Evaluation of the toxicity of the medicinal plant Peumus Boldus during the gestational period in Wistar rats

Avaliação da toxicidade da planta medicinal Peumus Boldus durante o período gestacional de ratas Wistar

DOI:10.34117/bjdv7n6-550

Recebimento dos originais: 24/05/2021
Aceitação para publicação: 24/06/2021

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ABSTRACT

Medicinal plants are sources of numerous compounds used in the production of drugs to treat a wide variety of symptoms. It is known that Peumus boldus, traditionally known as Boldo, has therapeutic properties applied to several digestive and liver disorders. However, as with all natural extracts, little is known about its effects on pregnancy. The literature suggests abortifacient and teratogenic effects in pregnant animals treated with P. boldus. In this sense, the present study verified the effects of commercial P. boldus extract during the gestational period in Wistar rats. The animals were divided into two groups: Control (n=3; 1 ml of distilled water) and Treated (n=6; 1.7mL/300g/day of P. boldus). Oral administration of distilled water or extract took place on days 1, 5, 10 and

15 of gestation in the rats. After 18 days of gestation, a cesarean section was performed, and the reproductive capacity of the rats and the vitality of the pups were evaluated. The placentas and fetuses were measured, and the fetuses were fixed (24h) in 10% formaldehyde solution for analysis of the anatomical parameters and measurements: anteroposterior of the skull, lateral-lateral of the skull, anteroposterior of the thorax, lateral-lateral of the thorax, craniocaudal and caudal. Other parameters were also evaluated, such as: eye and ear implantation, upper and lower limbs, finger configuration, and anal orifice. Next, the fetuses were dehydrated in increasing ethanol series, clarified in xylene and embedded in paraffin for microtomy (5µm) and stained with hematoxylin and eosin. The tissues of the following organs were observed in the histological analysis: brain, heart, lung, liver and kidney. Reproductive capacity, vitality of the pups, weights of fetuses and placentas and macroscopic analysis of morphological parameters of the pups showed no significant difference when compared to the control group ($p>0.05$). In the histopathological analysis, there were no significant changes when compared to the control group ($p>0.05$). Therefore, it was concluded that the commercial extract of P. boldus, at the dose studied, did not induce maternal and reproductive toxicity, since it did not present deleterious effects to the pregnant woman or to the embryo and fetus of the rats exposed to this phytotherapeutic. However, further studies are needed, with higher doses of P. boldus administered over the long term, to ensure its safe use in pregnant women.

Keywords: Peumus Boldus, Histopathology, Reproductive Capacity, Fetal Vitality, Boldus.

RESUMO

As plantas medicinais são fontes de inúmeros compostos utilizados na produção de medicamentos para tratamento de sintomas dos mais variados. Sabe-se que, o Peumus boldus tradicionalmente conhecido por boldo, apresenta propriedades terapêuticas aplicadas a diversos distúrbios digestivos e hepáticos. Entretanto, como todo extrato natural, pouco se sabe sobre seus efeitos na gestação. A literatura sugere efeitos abortivo e teratogênico em animais prenhes tratados com P. boldus. Neste sentido, o presente estudo verificou os efeitos do extrato comercial de P. boldus durante o período gestacional de ratas Wistar. Os animais foram divididos em dois grupos: Controle (n=3; 1 ml de água destilada) e Tratado (n=6; 1,7mL/300g/dia de P. boldus). A administração oral de água destilada ou extrato aconteceu no 1º, 5º, 10º e 15º dias do período gestacional das ratas. Transcorridos 18 dias de gestação foi realizada a cesárea, bem como a avaliação da capacidade reprodutiva das ratas e vitalidade dos filhotes. As placentas e os fetos foram mensurados, e os fetos fixados (24h) em solução de formaldeído à 10% para análise dos parâmetros anatômicos e medidas: ântero-posterior do crânio, lateral-lateral do crânio, ântero-posterior do tórax, lateral-lateral do tórax, craniocaudal e caudal. Outros parâmetros também foram avaliados, tais como: implantação dos olhos e orelhas, membros superiores e inferiores, configuração dos dedos e orifício anal. A seguir, os fetos foram desidratados em série crescente de etanol, clarificados em xilol e incluídos em parafina para microtomia (5µm) e corados com hematoxilina e eosina. Na análise histológica foram observados os tecidos dos seguintes órgãos: cérebro, coração, pulmão, fígado e rim. A capacidade reprodutiva, vitalidade dos filhotes, pesos dos fetos e placentas e análise macroscópica dos parâmetros morfológicos dos filhotes não demonstraram diferença significativa quando comparada com o grupo controle ($p>0,05$). Na análise histopatológica, não foram verificadas alterações significativas quando comparada ao grupo controle ($p>0,05$). Portanto, concluiu-se que o extrato comercial de P. boldus, na

dose estudada, não induziu toxicidade materna e reprodutiva, uma vez que não apresentou efeitos deletérios para a gestante nem tampouco para o embrião e feto das ratas expostas a este fitoterápico. Porém, faz-se necessário estudos complementares, com doses maiores de P. boldus administrados a longo prazo, para garantir segurança no seu uso em gestantes.

Palavras-chave: Peumus Boldus, Histopatologia, Capacidade Reprodutiva, Vitalidade dos Fetos, Boldo.

1 INTRODUCTION

The use of alternative therapies is widespread in the world population to treat, cure and prevent diseases, being considered the oldest medicinal practice. According to the Alma-Ata declaration (Rosa et al., 2011), the World Health Organization (WHO) recognizes that 85% of traditional practices used by the population in developing countries include medicinal plants, however the belief that plant-based products are free of toxic effects and adverse reactions is the main concern about the use of medicinal plants (Clarke et al., 2007). Embryotoxicity, teratogenicity and abortifacient are the most worrying adverse reactions arising from the indiscriminate use of herbal medicines, since the organic components present in the extracts of these plants can cross the hematoplacental barrier and cause damage to the fetus, especially in the first gestational trimester (Araújo et al., 2010).

According to Rodrigues (2011) several plants commonly used by the Brazilian population have abortifacient, embryotoxic, and teratogenic effects, such as rosemary (Rosmarinus officinalis), rue (Ruta chalepensis; Ruta graveolens), leather hat (Echinodorus macrophylus), eucalyptus (Eucaliptus globulus), and cinnamon (Cinnamorum vernun). Among the widely used plants, Peumus boldus stands out because it has antidiseptic, choleretic, and cholagogue actions, among others (Newall et al., 2002). The first compound isolated was the alkaloid boldine, and many studies have emerged since then (Tavares and Takahashi, 1994; Kringstein and Cederbaum, 1995) followed by eighteen other alkaloids, such as secoboldine, N-methylsecoboldine, glaucine, laurolitsine, anorecticuline, N-methyl-urotetanine, laurotetanine, coclaurine, isoridine, as well as essential oils, tannins and coumarins (Fuentes et al, 2018).

Studies by Almeida et al. (2000) performed toxicological evaluation of the hydroalcoholic extract of P. boldus and boldine in pregnant rats and observed blastocyst-toxicity, hepatic histological and anatomical changes in their fetuses, suggesting moderation and caution in the administration of boldo, especially in the first three

gestational months. In this same vein, Moraes et al. (2018) also detected toxic effects in the fetuses of female mice that received boldo extract up to the 18th gestational day. Therefore, toxicological assays with medicinal plants are extremely important to ensure the safety of their use in pregnancy. In this sense, this work aimed to study the toxic effects induced by commercial extract of boldo during the gestational period of rats.

2 MATERIALS AND METHODS

Female and male Wistar rats aged 90 days, weighing between 200-250g, kept in cages supplied with water and feed ad libitum, in an environment with constant temperature and controlled lighting (12h light-dark cycle). All procedures were approved by the CEUA of PUC-SP, under protocols n° 2018/80 and n° 2019/118. The herbal medicine was purchased commercially, hydroalcoholic extract with 0.1% of Peumus boldus Molina, lot 14.0010-1101.

To evaluate the fertility and reproductive performance of the females, the harem method was applied and pregnancy was confirmed by the presence of spermatozoa in the smear, after 18 days the cesarean section was performed. The animals were divided into two experimental groups: treated group (n=6) with single oral administration of P. boldus (gavage, 1.7mL of the extract/300g of the pussy's weight) on the 1st, 5th, 10th and 15th days of gestation. These periods were predetermined by the importance of embryogenesis (1st to 5th day of gestation) and organogenesis (10th to 15th day of gestation) (Wilson and Warkanny, 1965). The control group (n=3) received only distilled water, under the same conditions as the treated group.

The cesarean section was performed on the 18th day of gestation when the rats were anesthetized with halothane (inhalation anesthetic) and after the surgical procedure the following parameters were evaluated to determine reproductive performance: number of corpora lutea and implantations (pre- and post-implantation loss); number of live fetuses; weight of fetuses and corresponding placentas. Once the surgery was completed and after macroscopic inspection of the fetuses, they were sacrificed with deep inhalation anesthesia.

The macroscopic analysis of the morphological parameters of the pups was performed as follows: the fetuses (n = 92) were fixed in 10% formaldehyde solution for approximately 24 hours and transferred to 70% ethanol. With the aid of a digital pachymeter the following anatomical measurements were made: anteroposterior and laterolateral skull, anteroposterior and laterolateral thorax, craniocaudal and caudal. Other

parameters were also evaluated, such as: eye and ear implantation, upper and lower limbs, the toes of the front and rear legs, tail insertion and the presence of the anal opening.

For histopathological analysis, the fetuses were dehydrated in successive concentrations of ethanol (80%, 90% and 100%), submitted to a xylene clarification process using an automatic tissue processor (Lupetec PT 09 TS) and embedded in paraffin using a paraffin dispenser (Lupetec DP 2010) and a refrigerated plate (Lupetec PR 2010D). The blocks were subjected to microtomy on a microtome (Lupetec MRPO3) and the 5 µm thick longitudinal sections collected on glass slides and adhered to it with the aid of a heated plate (Lupetec PA 2012).

Histological slides were deparaffinized in xylene, hydrated in decreasing concentrations of ethanol (100%, 95%, 80%, and 70%), hydrated in distilled water, and stained in Hematoxylin for 5 minutes. Again, the slides were washed in tap water, stained in eosin for 3 minutes, dehydrated in increasing concentrations of ethanol and in xylol, and mounted with a slide and synthetic Canada balsam for analysis under the light microscope (Nikon SE).

The results, when appropriate, were analyzed in specific program (GraphPad Prisma 8) and presented as Mean of experiments (M) ± Standard Deviation of the Mean (SDM). ANOVA and Chi Square tests were used to compare the treatments. The significance level adopted was 5% to reject the null hypothesis.

3 RESULTS

The confirmation of the rats' pregnancy was confirmed by the presence of spermatozoa in suspension in the smear from the vaginal lavage, as reported by Aguilar et al. (2014).

During the gestational period of the rats, weighing was performed on the 1st, 5th, 10th, 15th days of gestation and also on the 18th day of gestation before performing cesarean section in both control and treated groups. The mean weight gain of pregnant rats in the control group (n=3) were: 23.39±4.89g (day 1-5); 8.45±2.22g (day 5-10); 24.43±6.78g (day 10-15) and 25.08±4.65g (day 15-18). The mean weight gain of pregnant rats in the treated group (n=6) were: 11.75±7.13g (1st - 5th day); 8.75±4.11g (5th - 10th day); 22.25±3.4g (10th - 15th day) and 17.25±7.8g (15th - 18th day) ($p>0.05$ when compared to the control group).

In the evaluation of fetal vitality it was observed that all fetuses of the control group (n=35) and treated group (n=57) were alive, therefore, vitality of 100%. The

corpora lutea of the control group was equal to 39 and the treated group was equal to 69, without signs of reabsorption or significant pre-implantation and post-implantation losses ($p>0.05$).

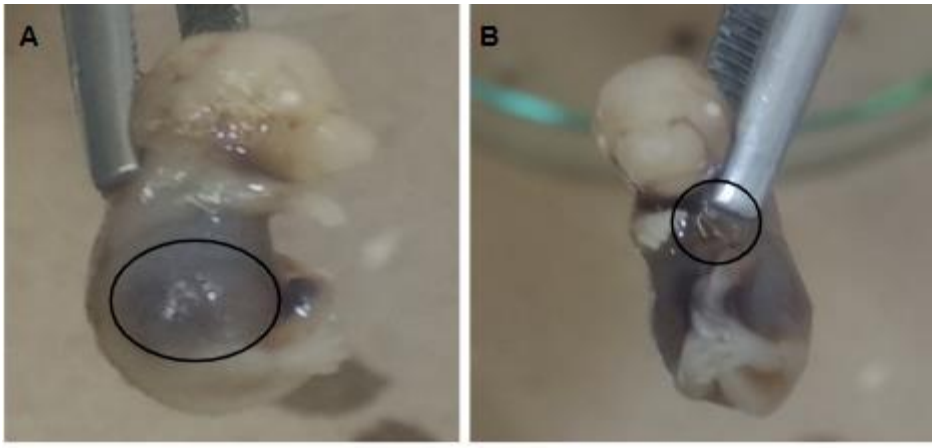
All fetuses and their respective placentas were weighed; the control group had mean fetal weights of 0.92±0.14g and placental weights of 0.40±0.07g. In the treated group, the mean weights of fetuses were 0.93±0.08g and placentas were 0.43±0.08g and both weights (placentas and fetuses) were not significantly different when compared to the control group ($p>0.05$).

Macroscopic analysis of the morphological parameters of the pups showed the following average measurements (cm) in the control group: anteroposterior skull 0.59±0.07; lateral-lateral skull 0.49±0.07; craniocaudal 1.67±0.11; caudal 0.49±0.08; anteroposterior thorax 0.51±0.09 and lateral-lateral thorax 0.48±0.07. In the treated group, the means were (cm): anteroposterior cranial 0.57 ± 0.05; laterolateral cranial 0.49 ± 0.06; craniocaudal 1.70 ± 0.13; caudal 0.50 ± 0.12; anteroposterior thorax 0.55 ± 0.10; and laterolateral thorax 0.52 ± 0.09 ($p>0.05$ when compared to the control group).

Regarding anatomy, no changes were observed in the progeny of the rats from the control group (n=35). Only 1 fetus presented agenesis, ectrodactyly of the left lower limb, left hind leg, corresponding to 2.85% of alteration in the group. The observed aspects, skull diameter, thorax size and tail dimensions, were homogeneous among the animals and no malformations were found. Other parameters such as eye and ear implantation, upper and lower limb implantation, finger configuration, and anus orifice were also evaluated and found to be unchanged.

In relation to the treated group (n=57), 3 fetuses presented hepatomegaly corresponding to 5.26% and 3 fetuses presented alterations in limbs, characterized as agenesis of the left lower limb (5.26%) and/or ectrodactyly of the left hind leg (1.75%), 3 fetuses presented hepatomegaly and 1 fetus presented ectrodactyly of the left upper limb (Figure 1), with no difference when compared to the control group ($p>0.05$).

Figure 1: External fetal anatomical parameters of pregnant rat pups from the treated group: liver size and finger formation.



Histological analyses of the brain, heart, lung, liver and kidney of the pups in the control and treated groups were unchanged. Both groups showed neural tissue (neurons and glia cells) with no evidence of apoptosis or tissue edema (Figure 2). Also the heart showed myocardium with unaltered cardiomyocytes (Figure 3). Analysis of the lung parenchyma of the animals in both groups showed the presence of bronchioles in formation, but without air sacs and/or alveoli (Figure 4). The kidneys are not yet completely differentiated at 18 weeks, presenting nephrons in formation in the future cortical region and the connective tissue is concentrated in the future medullary portion (Figure 5). In the livers of the animals we observed the presence of hepatocytes organizing themselves in radial arrangement, around the central-lobular vein, intermingled by sinusoidal capillaries, but the classic aspect of hepatic lobules cannot yet be observed in this gestational period (Figure 6). Even in animals with hepatomegaly the normal histological appearance was maintained.

Figure 2: Representative histological image of the animals' brains, overview and detail at higher magnification, from the control (A, B) and treated (C, D) groups, respectively. Note the normal distribution

of neurons and glia cells throughout the cerebral cortex in both groups. Magnifications: A, C = 100X; B, D = 400X.

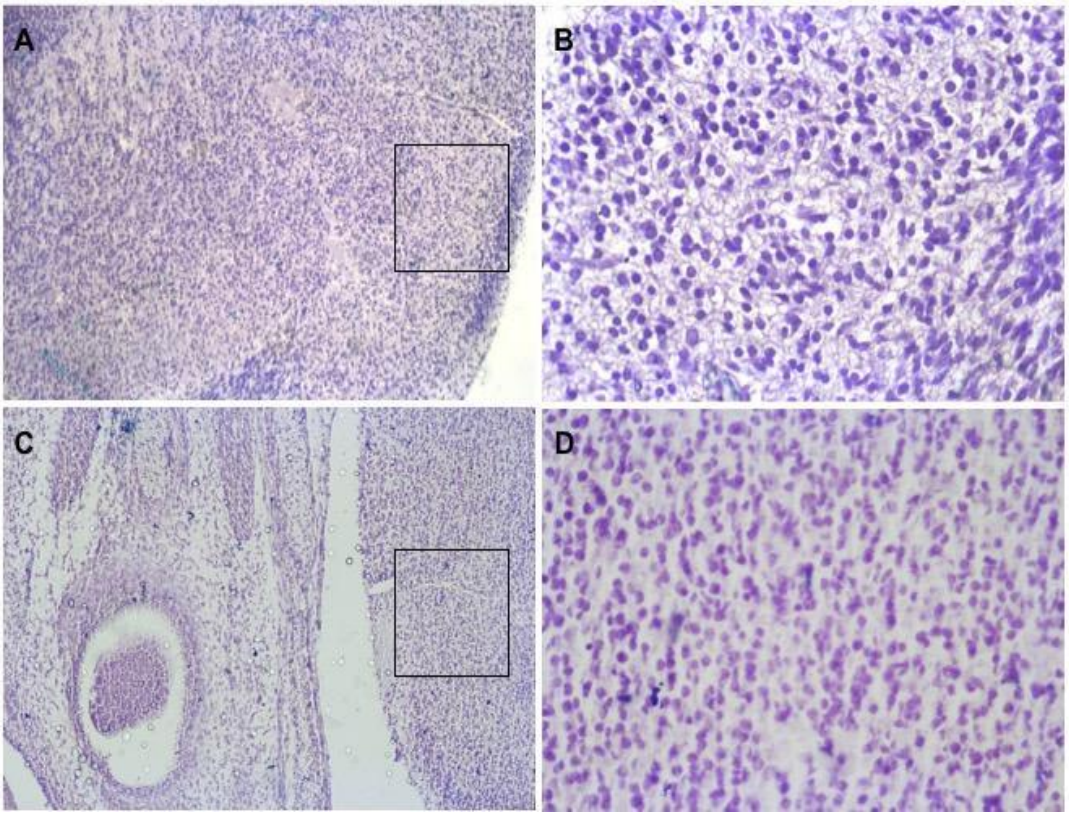


Figure 3: Representative histological image of the animals' hearts, overview, general view and detail at higher magnification, from the control (A, B, C) and treated (D, E, F) groups, respectively. Note the normal distribution of cardiomyocytes (arrow) throughout the myocardium in both groups. Magnifications: A, D = 40X; B, E = 100X; C, F = 400X.

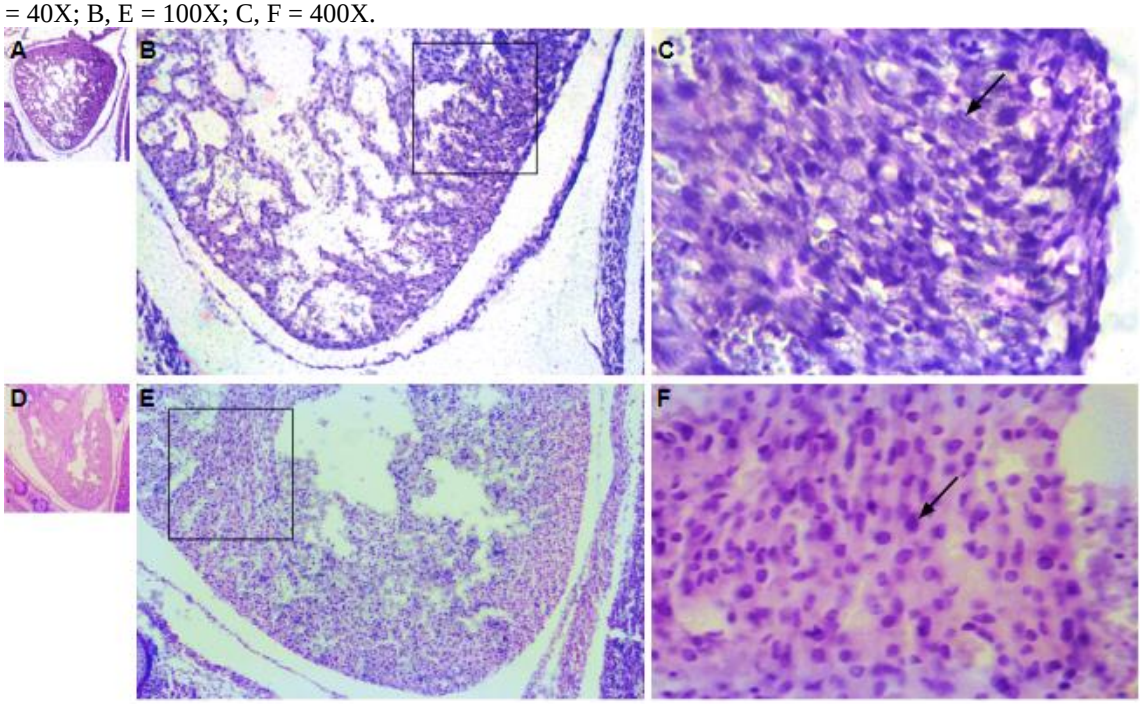


Figure 4: Representative histological image of the animals' lungs, overview and at higher magnification, from the control (A, B) and treated (C, D) groups, respectively. Note the normal distribution of bronchioles throughout the lung parenchyma in both groups. Magnifications: A, C = 100X; B, D = 400X.

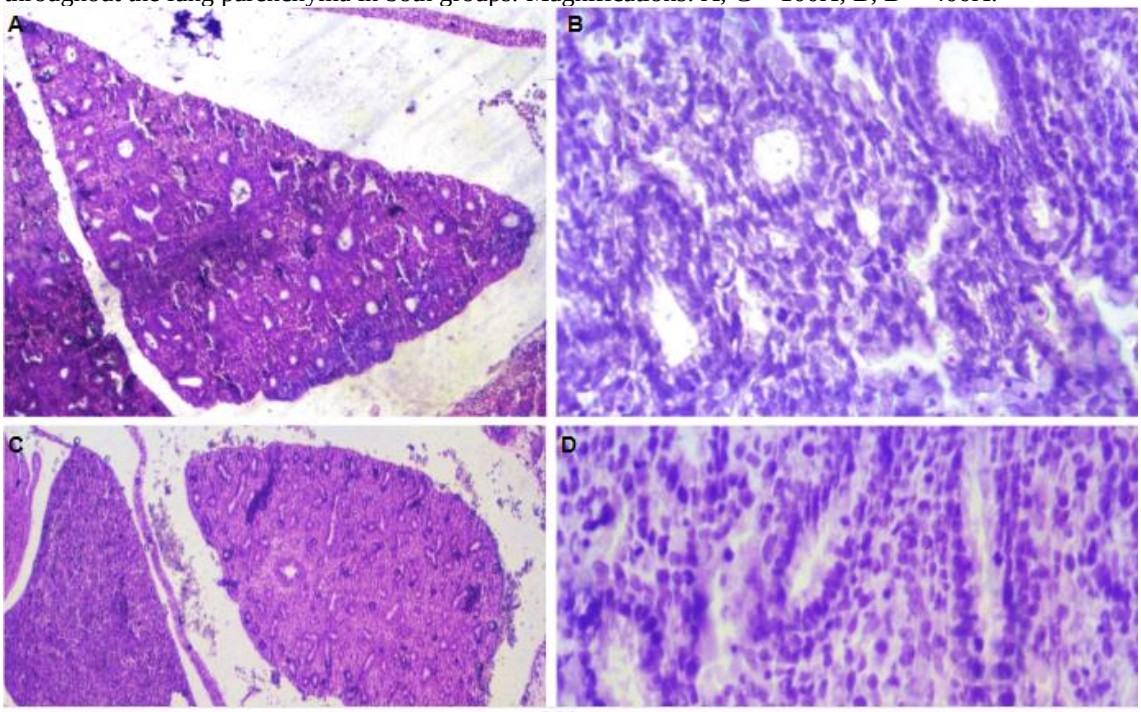


Figure 5: Representative histological image of the animals' kidney, overview and at higher magnification, from control (A, B) and treated (C, D) groups, respectively. Note the normal distribution of the nephrons (B, D) throughout the renal cortex and the formation of the medullary portion (M) in both groups. Magnifications: A, C = 100X; B, D = 400X.

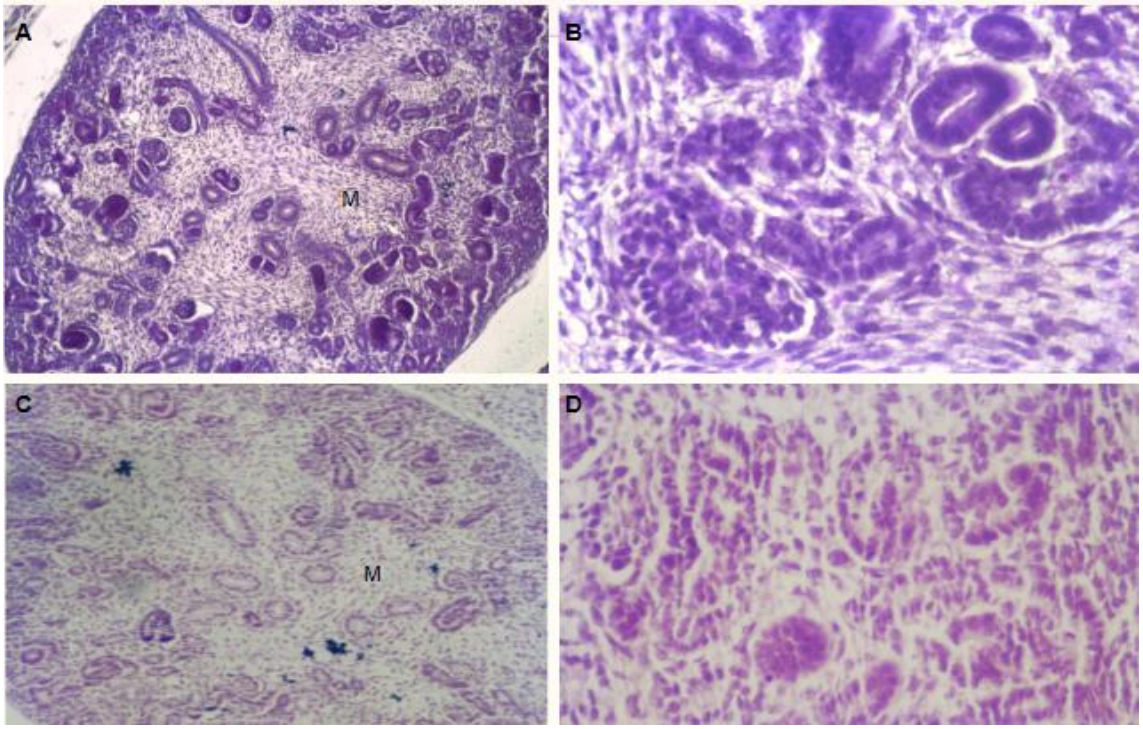
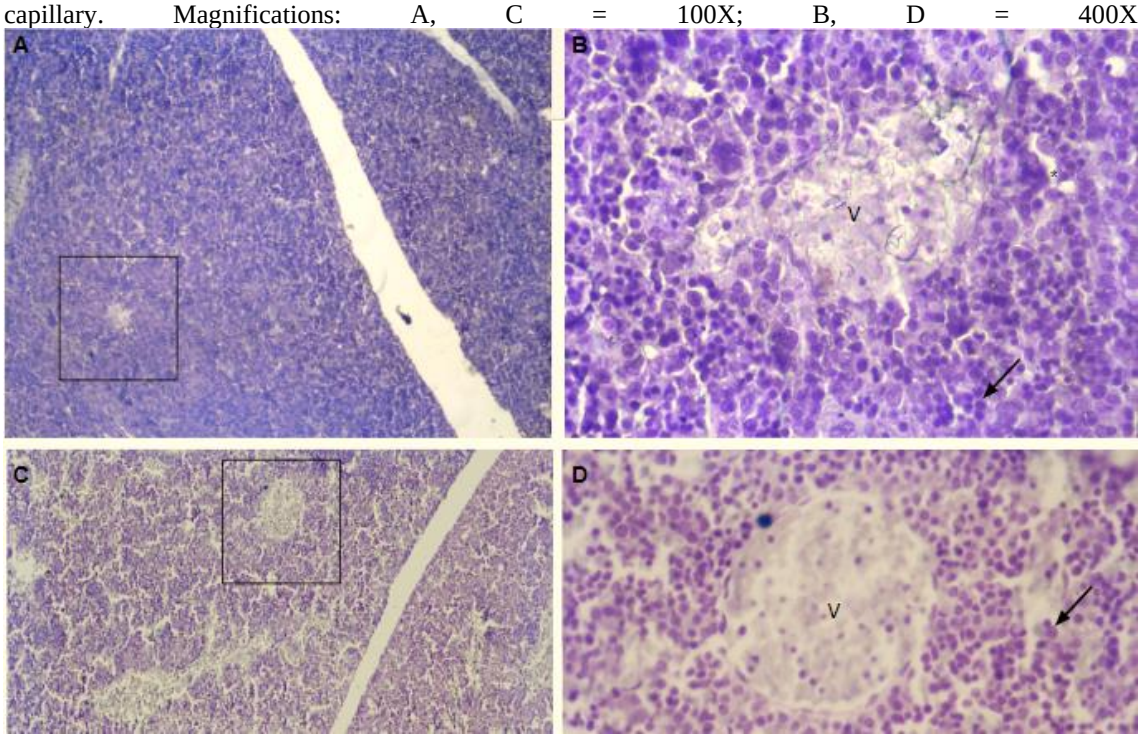


Figure 6: Representative histological image of the animals' livers, overview and detail at higher magnification, from the control (A, B) and treated (C, D) groups, respectively. Note the normal distribution of hepatocytes (arrow) throughout the parenchyma in both groups. V = central-lobular vein; * = sinusoidal capillary. Magnifications: A, C = 100X; B, D = 400X



4 DISCUSSION

The use of medicinal plants and herbal medicines is a widespread practice worldwide and is encouraged by the World Health Organization, especially in developing countries such as Brazil (Newall et al., 2002). In 2006, the Ministry of Health launched the National Policy for Integrative and Complementary Practices (PNPIC), which offers herbal medicine to users of the Unified Health System (SUS) (Brazil, 2006).

After the implementation of PNPIC, an increase in the number of municipalities offering phytotherapy in Basic Health Units was observed. However, it is evident that the users of this alternative therapy need better orientations based on adequate prescriptions related to dosage, possible drug interactions and toxic effects, because the fact that medicinal plants are of natural origin creates the myth that their indiscriminate use is free of side effects (Leite e Schor, 2005).

Self-medication is something very common in the Brazilian population, especially among pregnant women. Studies report that there are many plant actives that can have toxic, genotoxic and carcinogenic effects when used continuously. The mistaken idea that medicinal plants and phytotherapics do not cause side effects represents a danger, because there are no scientific studies that prove the effectiveness of most plants and the effects that different doses and associations can cause in the human body (Mello et al., 2001).

There are numerous substances present in medicinal plants that can cause reproductive toxicity, such as rosemary (Rosmarinus officinalis), rue (Ruta chapelensis), boldo (Peumus boldus) (Almeida et al., 2000; Campesato, 2005; Gonzales et al., 2007; Rodrigues et al., 2011; Jardim, 2017), among others. While the constituents have relevant pharmacological effects, they can also present deleterious effects in a certain gestational period.

The methodology of this study is similar to the studies conducted with hydroacetone extract of Mayatenus ilicifolia (Laura, 2009), guaco extract (Mikania glomerata) (Mendes, 2012) and the aqueous extract of chamomile (Chamomilla recutita L.) (Arruda et al., 2013).

The gestational period is characterized by rapid weight gain in these animals due to the accumulation of subcutaneous tissue. The analysis of their weight gain is of paramount importance, as it is one of the parameters often used as a way to analyze and determine the toxicological effects of chemicals. This experimental design evaluated the toxicity of the extract through the variation in the ponderal gain of pregnant rats, where both control and treated groups obtained ponderal gain throughout the gestational period, although more evident in the control group ($p>0.05$); similar to the results obtained in the study by Salinas et al. (2013).

The toxic effects of plant extracts can directly influence the fertility of the animal and the subsequent pregnancy, because if embryonic loss is observed, either by acceleration or delay of uterine nidations, it proves the toxic effects of the substance studied (Almeida et al., 2000). The correlation between the numbers of implanted blastocysts and those that did not develop is established from the evidence of resorption and post-implantation loss. Thus, the greater the number of resorptions, the greater the proportion of fetuses that had their development interrupted (Almeida et al., 2000; Nepomuceno et al., 2005).

Thus, pre- and post-implantation loss of blastocysts is of fundamental importance to analyze the possible toxic effects caused by P. boldus extract during gestation, since it is possible to distinguish embryotoxic effects and uterine toxicity caused by this phytotherapeutic. In this study, no signs of significant pre- and post-implantation resorption or loss were observed at the elected concentration, similar to the results obtained in the studies of Nepomuceno et al. (2005), but distinct from the findings of Almeida et al. (2000) who pointed out a significant increase in embryonic loss induced by hydroalcoholic extract of P. boldus and boldine. Jardim (2017) analyzed the

administration of P. boldus and boldine tea during the gestational period of rats and observed changes in the reproductive performance of females in relation to pre- and post-implantation loss, therefore concluding that the use of these substances should be avoided in pregnant women.

Regarding corpora lutea, the results showed no significant changes in the amount of corpora lutea between the control and treated groups. This also corroborates the findings of other studies that used Hypericum perforatum (Nepomuceno et al., 2005) in which no alterations were observed in the implantation process.

The weights of the placentas and fetuses showed no significant difference when compared to the control group, also corroborating Almeida et al. (2000). Other studies using different phytotherapeutics, observed significant differences in fetal and placental weights of the groups treated with Mikania glomerata (Mendes, 2012) or with Chamomilla recutita (Arruda et al., 2013).

The fetuses were macroscopically analyzed using external morphological parameters and showed no significant changes when compared to the control group. Differently from the results observed here, other studies verified significant alterations caused by extracts of Plathymenia reticulata Benth by Albuquerque (2009) and Mikania glomerata Sprengel by Mendes (2012). Magalhães et. al. (2020) observed that fetuses obtained from pregnant rats that received the hydroalcoholic extract of Cissus sulcicaulis (parreira brava) showed changes in all parameters evaluated in skeletal and visceral analysis.

Some studies with plant extracts revealed that their components (vegetable oils) showed histo- and immunopathological alterations, such as inflammatory parenchymal or chronic liver tissue lesions of small and medium intensity, evidencing Kupffer cell hyperplasia, incipient fibrosis, and microvesicular steatosis. Among these plants are Heliotropium indicum L. (crista de galo), presents in its composition alkaloids and pyrrolizidines, causing chronic liver affection (Mattos et al., 2018) and Symphytum officinalis L. (confrei) which also presents in its composition alkaloids and pyrrolizidines, causing abdominal pain and hepatocyte destruction, besides stimulating uterine motility (Dias et al., 2013).

In the present study, microscopic analyses compared the nerve tissue, cardiac striated muscle tissue, lung, liver and kidneys of the fetuses. Only in one of the fetuses was the absence of fingers on one of the legs observed (ectrodactyly) and in three fetuses hepatomegaly without tissue alteration was observed. The malformations found were

related to the phases of embryogenesis and organogenesis, such as the occurrence of differentiation of the sprouts to form the upper and lower limbs, from the mesoderm, and the hepatobiliary vesicle, which has an endodermal origin. Histological analyses showed similar tissue and cellular characteristics between the fetuses of the control and treated groups that were not affected by the dose of P. boldus studied. Almeida et al. (2000), however, observed anatomical alterations in their fetuses and liver histology, suggesting moderation in the administration of boldus in the first three gestational months.

Based on the experiments performed in this study, it was concluded that the commercial extract of P. boldus, at the dose studied, did not induce maternal and reproductive toxicity, since it did not present deleterious effects to the pregnant woman or to the embryo and fetus of the rats exposed to this herbal medicine. However, further studies are necessary, with larger doses of P. boldus administered over the long term, to ensure duly proven safety, quality and efficacy, contributing to the therapeutic consolidation of its use in pregnant women.

REFERENCES

AGUILAR, L.; LOPES, C.M.; BARBIERI, D.S.; ROCHA, T.; RANDAZZO, M. P. Toxic effects of glibenclamide in fetuses of normoglycemic rats: an2211' alternative therapy for gestational diabetes mellitus. **Open Veterinary Journal**, v. 4, p. 59–64, 2014.

ALBUQUERQUE, L. B. L. **Estudos in vitro e in vivo da Plathymenia reticulata Benth.** Dissertação de Mestrado. Universidade de Sorocaba, 2009.

ALMEIDA, E. R.; MELO, A. M.; XAVIER, H. Toxicological evaluation of the hydro-alcohol of the dry leaves of Peumus boldus and boldine in rats. **Phytotherapy Research**, v. 14, p. 99–102, 2000.

ARAUJO, R. C. Estudo Toxicológico das Drogas. Correlação Clinicopatológica. In: PENILDON, S. **Farmacologia. 8ª ed.** Rio de Janeiro: Guanabara Koogan, p. 178–195, 2010.

ARRUDA, J. T.; APPOBATO, F. C.; MAIA M. C. S.; SILVA, T. M.; APPOBATO, M. S. Efeito do extrato aquoso de camomila (Chamomilla recutita L.) na prenhez de ratas e no desenvolvimento dos filhotes. **Rev. Bras. Plantas Med.** v. 15, p. 103–154, 2013.

BRASIL. Política Nacional de Práticas Integrativas e Complementares no SUS - PNPIC-SUS. Ministério da Saúde. 2006. 92 p.

CAMPESATO, V. R. **Uso de plantas medicinais durante a gravidez e risco para malformações congênitas.** Dissertação (Doutorado). Universidade Federal do Rio Grande do Sul. 2005. v. 1, p. 40–44, 2005.

CLARKE, J. H. R.; RATES, S. M. K.; BRIDI, R. Um alerta sobre o uso de produtos de origem vegetal na gravidez. **Infarma**, v. 19, p. 41–48, 2007.

DIAS, T. R.; TOMÁS, G.; TEIXEIRA, N. F.; ALVES, M. G.; OLIVEIRA, P. F. White Tea (Camellia sinensis (L.)): Antioxidant Properties and Beneficial Health Effects. **Int J Food Sci Nutr Diet**. v. 2, p. 19–26, 2013.
FUENTES, B. G., CASTRO, S. S. L. L.; ACEVEDO, F. W.; TIRAPEGUI, C.; MATTAR C.; CASSEL, B.K. Variação de teor Alcalóide de Peumus Boldus. **Fitoterapia**, v. 127, p. 179–185, 2018.

GONZALES, J. R.; BENAVIDES, V.; ROJAS, R.; PINO, J. Efecto embriotóxico y teratogénico de Ruta chalepensis L. « ruda », en ratón (Mus musculus). **Rev. Peru Biol**., 13, 223–225, 2007.

KRINGSTEIN, P.; CEDERBAUM, A. I. Boldine prevents human liver microsomal lipid peroxidation and inactivation of cytochrome P4502E1. **Free Radical Biol. Med.**, v. 18, p.559–563, 1995.

JARDIM, L. H. **Avaliação da administração do alcaloide boldina em ratas Wistar durante o período gestacional sobre variáveis reprodutivas e comportamentais.** Dissertação de Mestrado. Universidade Federal do Rio Grande do Sul, 2017.

LAURA, A. L. C. **Efeitos da ingestão de extrato hidroacetônico de Maytenus ilicifolia e hidroetanólico de Achyrocline alata em ratas prenhes e seus fetos.** Tese de Doutorado. Universidade Federal de Mato Grosso do Sul, 2009.

LEITE, S. N.; SCHOR, N. Fitoterapia no Serviço de Saúde: significados para clientes e profissionais de saúde. **Saúde em debate**, v. 29, p. 78–85, 2005.

MAGALHÃES, S. A.; MARCONDES, L. Z.; DUARTE, B. R.; CRUZ-LOPES, L.; GERENUTTI, M. Neurotoxicological study about a medicinal plant of Cissus sulcicaulis Baker in pregnancy rats. **Braz. J. of Develop.**, v. 6, p. 8167–8187, 2020.

MATTOS, G.; CAMARGO, A.; SOUSA, C. A.; ZENI, A. L. B. Plantas medicinais e fitoterápicos na Atenção Primária em Saúde: percepção dos profissionais. **Cien. Saúde Colet.**, v. 23, p. 3735–3744, 2018.

MELLO, J. R. B.; LANGELOH, A.; HABERMEHL, G.; KREBS, H. C.; BATATINHA, M. J. M.; ALMEIDA, C. R. C.; BASTOS, F. C.; BASSANI, M. L.; BARUFALDI, C. M.; ALVARES, F. T.; FRANCISCO, D**. Avaliação do extrato aquoso de Crotalaria retusa Leguminosae sobre a fertilidade de ratas.** Faculdade Veterinária UFRGS, v. 25, p. 34–42, 2001.

MENDES, C. F. **Efeito do extrato de Mikania glomerata Sprengel (guaco) sobre a implantação e o desenvolvimento embrionário e placentário em camundongos.** Dissertação de Mestrado. Universidade de São Paulo, 2012.

MORAES, S. G.; FREIRIA, G. F.; ZAMBOTTI, T. R. Efeitos dos extratos de Peumus boldus e Foeniculum vulgare sobre o desenvolvimento embrionário e placentário em camundongos. Rev. da Fac. Ciências Médicas Sorocaba. 2018; 20 (Supl.).

NEPOMUCENO, F.; LAS CASAS, L.; PETERS, V. M.; GUERRA, M. O. Desenvolvimento embrionário em ratas tratadas com Hypericum perforatum durante o período de implantação. **Rev. Bras. Farmacogn.**, v. 15, p. 224–228, 2005.

NEWALL, C. A.; ADERSON, L. A.; PHILLIPSON, J. D. **Fitoterapia-Plantas Medicinais: Guia para Profissionais da Saúde.** São Paulo: Premier, p.57–58, 2002.

RODRIGUES, H. G. MEIRELES, C. G.; LIMA, J. T. S.; TOLEDO, G. P.; CARDOSO, J. L.; GOMES, S. L. Efeito embriotóxico, teratogênico e abortivo de plantas medicinais. **Rev. Bras. Plantas Med.** v. 13, p. 359–366, 2011.

ROSA, C, DA, CÂMARA, S. G.; BÉRIA, J. U.; Representações e intenção de uso da fitoterapia na atenção básica à saúde. **Cien. Saúde Colet.**, v. 16, p. 311–318, 2011.

SALINAS, H. R.; VIELMA, A. Z.; ARISMEDI, M. N.; BORIC, M. P.; SÀEZ, J. C.; VERLADE, V. Boldine prevents renal alterations in diabetic rats. **J. Diabetes Res.,** v. 2013, p. 7–9, 2013.

TAVARES, D. C.; TAKAHASHI, C. S. Avaliação do potencial genotóxico do alcalóide boldina em sistemas celulares de mamíferos in vitro e in vivo. **Mutat. Res.**, v. 321, p. 139–145, 1994.

WILSON, J. C.; WARKANY J. **Teratologia: princípios e técnicas Chicago.** University of Chicago Press., p. 47–74, 1965.