The effects of aqueous extract of babassu (*Orbignya phalerata*) on the pleura and lung parenchyma in rats[1]

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ABSTRACT

PURPOSE: To evaluate macro and microscopically, changes following the use of the aqueous extract of babassu (*Orbignya phalerata*) in the lung parenchyma and pleura of rats.

METHODS: Sixty adult male rats with average weight of 350 g, were randomized into two groups of 30 animals (experimental and control) further divided into sub-groups of 10 to be sacrificed at 48 h, 72 h and 21 days. The substance was injected into the right pleura of the animals.

RESULTS: There was intense pleuropulmonary macroscopic reaction with statistically significant differences between groups respectively ($p<0.05$, $p<0.02$, $p<0.03$). Microscopically, no statistically significant difference was evident ($p>0.05$).

CONCLUSION: The aqueous extract of babassu (*Orbignya phalerata)* was found to be highly irritating to the pleura and lung of rats, evidenced macroscopically by numerous adhesions and inflammation while no major changes were evident microscopically.

Key words: Plants, Medicinal. Phytotherapy. Pleura. Lung. Rats.

Introduction

Pleurodesis is a well-established treatment for patients with spontaneous pneumothorax or malignant pleural effusion of multiple causes. The objective of pleurodesis is to promote the symphysis of the parietal and visceral pleura by inducing fibrosis, with consequent obliteration of the pleural space, thereby preventing the accumulation of air and fluid in the pleural cavity[1-5].

The ideal agent for pleurodesis has yet to be found. Several substances have been studied both clinically and experimentally, such as ethanolamine oleate, diazepam, erythromycin, macrolides, quinolones, povidone-iodine, in search of an agent with the following characteristics: effective, widely available, low cost, and few side effects[6]. Currently, the most commonly used agents are talc instilled into the pleural cavity by aspersion or dilution in saline solution, tetracycline derivatives (minocycline and doxycycline), and bleomycin[7-10]. In the 1980s, tetracycline was the preferred agent for chemical pleurodesis. However, manufacture of tetracycline has ceased and is therefore no longer available for this application. Bleomycin is very high cost and less effective in the treatment of pleurodesis compared to other medications[11].

Babassu represents an abundant, economically important plant. The mesocarp of the fruit is rich in carbohydrates and minerals and exhibits anti-inflammatory activity[10]. Various publications have reported the use of babassu in a variety of different situations. Its use in bladder healing in rats has shown a microscopically favorable effect[11]. Also in rats, a similarly positive effect was observed both macro- and microscopically in cutaneous wounds, with action most evident during the first healing phase[12]. When administered intraperitoneally at a dose of 50 mg/kg of bodyweight, full coaptation of the edges of gastric wounds[13,14] and enhanced healing in the abdominal alba line were observed[15].

Regarding pleuropulmonary changes, no studies are available investigating the use of babassu. Westphal FL et al., studying changes after injection of *copaíba* (*Copaifera* sp), observed major irritation in the rat´s pleura[16].

Pharmacological trials using the mesocarp of babassu have proven the potent anti-inflammatory action of powder extract in several experimental models of inflammation, confirming its action as a healing and anti-inflammatory agent[17].

The objective of this study was to verify if the aqueous extract of *Orbignya phalerata* can induce changes in the pleura and lung parenchyma of rats.

Methods

This study was carried out at the Central Animal House, Universidade Federal do Maranhão (UFMA). The study had an experimental, prospective, randomized design and was based on the following criteria: animals that are easy to breed and keep; low-cost and painless anesthesia technique; minimally invasive surgical approach; and requiring no special care postoperatively.

The experimental protocol of this study was approved by the Ethics Committee on Animal Use (CEUA 23115.017952/2013-01) of the Federal University of Maranhão.

During the operative procedure, the animals breathed spontaneously without use of respiratory support.

Study design and animal handling

In accordance with the established criteria, 60 *Rattus norvegicus albinus,* Rodentia mammalia*,* Wistar, male, adults, with an average weight of 350 g were selected. Animals were treated and housed in individual drawer-type cages of stainless steel wire mesh with hole size of 10 mm, measuring 23x20x25 cm, the rear of which was protected by 10x10mm square mesh screen. The cage contained a stainless steel feeder and a 250 ml polypropylene drinking vessel fitted with anti-acid rubber stopper and stainless steel drinking nipple. Cages were kept under a controlled environment with control of luminosity intensity and duration, temperature, ventilation, noise and odors with water and feed provided ad libitum. Animals were randomized into two groups of 30 as follows: control group (treated with 0.9% physiological saline solution) and babassu group (treated with pure aqueous extract). Each group was then divided into three sub-groups of 10 animals, according to the pre-established timepoints for application of the substances, observation of the animals and histological analysis at 48 h, 72 h and 21 days.

Preparation of extract and operative procedures

For the preparation of the aqueous extract, the powder was weighed and diluted in distilled water to a concentration of 25 mg/ml (10 g/400 ml distilled water) for the 50 mg/kg (concentration of 25 mg/ml) dose used directly in the pleural space. Thus, a dose of 0.8 ml of solution was administered for every 400 g of bodyweight of the animal.

The mesocarp of the coconuts was removed using a wooden club. The top of the coconuts were beaten by hand until

rupture of the shells. The exposed material (mesocarp) was then removed with a spatula and placed on the bench top for three days to dry. When dried, the material was placed in a drying oven at a temperature of 45-50º C for 24 hours to complete the removal of moisture. The dried material was subjected to a grinding process in an electric mill at the Technological Pavilion Laboratory, UFMA, yielding a flour powder.

All procedures were performed under intramuscular anesthesia using 5% ketamine hydrochloride at 60 ml/kg of bodyweight in association with 2% xylazine hydrochloride at 10 mg/kg of bodyweight. The route of administration was via the posterior face of the thigh, preceded by local asepsis, in the subxiphoid region. At the site, hair was removed for 1 cm^2 and the area disinfected with povidone-iodine. The product was injected trans-diaphragmatically into the right pleural cavity using a BD12F peridural anesthesia needle. For more rapid recovery and a lower level of suffering, stress and mortality, the rats were submitted to post-anesthetic oxygen therapy for approximately 10 min before being placed in their respective cages.

The animals were sacrificed at 48 hours, 72 hours and 21 days after injection of the selected substance.

Histopathological analysis was performed by an experienced pathologist, ensuring blinded assessment, under an optical microscope of 5 mm-thick histological slices stained with H&E. The histopathological variables were studied over the whole histological section.

The semiquantitative method was employed to analyze the intensity/degree of inflammatory reaction based on a score of 0 to 3. A score of 0 indicated the inflammatory response was "absent" (inflammation parameters undetected); 1 "mild" (inflammation parameters in up to 20% of sample); 2 "moderate" (inflammation parameters between 20% and 50% of sample); and 3 "severe" (inflammation parameters in over 50% of the sample). For grading, the presence of the following histopathological findings was considered: vascular congestion (blood flow increase/vasodilatation), edema ("negative image" pleural/interstitial/alveolar wall) and inflammatory pleural/intra-alveolar/interstitial/peribronchial cell infiltration (leukocytes mono and/or polymorphs).

Macro and microscopic assessments

The following parameters were adopted for the macroscopic analyses and classified as described by Tonietto *et al*.[16]: grade 0=no macroscopic changes; grade 1=presence of exsudate, without fibrinous reaction and/or adhesions; grade 2=presence of exsudate, with mild fibrinous reaction and slight adhesions; grade 3=presence of exsudate, with moderate fibrinous reaction and moderate adhesions; grade 4=absence of exsudate, lung stuck by intense fibrinous reaction and numerous adhesions.

The following two classifications were used in the analysis of microscopic changes: one denoting the grade of inflammatory reaction and the other categorizing the type of reaction as acute or chronic. The neutrophilic infiltrate, interstitial edema and vascular congestion were the indicators of the acute inflammatory process. The presence of vascular proliferation, mononuclear cell infiltration, fibroblast proliferation, fibrosis (collagen deposition) were chronic process. The following grades were used to assess the reaction: grade 0=no microscopic change; grade 2=mild inflammatory reaction; grade 2=moderate inflammatory reaction; grade 3=severe inflammatory reaction.

Statistical analysis

The data were assessed using the statistics program IBM SPSS Statistics 20 (2011). Graphs and tables depicting the frequency of variables were first produced. Subsequently, the Chi-square ($\chi 2$) test of independence was applied to assess the association of grade of reaction, type of reaction, pneumonitis and macroscopic analysis for the two groups (control and babassu). Non-parametric Mann-Whitney test was employed to assess the effect of babassu on each day and overall on the ordinal variables grade of reaction, reaction type and macroscopic analysis where the non-parametric Kruskall-Wallis and Student-Newman-Keuls post-hoc test for pair-wise comparisons were used to assess the time effect (48 h, 72 h and 21 days) within each group (control and babassu). A 5% level of significance (α) was adopted and considered significant for values of $p<0.05$.

Results

The variables grade of reaction, type of reaction and pneumonitis showed no significant difference ($p>0.05$) between groups. However, the groups differed significantly ($p<0.05$, Table 1) on macroscopic analysis, with 73.3% of the babassu group exhibiting some degree of macroscopic change and 90% of the control group showing no changes (Table 2).

TABLE 1 – Distribution according to grade and type of reaction, presence of pneumonitis and macroscopy by group.

Variable	Group					p
	Control	%	Babassu	%	Total	
Grade of reaction						
Grade 0	19	63.3	12	40.0	31	
Grade 1	11	36.7	15	50.0	26	0.074
Grade 2	0	0.0	3	10.0	3	
Type of reaction						
Absent	19	63.3	12	40.0	31	
Acute (A)	7	23.3	6	20.0	13	0.059
Chronic (B)	4	13.3	12	40.0	16	
Pneumonitis						
Yes	5	16.7	11	36.7	16	0.080
No	25	83.3	19	63.3	44	
Macroscopy						
Grade 0	27	90.0	8	26.7	35	
Grade 1	1	3.3	9	30.0	10	
Grade 2	0	0.0	3	10.0	3	0.000
Grade 3	0	0.0	2	6.7	2	
Grade 4	2	6.7	8	26.7	10	
Total	30	100.0	30	100.0	60	

Chi-square test.

TABLE 2 – Comparison of variable grades of reaction, type of reaction and macroscopy by group Mann-Whitney.

Variable	Group	n	Mean rank	Median	p
Grade of reaction	Control	30	26.5	Grade 0	0.042
	Babassu	30	34.6	Grade 1	
Type of reaction	Control	30	26.0	Absent	0.029
	Babassu	30	35.0	Acute (A)	
Macroscopy	Control	30	21.3	Grade 0	0.0001
	Babassu	30	39.8	Grade 1	

At 48 h and 72 h post-intervention, a significant favorable effect ($p<0.05$) of babassu was seen only on the macroscopic analysis; by contrast, at the 21° day post-intervention a significant damaging effect of babassu ($p<0.05$) was evidenced by the three variables (Tables 3 and 4).

TABLE 3 - Comparison of variable grades of reaction, type of reaction and macroscopy by group at each timepoint using Mann-Whitney test.

Time-point	**Variable**	**Group**	**Mean rank**	**Median**	**p**
48 h	Grade of reaction	Control	26.5	Grade 0/1	0.830
		Babassu	34.6	Grade 0	
	Type of reaction	Control	26.0	Absent/ Acute (A)	0.702
		Babassu	35.0	Absent	
	Macroscopy	Control	21.3	Grade 0	0.045
		Babassu	39.8	Grade 1	
72 h	Grade of reaction	Control	9.5	Grade 0	0.342
		Babassu	11.5	Grade 0	
	Type of reaction	Control	9.5	Absent	0.335
		Babassu	11.5	Absent	
	Macroscopy	Control	6.9	Grade 0	0.002
		Babassu	14.2	Grade 1	
21 days	Grade of reaction	Control	7.1	Grade 0	0.003
		Babassu	13.9	Grade 1	
	Type of reaction	Control	6.7	Absent	0.001
		Babassu	14.3	Chronic (C)	
	Macroscopy	Control	7.1	Grade 0	0.003
		Babassu	14.0	Grade 4	

TABLE 4 – Comparison of variable grades of reaction, type of reaction and macroscopy for timepoint within each group using Kruskal-Wallis test.

Timepoint	Variable	Timepoint	Mean rank	Median	p
Control	Grade of reaction	48 h	17.5	Grade 0/1	0.379
		72 h	13.0	Grade 0	
		21 days	16.0	Grade 0	
	Type of reaction	48 h	17.1	Absent/ Acute (A)	0.470
		72 h	13.2	Absent	
		21 days	16.3	Absent	
	Macroscopy	48 h	15.6	Grade 0	0.996
		72 h	15.4	Grade 0	
		21 days	15.6	Grade 0	
Babassu	Grade of reaction	48 h	12.8 [b]	Grade 0	0.008
		72 h	11.9 [b]	Grade 0	
		21 days	21.8 [a]	Grade 1	
	Type of reaction	48 h	11.0 [b]	Absent	0.001
		72 h	11.9 [b]	Absent	
		21 days	23.6 [a]	Chronic (C)	
	Macroscopy	48 h	11.6 [b]	Grade 1	0.019
		72 h	13.4 [b]	Grade 1	
		21 days	21.6 [a]	Grade 4	

[a,b]Different letters indicate $p<0.05$ on the Student-Newman-Keuls test.

Discussion

This is the first report describing an experimental pleurodesis model.

In the present study, a natural substance known popularly and scientifically for its anti-inflammatory and healing actions was used. Factors influencing the decision to use babassu were its ease obtainment, applicability to animals, low cost and the refining experience of the university staff[9,12,14].

In this study, the aqueous extract of *Orbignya phalerata,* at the dose administered, promoted no adverse reactions like other drugs in literature. Godazandeh *et al.*[11], when using povidone-iodine, noted dyspnea, cough and chest pain, symptoms not observed in the use of babassu.

No previous experimental studies administering *Orbignya phalerata* to rat pleura were found in the related literature. Babassu is a plant of the *palmae* family native to the mid-north of Brazil and found predominantly in the state of Maranhão[25].

Previous studies on the healing of the stomach and bladder after intraperitonial use of aqueous extract of the substance showed an enhancing action in macro and microscopic healing[14].

To prove efficiency, semiquantitative scoring methods are widely used for obtaining histological information in biomedical research. These methods are employed in experimental studies on animals. Klopfleisch[18] in a systematic review of semiquantitative score systems and multiparameters for histopathological evaluation of various organs and tissues in rats, reinforced the validity of this methodology and cited several articles, employing the 0-3 rating scale for several parameters, including assessment of inflammatory response in lung tissue. With regard to the "quality/type of inflammation", this was classified as "acute" or "chronic" based on the predominant cell type in the inflammatory infiltrate or tissue samples. Tissues with a predominance of polymorphonuclear leukocytes (neutrophils) were categorized as "acute inflammation" whereas samples with predominance of leukocyte/mononuclear cells (lymphocytes, plasma cells and macrophages) were categorized as "chronic inflammation". This stratification is based on the concepts of classic inflammatory reaction mechanisms, as described by Robbins *et al*.[19].

Based on the paper published by Batista *et al*.[26], with babassu in the stomach of rats, the peritoneum and the pleura seemed similar and thus may have the same effect on the formation of adhesions.

The variable grades of reaction, type of reaction and pneumonitis showed no significant difference ($p>0.05$) between groups, whereas a difference was found on macroscopic analysis with 73.3% of the babassu group exhibiting some degree of macroscopic change and the control group showing no changes[22].

For the groups at two and three days, only a weak action was observed in the experimental group – much lower than that seen at 21 days -, where this was attributed to short period of use of the substance; however, in the experimental and control groups at 21 days, moderate inflammatory changes were evident. Overall, there was a significant favorable effect of babassu on the variable grades of reaction, type of reaction and on macroscopic analysis. Compared to Teixeira *et al*.[23] results, babassu extract showed no significant difference.

The findings of babassu action on the pleural cavity differ to other substances in which the majority of rats exhibited

slight adhesions, as well as acute and mild inflammatory changes macroscopically at study endpoint[24].

There are many studies in the literature reporting the successful use of a variety of substances in pleurodesis[9,23,26]. However, substances such as talc, povidone, OK-432 and cytokine, are already in medical use, but no studies are available with phytotherapics, in this case with the use of *Orbignya phalerata* (babassu) or other plants from Brazilian flora.

Conclusion

The aqueous extract of babassu *(Orbignya phalerata)* was highly irritating to the pleura and pulmonary parenchyma of rats, evidenced macroscopically by numerous adhesions, while no major changes were evident microscopically

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