10/group) in a single dose of 1000, 3000 and 5000 mg/kg, by gavage. General behavior adverse effects and mortality were determined for up to fourteen days. Selected biochemical parameters including glucose, triacylglyceride, cholesterol, urea, alkaline phosphatase and creatinine were determined by colorimetric assay. The heart, liver, spleen, kidneys and brain were weighted and evaluated macro and microscopically. The median lethal dose (LD50) of BME was greater than 5000 mg/kg. No behavior or body weight alterations were detected after the treatment. The acute treatment with BME has no effect on macroscopic and microscopic aspect of examined organs. Instead, BME increased the alkaline phosphatase and reduced the urea concentration in all groups. A significant increase on triacylglyceride was detected in the group BME1000. In conclusion, the acute treatment with high doses of BME can affect some biochemical parameters with a long lasting effect, although any change was detected at tissue level or body and organ weight.

Introduction

Babassu is the popular name of *Orbignya phalerata* Mart. (syn. *Attalea glasmanii* Zona Palms) [Arecaceae (Palmae)]. This palm is found in the North, Northeast and Center-west regions of Brazil and it has been used by Apinaye and Guajajara Indians from northeastern Brazil, yielding a variety of important products. Babassu palms provide food, fuel, shelter, fiber, construction materials, medicine, and other basic necessities of life for these people (Balick, 1988). The babassu mesocarp is extracted, powdered and suspended in water for use by the Brazilian people as food supplement, since it is rich in carbohydrates and mineral salts (Gaitan et al., 1994). In the same way, it is also used in the folk medicine in the treatment of dysmenorrheal, constipation, obesity, rheumatism, tumor such as leukemia and venous disease (Silva & Parente, 2001; Caetano et al., 2002a; Agra et al., 2007) ulcerations, inflammatory related diseases such as colitis and arthritis and finally, and infectious diseases (Souza et al., 2011).

Preclinical evaluation of the therapeutic effects of babassu mesocarp has shown that this product possesses healing (Batista et al., 2006, Maciel et al., 2007), antitumor (Rennó et al., 2008; Fortes et al., 2009), anti-inflammatory (Ferreira et al., 2004; Nascimento et al., 2006), and antimicrobial properties (Caetano et al., 2002b). In addition, the mesocarp has a significant anti-thrombotic activity what seems to be related to a slow coagulation process and an enhanced ability of macrophages to produce nitric oxide after stimulation (Azevedo et al., 2007). Despite the biological activities of babassu mesocarp it is necessary to consider that this product is rich in some toxic compounds as tannins and saponins (Bandeira et al., 1986), which could be potentially toxic when used in high doses or continuously. Hence, in view of the results previously obtained by our group and others it is essential to evaluate the toxicity of high doses of this product to really assure the safety of the use and dissemination of babassu mesocarp as a natural therapeutic possibility.

Materials and Methods

Animals

Male and female C3H/HePas mice (10/group),

8-12 weeks old, 25 g weight were used, supplied by the Central Animal Facilities of Universidade Federal do Maranhão (UFMA) 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.

Plant material and babassu ethanolic extract (BME)

Babassu (*Orbignya phalerata* Mart., Arecaceae) mesocarp was obtained from mature coconuts. The fruits were opened and the mesocarp was separated with a spatula. The material obtained was dried at room temperature (±25 ºC) for three days and then at 45 ºC for 24 h. Once dry, the mesocarp was ground to a powder, called babassu mesocarp flour. The mesocarp flour was submitted to analysis of authenticity, integrity and purity, by physical-chemical tests such as standard chromatographic techniques. An authenticated voucher specimen is kept on file in Herbário Ático Seabra under the number 1135.

The babassu mesocarp flour (50 g) were suspended in 1 L of ethanol PA (Merck, Ma, Brazil). This suspension was shaken for 2 h, and then it was maintained for 30 min at 4 ºC. The suspension was centrifuged at 2000 x *g*. The supernatant was collected, filtered and diluted in PBS (Phosphate Buffered Solution, pH 7.2) to obtain the doses for mice treatment (1000, 3000 and 5000 mg/kg).

Treatment with BME

C3H/HePas mice received, by gavage, single doses of BME at 1000, 3000 and 5000 mg/kg. These doses were chosen based on the therapeutic dose ranging from 5 to 500 mg/kg, as previously described by Azevedo et al. (2007). Therefore, the doses used here were 10 to 100 x more concentrated that the high therapeutic dose. The control group received only PBS.

Acute toxicity assay

The mortality and the acute toxicity signs were analyzed according to the methodology described by Malone (1977) and observed until fourteen days after the treatment with BME. The weight was measured using an analytical scale (Marte). Blood samples were collected fourteen days after the treatment from the retro-orbital plexus and centrifuged at 1500 x *g*, for 10 min. The biochemical analysis was performed in serum for determining the concentration of glucose, urea, creatinine, alkaline phosphatase, total cholesterol and triacylglycerides using commercial kits (Labtest, Brazil). All individual samples were tested in triplicate.

The animals were sacrificed by cervical dislocation for removal of the heart, liver, spleen, kidney and brain. All organs were weighted and submitted to macroscopic analysis. Histological analysis was performed in all organs recovered, fixed with formalin and placed in paraffin. Sections with 5 µm of diameter 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 400x magnification.

Statistical analysis

Data are reported as means±standard deviation of ten mice/group. The results were analyzed statistically using the graph pad Prism software, version 5.0, by analysis of variance (ANOVA), followed by the Tukey-Kramer test, with $p<0.05$.

Results and Discussion

The correct application of therapeutic plants by the population requires the use of plant species whose efficacy as well as therapeutic safety has been validated scientifically. Preclinical studies conducted on mice have shown that the extract of babassu mesocarp produces both beneficial (Silva & Parente, 2001; Nascimento et al., 2006; Azevedo et al., 2007) and adverse effects (Gaitan et al., 1994), which need to be clearly established in order to guarantee the safe use of this product as medicine.

In this study, no death was observed fourteen days after acute treatment with high doses (1000, 3000 and 5000 mg/kg) of BME, so it was not possible to calculate the LD50, considered here as greater than 5000 mg/kg. The extract showed no significant toxicity on body weight, or on cutaneous, neurological or behavioral alterations based on the macroscopic observation according Schuchman (1984). Indeed, macroscopic and microscopic examination revealed the preservation of the heart, spleen, kidneys, liver and brain of BME-treated animals (Figure 1).

However, to clarify if the treatment has no adverse effect some biochemical analysis was performed (Table 1). It was found that serum alkaline phosphatase (AP) concentration was higher in almost all groups treated with BME. Only in BM1000 the values remain unchanged the when compared to control, indicating that acute treatment can affect this liver enzyme production. Since young mice (2 to 3 months) were used in this study, the alterations in alkaline phosphatase concentration might have been influenced by the age of the animal According to Emanuelli et al. (2008),

in young animals plasma concentrations of alkaline phosphatase might be altered by the kind and amount of the diet. Akaline phosphatase is a biochemical serum marker of cholestasis process and hepatic disorders (Emanuelli et al, 2008). No alteration was observed on microscopic examination indicating what phosphatase alkaline alteration is not sufficient to cause hepatic degeneration.

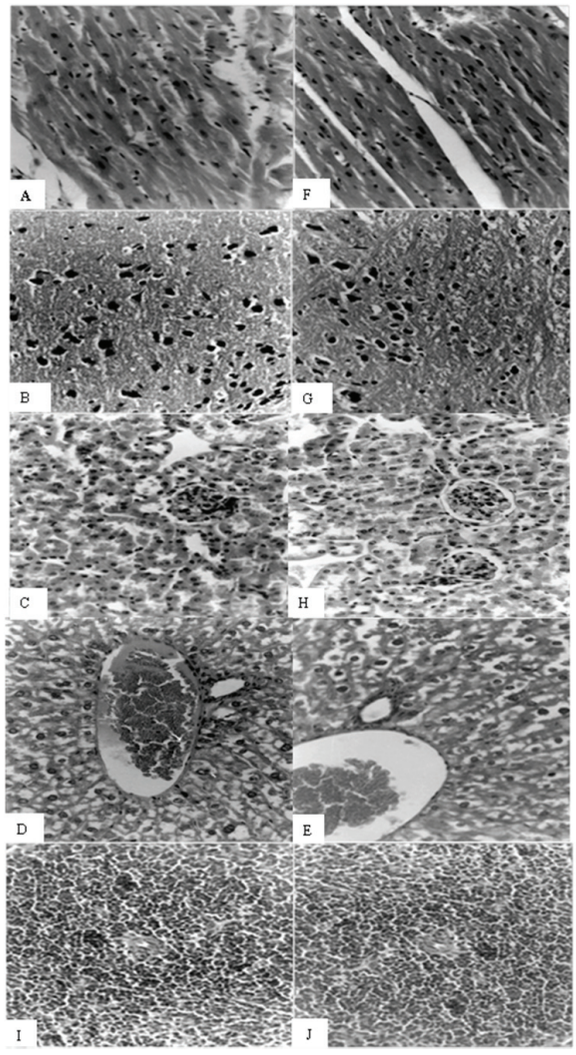

Figure 1. Histological analysis in organs from babassu mesocarp-treated mice. Microscopic analysis of histological sections obtained from control mice (A, B, C, D and E) and from mice treated with a single oral dose of BM (5000 mg/kg) (F, G, H, I and J). Hematoxylin-eosin-stained sections obtained from the heart (A, F), brain (B, G), kidney (C, H), liver (D, I), and spleen (E, J) were analyzed The photos show no alteration in all the organs (400 x magnification).

The BME treatment also caused a significant reduction of serum urea concentration. Since the babassu mesocarp contains only 1.54% of proteins (Rosenthal, 1975), our first hypothesis was that the low protein could be related to the low production of urea. However, this possibility could be ruled out since BME-treated animals had free access to the conventional food and the ingestion was quite similar to that observed in control group. It is known that, under normal metabolic conditions, urea is a final product of the degradation of excess amino acids and protein oxidation. Thus, the production and excretion of urea play an important role in the utilization of nitrogen by animals (Badaloo et al., 1999), therefore the mammals are unable to store amino acids or proteins. The fact that both carbohydrates and urea participate in protein metabolism suggests that the high carbohydrate concentration found in babassu mesocarp, about 68.3% (Rosenthal, 1975), may be related to either a low production or a high excretion of urea. These results could indicate a beneficial effect of BME in patients with acute renal failure, especially considering that urea production rates depend on protein intake and catabolism of endogenous proteins (Younes et al 2001). However, we must consider that the main causes of alterations in urea concentrations occurs in consequence of primary or secondary urea cycle disorders, at least in humans. The availability of carbohydrate due to its high content in babassu mesocarp may affect the nitrogen metabolism (Beames & Eggum, 1981; Mason, 1984; Macfarlane et al, 1986). When the intake of fermentable carbohydrate increases, this nitrogen may be insufficient for optimal bacterial growth. In such conditions, blood urea constitutes the largest and the most readily available nitrogen for bacterial protein synthesis in the cecum (Demigne & Rémésy, 1979; Viallard, 1984; Langran et al, 1992) and, as a result, fecal nitrogen excretion is significantly increased compared with fiber-free diets. (Younes et al, 1995; 1999). Thus, fermentable carbohydrate may exert a urea-lowering effect, in parallel to a decrease in urinary nitrogen excretion. So the decrease of urea levels in serum may be a direct effect of babassu mesocarp on urea-lowering effect or may be associated to other mechanisms rather than increased urea transfer in the large intestine due to its high content of carbohydrates. This point needs to be confirmed by other investigations.

Despite the serum urea levels can be used as a renal function indicator, this result must be added with the creatinine index. Creatinine index is more efficient to determine renal failure and is more constant than urea that is reabsorved on renal tubules (Schossler et al, 2001). On this work, the creatinine levels remains unchanged in all groups. In addition no microscopic alteration on kidney were detected.

Unexpected increase on triacylglyceride concentration was detected in the mice treated with the smallest dose of BME (1000 mg/kg). Carbohydrates are rapidly removed from the intestine to the blood,

Table 1. Biochemical profile in the serum of BM-treated mice. Serum concentrations of alkaline phosphatase, urea, triacylglycerides, glucose, cholesterol and creatinine were evaluated in mice treated by oral route with BM (1000, 3000 or 5000 mg/kg).

	Unit	Control[a]	BM 1000[a]	BM 3000[a]	BM 5000[a]
Alkaline phosphatase	U/L	6±1	5±1	23±2*	21±2*
Urea	mg/dL	40±4	27±2*	26±2*	26±2*
Triacylglycerides	mg/dL	54±6	104±8*	72±7	57±12
Glucose	mg/dL	100±6	83±5	82±7	83±5
Cholesterol	mg/dL	157±14	157±13	150±9	180±19
Creatinine	mg/dL	0.4±0.1	0.5±0.1	0.3±0.1	0.5±0.1

[a]Mean±SD of ten animals per group. *$p<0.05$ when compared to the control group.

and as a result, occurs an increase of lipogenesis what can be a risk cofactor for the future development of atherosclerosis and in a consequence can contributes to atherogenesis. In fact, there is a great content of carbohydrates in babassu mesocarp, so the increase of triacylglycerides can be a consequence of this excess of carbohydrate intake, wherever it can also be an isolated result because it did not occur with the other groups.

Despite the alterations mentioned above, serum glucose, cholesterol or creatinine concentrations remain unchanged after BME treatment at any of the doses tested (Table 1). This finding supports the hypothesis that the ethanolic extract of babassu mesocarp presents low acute toxicity.

With few exceptions, no consistent pattern of altered toxicological endpoints was identified in this evaluation of acute toxicity study in mice. Changes from control values were seen in biochemistry parameters with increases on alkaline phosphatase and reductions in urea. In all cases, no consistent, dose-related change was seen across groups within the same sex. It is important to emphasize that no organic microscopic were detected despite some the biochemical altered results, possible due to the low toxicity of the extract. Thus, these findings were considered incidental and unrelated to treatment. In conclusion, the administration of a single high dose of the BME induced very low acute toxicity or at least it has a great safety margin.

Acknowledgments

We thanks to CNPq for the financial support (Proc. Nº 478351/2006-2 and 477714/2007-2), for the postdoctoral fellowship to RNMG, for the researcher fellowship (Proc. N° 311339/2006-9) to FRFN, for Master fellowships to LAS, and MTP fellowships. We are also grateful to Fapema for master fellowship to MCGM and for scientific initiation fellowships to FB and PB.

References

Agra MF, Freitas PF, Barbosa-Filho JM 2007. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. *Rev Bras Farmacogn 17*: 114-140.

Azevedo AP, Farias JC, Costa GC, Ferreira SCP, Aragão-Filho WC, Sousa PRA, Pinheiro MT, Maciel MCG, Silva LA, Lopes AS, Barroqueiro ESB, Borges MOR, Guerra RNM, Nascimento FRF. 2007 Anti-thrombotic effect of chronic oral treatment with Orbignya phalerata Mart. *J Ethnopharmacol 111*: 115-159.

Badaloo A, Boyne M, Reid M, Persuad C, Forrester T, Millward DJ, Jackson AA 1999. Dietary protein growth and urea kinetics in severely malnourished children and during recovery. *J Nutr 129*: 969-979.

Balick MJ 1988. The use of palms by the Apinayé and Guajajara indians of northeastern. *Brazil Adv Econc Botany 6*: 65-90.

Bandeira MAM, Matos MEO, Matos FJA, Maia MBS 1986. Contribuição ao estudo químico do côco babaçu. *Rev Bras Farmacogn 1*: 27-28.

Batista CP, Torres OJM, Matias JEF, Moreira ATR, Colman D, Lima JHF, Macri MM, Rauen JR, RJ, Ferreira LM, Freitas ASCT 2006. Effect of extract of *Orbignya phalerata* (babassu) in the gastric healing in rats: morphologic and tensiometric study. *Acta Cirurg Bras 21*: 26-32.

Beames RM, Eggum BO 1981. The effect of type and level of protein, fiber and starch on nitrogen excretion patterns in rats. *Br J Nutr 46*: 301-313.

Caetano N, Saraiva A, Pereira R, Carvalho D, Pimentel MCB, Maia MBS 2002a. Determinação de atividade antimicrobiana de extratos de plantas de uso popular como antiflamatório. *Rev Bras Farmacogn 12*: 132-135.

Caetano N, Saraiva A, Pereira R, Carvalho D, Pimentel MCB, Maia MBS 2002b. Determinação de atividade antimicrobiana de extratos de plantas de uso popular como antiflamatório. *Rev Bras Farmacogn 12*: 132-135.

Demigné C, Rémésy C 1979. Urea cycling and ammonia absorption *in vivo* in the digestive tract of the rat. *Ann Biol Anim Biophys 19*: 929-935.

Emanuelli MP, Lopes STA, Maciel RM, Garmatz BC, Tavares MO 2008. Concentração sérica de fosfatase alcalina gama-glutamil transferase uréia e creatinina em

coelhos (*Oryctolagus cuniculus*). *Cienc Animal Brasil 9*: 251-255.

Ferreira SCP, Frazão JB, Silva LA, Maciel MCG, Barroqueiro ESB, Azevedo APS, Lopes AS, Guerra RNM, Nascimento FRF 2004. Efeito do mesocarpo do babaçu sobre a inflamação tardia. *Rev Cienc Saude 5*: 11-17.

Fortes TS, Fialho EMS, Reis AS, Assunção AKM, Azevedo APS, Barroqueiro ESB, Guerra RNM, Nascimento, FRF 2009. Desenvolvimento do tumor de Ehrlich em camundongos após tratamento *in vitro* com mescocarpo de babaçu. *Rev Cienc Saude 11*: 101-105.

Gaitan E, Cooksey RC, Legan J, Lindsay RH, Ingbar SH, Mede*iros-Neto G 1994. Antithyroid effects in vivo and in vitro* of babassu and mandioca: A staple food in goiter areas of Brazil. *Eur J Endocrinol 131*: 138-144.

Langran M, Moran BJ, Murphy JL, Jackson AA 1992. Adaptation to a diet low in protein: Effect of complex carbohydrate upon urea kinetics in normal man. *Clin Sci 82*: 191-198.

Macfarlane GT, Cummings JH, Allison C 1986. Protein degradation by human intestinal bacteria. *J Gen Microbiol 132*: 1647-1656.

Maciel MCG, Barroqueiro ESB, Azevedo APS, Silva LA, Guerra RNM, Nascimento FRF 2007. Avaliação do efeito cicatrizante do mesocarpo de babaçu (*Orbignya phalerata*) em feridas de pele induzidas em camundongos. *Rev Cienc Saude 9*: 11-18.

Mason VC 1984. Metabolism of nitrogenous compounds in the large gut. *Proc Nutr Soc 43*: 45-53.

Malone MH 1977. Pharmacological approaches to natural products screening and evaluation. In: Wagner H, Wolf P (eds), *Natural products and plant drugs with pharmacological, biological or therapeutical activity.* Springer-Verlag, Berlin, p. 23-53.

Nascimento FRF, Barroqueiro ESB, Azevedo APS, Lopes AS, Ferreira SCP, Silva LA, Maciel MCG, Rodriguez D, Guerra RNM 2006. Macrophage activation induced by *Orbignya phalerata. Mart J Ethnopharmacol 103*: 53-58.

Rennó MN, Barbosa GM, Zacan P, Veiga VF, Alviano CS, Sola-Penna M, Menezes FS, Holandino, C 2008. Crude ethanol extract from babassu (*Orbignya speciosa*) cytotoxicity on tumoral and non-tumoral cell lines. *Ann Braz Acad Sci 80*: 467-476.

Rosenthal FRT 1975. O amido do coco de babaçu algumas propriedades dos grânulos e das pastas. *Rev Bras Tecnol 6*: 6-29.

Schossler D, Alievi MM, Emanuelli MP, Schossler JP 2001. Função renal de cães tratados com doses terapêuticas de flunixin meglumine e ketoprofeno durante e trans e pós-operatório. *Acta Cirurg Bras 16*: 46-51.

Schuchman SM 1984. Cuidados e tratamento individual de coelhos, camundongos, ratos cobaios, *hamsters* e gerbilos. In: Kirk RW. Atualização terapêutica veterinária de pequenos animais. São Paulo: Malone. p. 825-855.

Silva BP, Parente JP 2001. An anti-inflammatory and immunomodulatory polysaccharide from *Orbignya phalerata. Fitoterapia 72*: 887-893.

Souza MHSL, Monteiro CA, Figueredo PMS, Nascimento FRF, Guerra RNM 2011. Ethnopharmacological use of babassu (*Orbignya phalerata* Mart) in communities of babassu nut breakers in Maranhão, Brazil. *J Ethnopharmacol 133*: 1-5.

Viallard V 1984. Endogenous urea as a nitrogen source for microorganisms of the rabbit digestive tract. *Ann Nutr Metab 28*: 151-155.

Younes H, Alphonse JC, Behr SR, Demigné C, Rémésy C 1999. Role of fermentable carbohydrate supplements with a low protein diet in the course of chronic renal failure: Experimental bases. *Am J Kidney Dis 4*: 1-15.

Younes H, Alphonse JC, Hadj-Abdelkader M, Rémésy C 2001. Fermentable carbohydrate and digestive nitrogen excretion. *J Renal Nutr 11*: 139-148.

Younes H, Garleb K, Behr S 1995. Fermentable fibers or oligosaccharides reduce urinary nitrogen excretion by increasing urea disposal in the cecum. *J Nutr 125*: 1010-1016.

***Correspondence**

Rosane N. M. Guerra
Laboratório de Imunofisiologia, CCBS, Bloco 1, sl 1ª, Universidade Federal do Maranão
Av dos Portugueses s/nº, Campus do Bacanga, 65085-580 São Luís-MA, Brazil
rnmguerra@pq.cnpq.br
Tel. +55 98 3301 8548
Fax: +55 98 3231 6844