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Hepatic toxicity caused by PLGA-microspheres containing usnic acid from the lichen C*ladonia substellata* (AHTI) during pregnancy in Wistar rats

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ABSTRACT

This study aimed to evaluate the teratogenic and hepatotoxic potential of the usnic acid encapsulated into PLGA-microspheres. In total, 12 female Wistar rats in pregnancy were randomly distributed in the control group (n= 6) that received 1.0 mL of physiological solution and treatment group (n= 6) that received 25 mg/kg of encapsulated usnic acid by oral administration. All females were euthanized at day 20 of pregnancy and their fetuses were removed and analyzed. During the pregnancy was observed a reduction in weight gain. There was no difference in serum transaminases levels analyzed as well as any difference in liver weight in both groups. The histomorphometric analysis of the liver from the treatment group revealed an increase in number of hepatocytes and a decrease in nuclear area of these cells. Moreover, no alteration was observed in cell area of hepatocytes or number of Kupffer cells. The fetuses had an increase in total number of hepatocytes and a reduction in the amount of megakaryocytes. These results show the hepatotoxic potential of usnic acid during pregnancy. However, its toxicity can be minimized by encapsulation in microspheres.

Key words: encapsulation, fetotoxicity, hepatotoxicity, secondary metabolite.

INTRODUCTION

Usnic acid [2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyldibenzofuran-1,3-dione; $C_{18}H_{16}O_7$] is a chemical compound of natural origin, resulting from secondary lichen metabolites, which it is considered one of the most important biologically active metabolites produced by lichen (Muller 2001, Cocchietto et al. 2002).

Several studies have shown relevant pharmacological properties attributed to the usnic acid: Antibiotic (Segatore et al. 2012, Pompilio et

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al. 2013), antitumoral (Santos et al. 2006, Burlando et al. 2009), antiviral (Sokolov et al. 2012, Shtro et al. 2014), anti- inflammatory (Vijayakumar et al. 2000, Su et al. 2014), antioxidant (Behera et al. 2012, Suwalsky et al. 2015), wound healing properties (Nunes et al. 2011, Bruno et al. 2013), antifungal (Halama and Van 2004, Nithyanand et al. 2015), insecticide and larvicide (Sahib et al. 2008, Bomfim et al. 2009).

Although the usnic acid has remarkable pharmacological activity, its therapeutic application in the conventional manner is limited due to their unfavorable physicochemical properties, including low hydrosolubility, difficult interaction with biological barriers and high prevalence of hepatotoxicity (Cocchietto et al. 2002, Fraveau et al. 2002, Han et al. 2004, Pramyothin et al. 2004, Ribeiro-Costa et al. 2004, Sanchez et al. 2006, Santos et al. 2006).

Such limitation have driven the development of alternatives, an example is the encapsulation of usnic acid in controlled release systems, which reduces the hepatotoxic effects of the compound in the body, effectively preserve its active principle and potentiates its therapeutic application. A promising alternative related to the incorporation of new drugs on the market (Ribeiro-Costa et al. 2004, 2009, Santos et al. 2006, Siqueira-Moura et al. 2008, Grumezescu et al. 2014, Martinelli et al. 2014).

Encapsulated usnic acid in biodegradable polymeric microspheres, especially those microspheres constituted by Poly Lactic-co-Glycolic Acid (PLGA), have become an important means of drug release in order to reduce the liver toxicity (Ribeiro-Costa et al. 2004, 2009, Grumezescu et al. 2014).

Nowadays, despite the large investigation of the pharmacological use of usnic acid, data relating to human toxicity are very limited. Furthermore, its mechanism of action is not completely elucidated in the literature. Studies relate the mechanism of action of usnic acid with a similar effect to what happens to Carbon tetrachloride, acting as decoupling of the electron transport chain, affecting mitochondrial function and the cellular respiration (Fraveau et al. 2002, Han et al. 2004, Pramyothin et al. 2004, Joseph et al. 2009).

Studies on the evaluation of the toxicity of usnic acid, include the need for preclinical testing. Among them can be mentioned trials on the reproductive cycle to evaluate the effects of exposure during pregnancy. In addition, it is necessary to know the conditions for the safe use of usnic acid to the organism (Barros and Davino 2008, Lemonica 2008).

Considering the importance of understanding the effects of a particular drug in a biological system and the hepatotoxicity of usnic acid, this study aimed to evaluate the teratogenic and hepatotoxic potential of usnic acid encapsulated into PLGA- microspheres in female Wistar rats during pregnancy, as a way to neutralize its possible toxic effects during development of the organism.

MATERIALS AND METHODS

LICHEN MATERIALS: EXTRACTION, PURIFICATION AND CHARACTERIZATION OF USNIC ACID

The lichen *Cladonia substellata* (AHTI) was collected at the city of Mamanguape, Paraíba state, Brazil. The usnic acid (UA), the main substance of this study, was isolated, purified and characterized according to pre- established methodology at the laboratory of Natural Products on the Department of Biochemistry of the Federal University of Pernambuco (Asahina and Shibat 1954).

In order to obtain the Usnic Acid the lichen thallus of *Cladonia substellata* was totally macerated and subsequent a Soxhlet extractor was used for a refined extraction, per 72 hours in chloroform. After that the extracted material was submitted to rotary evaporator at 60 ºC until partial

evaporation of the solvent and later ending the total evaporation at room temperature. As a result of that, a yellow powder was obtained with impurities, which it was submitted to the purification and crystallization processes with chloroform/ice-cold ethanol used as solvents (1:3 v/v). Moreover, after 48 hours, this solution underwent the vacuum filtration process. Finally, the material trapped in the filter was recrystallized from *n*-Hexane, thus obtaining crystals of unic acid. Furthermore, the formation of those crystals was confirmed by Thin-Layer Chromatography (TLC). The highlighted bands were identified by retention factor (Rf) and subsequent it was compared to standard reference compound.

PLGA-MICROSPHERES PREPARATION AND USNIC ACID ENCAPSULATION

The microspheres of PLGA were prepared by multiple emulsion solvent evaporation method, according to the methodology established by Ribeiro-Costa et al. (2004). All those procedures were performed in partnership with the Laboratory of Immunopathology Keizo Asami (LIKA), Federal University of Pernambuco.

EXPERIMENTAL ANIMALS

12 female Wistar rats, virgins, weighing about 200-250 g at 60 days of age were utilized in this study. The animals were kept in cages lined in an air-conditioned room alternating light-dark periods of 12 hours and 12 hours at a controlled temperature of 25ºC ± 2ºC. In addition, all animals had free food and water access during the experiments. All procedures were carried out according to the international practices for animal use and care approved by the Ethics Committee on Animal Use – CEUA/UFPE, under protocol number 23076.029828/2013-94.

EXPERIMENTAL PROCEDURE

First of all, all females were submitted to the study of the estrous cycle in order to determine the fertile period. After confirming the time of ovulation, the females were paired with males (2:1) overnight and the mating was confirmed the next day with the presence of a vaginal plug (a white mass of sperm in the vaginal opening) or by the presence of sperm in the vaginal smear. The first 24 hours after confirmation of mating were considered as day zero of gestation (D0).

The females were randomly distributed in the control group (n=6) that received 1.0 mL of physiological solution and treatment group (n=6) that received 25 mg/kg of encapsulated usnic acid by oral administration. Dose based on the study of median lethal dose (or LD50). The administration happened once a day from the 6th to 15th day of pregnancy by oral gavage.

During the administration the females were weighed to evaluate the body mass gain at 0th, 6th, 10th, 14th and 20th days of pregnancy. After this period the females were anesthetized by Ketamine (80 mg/kg) and Xylazine (8 mg/kg) associated with Thiopental (100 mg/kg) by intramuscular injection, to collect 2 ml of blood through cardiac puncture. Then the animals were euthanized by deep anesthesia to access the uterus and to remove the fetuses. All fetuses were analyzed in the craniocaudal direction to evaluate the presence of external malformations. Subsequently, the fetuses and their placentas were weighed. After the Caesarean procedure, the fetus considered alive, in other words, those with movements in the hind limbs, following mechanical stimuli and respiratory signs, they were also euthanized.

BIOCHEMICAL ANALYSIS

Biochemical analyzes were conducted in all pregnant rats in late pregnancy to evaluate possible changes in liver function. Serum samples were

obtained from centrifugation the blood at 3500 rpm (KUBOTA, Tokyo, Japan) for 20 minutes, and subsequently subjected to conventional biochemical analysis. Serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were carried out by spectroscopy (Ultrospec 3000 Pro, Amersham Bioscience, Sweden) using biochemical assay kits (Katal Biotecnológica, Brazil).

HISTOMORPHOMETRIC ANALYSIS

In order to examine the hepatotoxicity, the liver of pregnant rats and their fetuses were dissected, weighed and subsequently subjected to histopathological analysis. Tissues were fixed in 10% neutral buffered formalin (NBF) for 24 hours, processed using routine histological techniques, and included in paraplast and sectioned in microtome into 4 μm thickness. Then, the tissue preparations were stained with hematoxylin and eosin (H.E.) and analyzed by light microscopy.

The photomicrographs were obtained at a magnification of 400 x using the *ScopePhoto* program. In order to obtain the photomicrographs it was used a digital capture camera (Moticam 2300), of 3.0 megapixels, coupled to an optical microscope (Nikon E-200). For morphometric measurements was used *ImageJ software* version 1.44 (*Research Services Branch, U.S. National Institutes of Health,* Bethesda, MD, USA). For fetuses, the following measurements were considered: Number of Hepatocytes (NH) and Number of Megakaryocytes (NM); while for the pregnant rats were considered: Number of Hepatocytes (NH), Number of Kupffer Cells (NKC), Cellular Area of Hepatocytes (CAH) and Nuclear Area of Hepatocytes (NAH). A total of 50 hepatocytes were accounted per animal for the measurement of CAH and NAH. Moreover, the areas of CAH and NAH were measured in square micrometer (μm^2).

STATISTIC

The values obtained of the average of each parameter analyzed were compared by *Student's t-test* and analysis of variance (ANOVA) of the software SPSS (*Statistical Package for the Social Sciences,* SPSS Inc. Chicago, EUA) version 15.0. The results were expressed as mean ± Standard Deviation (SD), with a significance level of 5% ($P < 0.05$).

RESULTS

PARAMETERS ATTRIBUTED TO PREGNANT RATS

During the oral administration of microspheres containing usnic acid at a dose of 25 mg/kg was not recorded any animal death during treatment until the end of the experiment. The pregnant rats in the experimental group showed a reduction of approximately 22% in body weight gain during pregnancy. Regarding the average weight of the liver was not detected any change (Table I).

There was no change in relation to serum levels of transaminase enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST). All values corresponding to animals were within the reference values, according to Lima et al. (2014) (Table II).

TABLE I
Body weight gain during pregnancy and liver weight of pregnant rats treated with usnic acid encapsulated into PLGA-microspheres at dose of 25 mg/kg.

variables	Control Group (Mean ± SD)	Treatment Group (Mean ± SD)	Significance of t-test
Body weight (g)	110.85 ± 10.03	86.12 ± 20.69*	0.025
Liver weight (g)	12.36 ± 1.56	12.94 ± 1.35	0.504

(g): grams. *Statistically significant difference by *t-test* at 5% ($P < 0.05$). Data expressed in mean ± standard deviation (SD).

TABLE II
Analysis of markers of liver function of pregnant rats after ten days of administration of usnic acid encapsulated into PLGA-microspheres at dose of 25mg/kg during pregnancy.

variables	Control Group (Mean ± SD)	Treatment Group (Mean ± SD)	Reference values	Significance of t-test
AST (U/L)	153.00 ± 17.61	122.00 ± 44.10	61-210	0.141
ALT (U/L)	75.17 ± 7.60	68.83 ± 11.96	38-82	0.299

(AST): Aspartate Aminotransferase; (ALT): Alanine Aminotransferase; (U/L): Units per liter. Data expressed in mean ± standard deviation (SD).

TABLE III
Usnic acid encapsulated into PLGA-microspheres effect on fetuses.

variables	Control Group (Mean ± SD)	Treatment Group (Mean ± SD)	Significance of t-test
Fetal weight (g)	4.85 ± 0.35	4.65 ± 0.41*	0.005
Liver weight (g)	0.35 ± 0.06	0.33 ± 0.05	0.112

(g): grams. *Statistically significant difference by *t-test* at 5% ($P < 0.05$). Data expressed in mean ± standard deviation (SD).

PARAMETERS ASSIGNED TO THE OFFSPRING

Fetal average weight with their respective placenta exhibited a decrease of approximately 4% compared to animals from the control group. Furthermore, no change was observed in relation to the average weight of the liver (Table III).

Regarding the external morphology it was observed that 100% of the fetuses showed up within normal patterns. However, one of the uteri from the pregnant rats exposed to usnic acid encapsulated into PLGA-microspheres exhibited a reduced placenta without the presence of the fetus (Figure 1b).

MICROANATOMICAL ANALYSIS OF THE LIVER

The liver of pregnant rats from the experimental group showed up consisted of hepatic lobes composed by anastomosing cords of hepatocytes with sinusoidal capillaries. The hepatic sinusoids

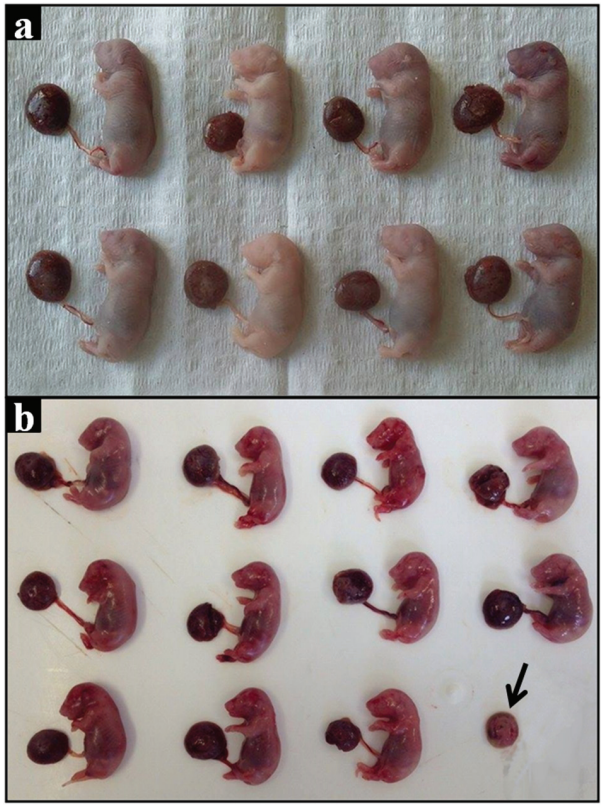

Figure 1 - Fetuses from the control group (a) and from the treatment group which received encapsulated usnic acid (b) during the period of organogenesis. Presence of reduced placental with interruption of fetal development (arrow).

are formed by sinusoidal endothelial cells with Kupffer cells located at the luminal surface. In addition, well-defined limits are displayed between the hepatic lobules with typical organizational structures of the portal space. In contrast, it was not possible to observe the space of Disse wherein the Ito cells are present (Figure 2b).

Regarding the offspring of the experimental group, the liver also was within normal patterns observed in the histological analysis. Liver cells were poorly organized in anastomosing cords of hepatocytes with sinusoidal capillaries. Therefore, it was not possible to identify the lobular organization. Furthermore, it was not possible to visualize the Kupffer cells and Ito cells. Moreover, the typical structures of the portal space were immature, which is considered normal at this gestational age (20 days). Several blood lineage cells were viewed between the liver cells, among them, the megakaryocytes was the easiest cell to identify (Figure 3b, d).

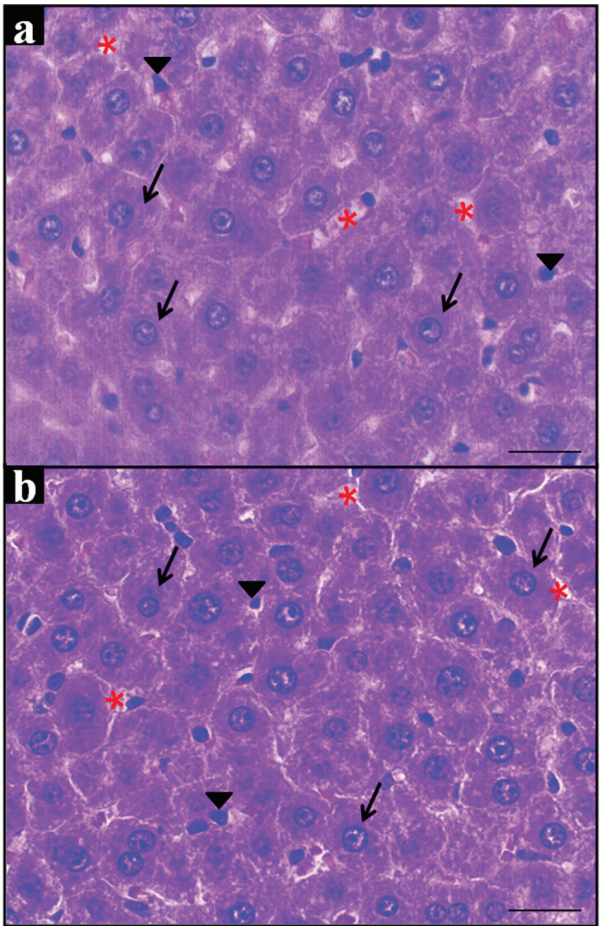

Figure 2 - Photomicrograph of the liver of the pregnant rats from the control group (a) and treatment group (b). Arrow: Hepatocytes; Arrowhead: Kupffer cells; Asterisk: Sinusoidal capillaries. H&E Stain. 400 x magnificent. Scale bar = 100 µm.

HISTOMORPHOMETRIC ANALYSIS

Histomorphometric analysis of the liver of pregnant rats exposed to usnic acid encapsulated into PLGA-microspheres revealed an increase of approximately 6% in the average of total number of hepatocytes per animal. There was no change in the average of cell area of these hepatocytes. However, a decrease of about 5% was observed for the nuclear area. The counting of Kupffer cells did not have any changes in the total number of such cells per animal (Table IV) (Figure 2b).

Regarding to the liver from the offspring of the experimental group, there was an increase of about 13% in the mean of total number of hepatocytes. Moreover, in relation to the counting of megakaryocyte there was a decrease of approximately 56% (Table V) (Figure 3b and d).

DISCUSSION

The gestation period is one of the stages very sensitive of the reproductive cycle, and exposure to some sort of compound can result in intrauterine growth retardation, congenital malformations, and

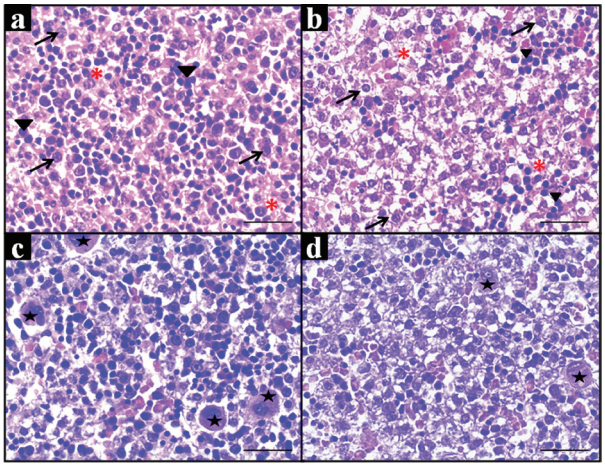

Figure 3 - Photomicrograph of the liver of the fetuses from the control group (a,c) and treatment group which received encapsulated usnic acid (b,d). Arrow: Hepatocytes; Asterisk: Sinusoidal capillaries; Arrowhead: Cells from the erythrocyte line; Star: megakaryocytes. H&E Stain. 400x magnificent. Scale bar = 100 µm.

TABLE IV
Histomorphometric analysis of the liver from the pregnant rats exposed to usnic acid encapsulated into PLGA-microspheres during pregnancy.

variables	Control Group (Mean ± SD)	Treatment Group (Mean ± SD)	Significance of t-test
NH	35.48 ± 6.00	37.56 ± 7.94*	0.049
CAH	386.26 ± 85.83	392.42 ± 9.98	0.363
NAH	58.25 ± 13.00	55.89 ± 13.00*	0.026
NKC	13.97 ± 3.97	15.02 ± 5.06	0.121

(NH): Number of Hepatocytes; (CAH): Cellular Area of Hepatocytes; (NAH): Nuclear Area of Hepatocytes; (NCK): Number of Kupffer Cells. *Statistically significant difference by *t-test* at 5% ($P < 0.05$). Data expressed in mean ± standard deviation (SD). CAH and NAH are values expressed in μm^2.

TABLE V
Histomorphometric analysis of the liver from the offspring born from the pregnant rats treated with usnic acid during pregnancy.

variables	Control Group (Mean ± SD)	Treatment Group (Mean ± SD)	Significance of t-test
NH	37.85 ± 8.86	43.31 ± 7.17*	0.001
NM	0.09 ± 0.37	0.04 ± 0.26*	0.002

(NH): Number of Hepatocytes; (NM): Number of Megakaryocytes. *Statistically significant difference by *t-test* at 5% ($P < 0.05$). Data expressed in mean ± standard deviation (SD).

even fetal death. The evaluation studies of drugs effects on organism including pre-clinical tests such as those investigated in this study, are very important because they seek to analyze the toxicity of on the maternal organism and their offspring (Wanderley et al. 2013, Roman et al. 2014).

According to Louzada et al. (2008), the indicative of maternal toxicity are related to the reduction of body weight, whether or not decrease in food consumption, by changes in weight and/or morphology of the organs and the occurrence of deaths during treatment. The reduction of approximately 22% in body mass gain during pregnancy may represent an indication of maternal toxicity by the compound in its encapsulated form. On the other hand, it should take into account the reports in the literature the action of usnic acid as a weight reducing, and it has been marketed in the United States as a dietary supplement, several cases of hepatotoxicity associated with weight loss were reported (Fraveau et al. 2002, Durazo et al. 2004, Neff et al. 2004, Sanchez et al. 2006). Despite the usnic acid in its encapsulated form have caused a decrease in body mass, this reduction was lower than that observed with its administration in suspension (Silva et al. 2017). Therefore, treatment with usnic acid in suspension was able to reduce by approximately 57% of the body weight gain during pregnancy.

The serum levels of transaminases AST and ALT are often regarded as excellent chemical markers in pre-clinical and clinical studies of liver function. Liver damage can be measured in relation to increased levels of these enzymes in the bloodstream, and this increase may be proportional to the number of affected hepatocytes (Hall 2007, Ozer et al. 2008). In the present study, the absence of alterations in the serum levels of transaminases analyzed, this confirms a reduction of hepatotoxicity caused by usnic acid, resulting from their encapsulation in PLGA-microspheres. These results corroborate those reported by M.S. Ferraz (unpublished data) after administration of 25 mg/kg of UA in the suspension form and encapsulated in PLGA-microspheres for 28 days. As a result a significant increase of 38% and 30% was observed in the serum levels of ALT and AST respectively, after treatment with UA in suspension. Therefore, these data suggest that the animals developed liver injury induced by exposure. In contrast, these enzymatic changes were not found after treatment with usnic acid encapsulated into PLGA-microspheres. Thus, confirming the reduction of hepatotoxicity caused by the UA due to its encapsulation.

In subchronic toxicity tests with the administration of 15 mg/kg of UA in suspension and in PLGA-nanocapsules during seven days,

there was a significant reduction of hepatotoxicity after administration of the compound in its nanocoated form. In addition, elevated plasma levels of approximately 91% and 66% of ALT and AST, respectively, were recorded for the treatment of animals with UA in suspension, suggesting a chronic liver dysfunction. In contrast, treatment with PLGA-nanocapsules reduced the hepatic damage caused by administration of usnic acid (Santos et al. 2006).

According to Barros and Davino (2008), the many alterations observed during the intrauterine development of the fetuses are especially due to exposure of mothers to chemical agents, without necessarily having a direct effect on them. The reduction of body weight by the offspring born from the pregnant rats exposed to usnic acid represented indicative of fetotoxicity, because it was able to cause a delay in the normal development of the conceptus.

One of the few researches of the potential toxicological of UA in suspension in the reproductive period, during organogenesis, showed a significant fetotoxicity signals with reduced body weight gain and fetal liver weight on offspring exposed to doses at 15 mg/kg and 25 mg/kg of UA. In addition, the dose considered more toxic was at 25 mg/kg (Silva et al. 2017). In contrast, our findings for the same dosage, with the UA in its encapsulated form, confirm once more that the administration of usnic acid encapsulated into PLGA-microspheres can minimize the damage caused by exposure critical periods of development.

According to Lemonica et al. (2008) during pregnancy the morphological abnormalities in fetuses usually occur during the period of organogenesis, which is the most vulnerable stage to the appearance of teratogenic effects on embryo-fetal development. Nevertheless, in this work the fetuses did not exhibit any abnormality during the anatomical development, even the usnic acid being considered as a possible teratogenic agent.

Although in the microanatomical analysis of the liver from all experimental animals was not observed any change in the tissue, such as liver damage, some studies have shown extensive necrotic areas in the liver of animals submitted to the UA in suspension. Therefore, this abnormality has been significantly reduced after treatment with nanocapsules and/or PLGA-microspheres (Ribeiro-Costa et al. 2004, Santos et al. 2006).

Abnormal proliferation of hepatocytes may occur in various pathological and/or experimental situations influenced by several factors. The administration of hepatotoxic drugs induces the proliferation of hepatocytes, which have the ability to regenerate the normal liver architecture in pathological conditions. Furthermore, these cells are the first to proliferate against aggression in the liver tissue and as the most important in parenchymal regeneration. Some studies have shown the effects of substances with hepatotoxic and teratogenic potential on the organization and structure of the hepatocytes (Domingues et al. 2009, Da Paz et al. 2010, Dos Santos et al. 2010, Aravinthan et al. 2013).

The results regarding the measurement of hepatocytes demonstrated that even in its encapsulated form the usnic acid, it causes a toxic effect on the liver of these animals. However, to minimize induced hepatotoxicity, hepatocytes entered into cellular proliferation for a possible regeneration of the liver tissue. In other words, that is a regenerative mechanism involving an increase in the number of hepatocytes. The administration of UA encapsulated might even have caused a disturbance in the tissue, but not to the point of causing necrosis. The lack of histomorphometric studies of the liver using natural substances, such as usnic acid, prevents us to make more accurate comparisons relative to the analyzed measurements.

A hepatotoxicity study of *Mikania hirsutissima*, a plant which has some pharmacological properties similar to the usnic acid, it was reported significant

decreases in relation to the nuclear area of hepatocytes in animals treated with the lowest doses from that plant (Da Paz et al. 2010). These results highlight the hepatotoxic potential of the plant as it was seen for usnic acid acid encapsulated into PLGA-microspheres.

The histomorphometry of the Kupffer cells in the experimental group indicates the absence of inflammatory processes as well as hepatic damage. According to Arii and Imamura (2000), these cells play a crucial role in maintaining of liver function in pathological and physiological conditions having as main functions phagocytosis of foreign particles. Moreover, that cell is important to remove harmful substances and to modulate immune response, playing a key role on the inflammatory response. The cell activation in inflammatory processes may be associated with morphological signs of activation and with an increase of cell population (Eguchi et al. 1991).

Nonetheless, evidence derived mainly from animal models suggest that the increase in Kupffer cells number is involved to the pathogenesis of some liver injury, due to exposure to toxins, chemicals, and pharmacological agents. These cells release biologically active substances, cytokines, which promote the pathological process after its activation (Luckey and Petersen 2001, Ito et al. 2003, Ono et al. 2004, Kolios et al. 2006). This information could explain the absence of lesions in the hepatic tissue, which may be associated to the morphology and quantity of Kupffer cells in animals treated with UA in its encapsulated form.

Reproductive toxicity studies during the period of organogenesis with beta- lapachone, compound of natural origin that has some properties similar to usnic acid, as low solubility and diverse biological activities, showed no histological changes in the liver of all fetuses exposed to doses of 40 mg/kg, 60 mg/kg and 160 mg/kg that compound. In contrast, the administration of beta-lapachone showed an abortifacient and teratogenic action (Almeida et al. 2009). Comparing these findings with our results, it can be noted that the usnic acid encapsulated into PLGA-microspheres are able to cause hepatotoxicity in fetuses, even at lower doses than those used in the study with beta-lapachone.

Silva et al. (2017) found a significant increase of approximately 25% of the total amount of hepatocytes, as well as a reduction of approximately 53% in the number of megakaryocytes in liver of offspring exposed to 25 mg/kg of usnic acid in suspension. Our findings show that these alterations in the hepatic tissue can be minimized by treatment with usnic acid encapsulated into PLGA-microspheres, especially damage in hepatocytes. On the other hand the UA encapsulated into PLGA-microspheres did not show to be effective against the damage caused on the multinucleated cells, the megakaryocytes. Thereby, maintaining toxicity on these cells, that may cause changes to the formation process of megakaryocytes, leading to a deficiency in initial coagulation cascade, because are those cells the responsible for originating the blood platelets.

CONCLUSIONS

The experimental model used in this study demonstrates that the administration of usnic acid acid encapsulated into PLGA-microspheres provokes toxic effects during pregnancy, including alterations in the hepatic tissue, which shows its potential hepatotoxic during organogenesis. However, these alterations were in much smaller proportions than those already found to usnic acid in its suspension form. Considering these findings, it is concluded that the toxicity of this compound can be minimized by means of its encapsulation.

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