Assessment of *Pradosia huberi* effects on the reproductive system of male rats

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Abstract

Pradosia huberi is a species found in the Amazon region and used as an antiulcerogenic and gastroprotective agent; however, phytochemical analysis has revealed the presence of compounds with potential toxic effects on the reproductive system. For the evaluation of the toxicity of *P. huberi* on male fertility, male Wistar rats were divided into four groups: one control (distilled water p.o.) and three treated (hydroalcoholic extract of the stem bark of *P. Huberi* (PH-HAE) at doses of 1.22, 6.1, and 30.5 mg/kg p.o.) once daily, for 63 days. In the last week of treatment (from the 57th to the 63rd day), the rats were mated with untreated virgin females ($n = 30/\text{group}$) and were killed on day 64. To investigate the toxic potential of PH-HAE on the reproductive system of rats the following parameters were evaluated: sperm production, genotoxicity, and general development. The production of gametes and their morphology did not differ between control and treated groups. Treatment with PH-HAE did not result in fewer vaginal plugs formed, indicating that the ability to mate was not impaired, but caused an increase of 14.3 and 10.8% in the preimplantation loss index, a reduction of 14.3 and 10.8% in the implantation index, and a reduction of 5.6 and 8.2% in the postimplantation loss index of female rats mated with rats treated with 6.1 and 30.5 mg/kg, respectively, indicating a possible toxic action of PH-HAE on the reproductive system of rats.

Keywords: *Pradosia huberi*, fertility, rats, reproductive toxicity, gametes, sperm

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Introduction

Medicinal plants are important sources of bioactive compounds, being used by humans since ancient times to treat their illnesses. The population's compliance to natural therapies has increased significantly, which justifies the interest of many researchers in investigating plants with relevant pharmacological properties, including toxicological testing of plant species.¹ There are reports of many plants potentially toxic to the reproductive system, evidenced by embryotoxic, teratogenic, and abortifacient effects^{2–4} besides changes in the quantitative and qualitative production of gametes.^{5,6}

Pradosia huberi (Ducke) (Sapotaceae)⁷ is a spanning tree 30–40 m tall,⁸ native to the Amazon, popularly known as “casca-doce,” “pau-doce,” or “paracauba” and widely

used in local popular medicine for the treatment of gastritis and gastric ulcer.^{9,10} Several substances were isolated from the ethanol extract of the stem bark of *P. huberi*, such as tannins, terpenoids, quinones, alkaloids, flavonoids, and saponins. Flavonoids were identified as 2,3-dihydromyricetin 3- α -L-rhamnoside, astilbin, and 2,3-engelitin dihydromyricetin, which have anti-inflammatory and antioxidant effects.^{11–13}

Preclinical studies showed the antiulcer activity of hydroalcoholic extract of the stem bark of *P. huberi* in mice,⁹ while other studies showed potent vasorelaxant activity on the endothelium superior mesenteric artery in rats.¹⁰ Acute toxicity tests with the hydroalcoholic extract of this species in rats have demonstrated the occurrence of adverse effects in the body due to reduced water and food consumption in males, affecting body weight. The

hematological analysis and red blood cells indices revealed decreased hematocrit, hemoglobin, and platelets. With regard to chronic treatment, increased levels of aspartate aminotransferase and alanine aminotransferase and histopathological changes (necrosis and influx of lymphocytes) were observed in the liver at the dose of 30.5 mg/kg, indicating hepatotoxic effect.¹⁴ Furthermore, rats treated with alcoholic extract of *P. huberi* during preimplantation showed decreased uterine implants and increased preimplantation losses, suggestive of toxicity on the reproductive system of these animals.¹⁵

Considering the popular use of *P. huberi*, the presence of bioactive compounds capable of interfering with fertility, and the lack of reproductive toxicity studies in males on this plant species, this study aimed at investigating the possible toxic effects of the hydroalcoholic extract of *P. huberi* on the reproductive system of mice.

Material and methods

Plant material

The stem bark of *P. huberi* was collected in the forest area of the Institute of Scientific and Technological Research of the State of Amapá (IEPA), Porto Grande—Amapá, where a voucher specimen is deposited in the Herbarium Amapaense (HAMAB) under No. 12519 at IEPA.

Animals

Adult Wistar rats (*Rattus norvegicus*) (aged 90 days) of both sexes weighing 250–300 g (males) and 150–250 g (virgin females) were obtained from the vivarium Prof. Thomas George, Federal University of Paraíba (UFPB) and used in the experimental protocols.

The animals were grouped in polyethylene cages (three rats per cage) without any medication and with free access to food (*pellets* of Purina®) and drinking water. They were housed under standard laboratory conditions with a light-dark cycle of 12 h and room temperature kept at $21 \pm 1^\circ\text{C}$. The animals were observed daily for clinical signs of toxicity, such as piloerection, changes in locomotor activity, and mortality.

All experimental procedures were performed in accordance with the principles of animal care and approved by the Ethics Committee on Animal Research (CEPA) of LTF/UFPB Protocol (CEPA/LTF: 0409/10).

Preparation of hydroalcoholic extract from the stem bark of *P. huberi* and solutions

The barks were dried at 45°C , crushed in mechanical grinder, powdered (3.3 kg), and macerated with 95% ethanol at room temperature for 10 days. The extraction solution was concentrated on a rotary evaporator at 45°C to yield 480 g crude ethanolic extract of *P. huberi* (PH-HAE).

The choice of doses was based on previous studies that evaluated the antiulcer activity of *P. huberi* in mice⁹ and its acute and chronic toxicity in rats,¹² and were determined by geometric progression, following the guidelines of National Health Surveillance Agency (ANVISA).¹⁶

PH-HAE was diluted in distilled water to obtain solutions of appropriate concentrations. Male rats were randomly distributed into four groups containing 15 animals each and were treated by gavage, once daily, as follows: treatment with PH-HAE T1—1.22 mg/kg, which is the recommended human equivalent dose, T2—6.1 mg/kg (five times the usual human dose), T3—30.5 mg/kg (25 times the usual human dose), and control C—10 mL/kg of distilled water.^{17–19}

Assessment of fertility and reproductive capacity of rats exposed to hydroalcoholic of *P. huberi*

The animals were treated for 63 consecutive days, period that corresponds to the duration of the spermatogenic cycle in Wistar rats plus the sperm transit time through the epididymis.²⁰ In the last week of treatment (from the 57th to the 63rd day), the rats were mated with untreated virgin females ($n=30/\text{group}$), following anesthesia with ketamine-xylazine solution of 0.2 mL/100 g (8.75 mL ketamine [100 mg/mL] and 1.25 mL xylazine [100 mg/mL]) and death by cervical dislocation on the 64th day.^{21,22}

Parameters evaluated in male rats

Body weight (g). For the assessment of body mass, body weight of the animals was recorded on the first day of administration PH-HAE, once a week and on the day of euthanasia.

Water and food consumption. The animals had access to water bottles and daily consumption (mL) was recorded. Daily food intake (g) was also monitored and recorded as the difference between a pre-established amount of food placed in a day and what was left the next day. Since the animals were housed in groups of three per cage, food and water intake was determined by the average consumption of each group.

Evaluation of the reproductive organs. After euthanasia, the animals underwent laparotomy for removal and weighing of the prostate, right epididymis, seminal vesicles, and testes. The organs were freed of adjacent fat and their absolute weight was measured in analytical balance (A&D, HR-120, Tokyo, Japan) followed by the determination of the respective relative weights (organ weight per 100 g of body weight). The organs were also examined macroscopically for assessment of changes in the appearance, size, color, and shape.

Fertility rate. Sperms were collected from the epididymal secretion in the left epididymis cauda. The secretion was placed in a 0.5 mL drop of saline solution and diluted in 20 mL distilled water. From this homogenate, a sample was taken and the number of sperm counted using a hemocytometer with improved double Neubauer ruling. The total number of spermatozoa was obtained by averaging two counts, corresponding to the upper and lower field in the Neubauer chamber.^{23,24}

Parameters evaluated in untreated female rats

Fertility and reproduction indices. The polygamous mating system was employed in which control and treated males were caged overnight with virgin females (in the estrous phase) in the ratio of 1:2.²⁵ In the following morning, the animals were placed in separate cages. Untreated female rats were mated with treated (T1, T2, and T3) and control (C) males ($n = 30$ females per group) and divided into two groups of 15 animals. The presence of spermatozoa in the vaginal smear and/or detection of vaginal plug indicated successful mating and were considered as the first day of gestation.

In group 1, females had the pregnancy interrupted on day 15 of gestation, being anesthetized with 0.2 mL/100 g ketamine-xylazine solution, euthanized by cervical dislocation and examined for analysis of the following reproductive parameters: total number of implanted embryos, number of resorptions, number of corpora lutea per pregnant female, mating index $[(\text{No. of inseminated}/\text{No. of mated females}) \times 100]$, gestation index $[(\text{No. of females with implants}/\text{No. of inseminated females}) \times 100]$, preimplantation loss index $[(\text{No. of corpora lutea} - \text{No. of implants}/\text{No. of corpora lutea}) \times 100]$, implantation index $[(\text{No. of implants}/\text{No. of corpora lutea}) \times 100]$, and occurrence of external malformations.^{26,27}

In group 2, the 15 remaining rats in each group were monitored throughout the gestational period (21 days) and killed on postnatal day 21; after euthanasia, the following variables were assessed: total number of births, number of males and females born, total number of animals weaned, number of males and females weaned, birth index (%) = $(\text{number of pups born alive}/\text{number of pups born}) \times 100$, viability index (%) = $(\text{No. of pups alive on day 4 of postnatal life}/\text{number of live births}) \times 100$, weaning index (%) = $(\text{No. of pups alive at weaning}/\text{number of pups born alive}) \times 100$, postimplantation loss index = $(\text{number of implantations} - \text{number of pups alive}/\text{number of implants}) \times 100$, and occurrence of external malformations.

Statistical analysis

Variables with interval measures and with normal distribution were analyzed by "one-way" or "two-way" analysis of variance. The differences between groups were determined by the Bonferroni test. Values were considered significantly different from each other at $p < 0.05$ and were expressed by mean and standard error (S.E.M.). The variables indicated as percentages or rates were analyzed by the chi-square test and Fisher's exact test. The statistical significance level of 5% ($\alpha = 0.05$) was used.

All data were analyzed using the GraphPad Prism® version 5.01 (GraphPad Software inc., USA).

Results

The occurrence of deaths and changes in locomotor activity, piloerection, or any other clinical signs of toxicity was not detected during the treatment period.

Body weight, water, and food consumption

The administration of 1.22, 6.1, and 30.5 mg/kg PH-HAE did not alter body weight during treatment as compared to control group (Figure 1(a)). Food intake and water consumption remained normal at all dose levels (Figure 1(b) and (c)).

Macroscopic analysis of the reproductive organs

There was no significant macroscopic change in appearance, size, color, and shape of the reproductive organs of rats treated with the three doses of PH-HAE.

The absolute and relative weights of testes, right epididymis, seminal vesicle, and prostate of the treated animals did not change significantly when compared to the control group (Table 1).

Evaluation of fertility index

The concentration of spermatozoa (Figure 2) and the proportion of normal and abnormal sperm (no tail, no head, and short or curled tail) (Table 2) did not differ between animals of the control and the PH-HAE-treated groups.

Evaluation of fertility and reproduction indices

Parameters assessed in female rats with interrupted pregnancy on day 15 of gestation. The PH-HAE administration did not affect mating and the presence of sperm was observed in the vaginal smear of all mated females (mating index = 100%). However, there was 14.3 and 10.8% increase in the preimplantation loss indexes and 14.3 and 10.8% reduction in the implantation indexes of female rats mated with males treated with 6.1 and 30.5 mg/kg PH-HAE, respectively. The number of corpora lutea, implantations, resorptions, and pregnancy rates was not significantly different between treated and control animals (Table 3). There were no external malformations.

Parameters assessed in females at the gestational period (21 days). None of the doses of PH-HAE affected the total number of implants (Table 3), births, and number of weaned animals (Table 4). The birth and viability indexes did not differ between control and PH-HAE-treated groups, but there was 5.6 and 8.2% increase in the postimplantation loss index of female rats mated with males exposed to the doses of 6.1 and 30.5 mg/kg PH-HAE, respectively (Table 5).

Discussion

The species *P. huberi* is widely used in folk medicine by the Amazonian communities for their gastroprotective and antiulcer activities. However, there are few studies on its toxicological activity, especially the reproductive toxicity area. This species has several secondary metabolites, such as flavonoids, saponins, steroids, and triterpenes, which appear to be responsible for the plant's therapeutic properties but are also capable of exerting adverse action in the body, including the reproductive system.^{9,10,24}

The evaluation of body weight during treatment with a particular substance provides information on the general

health of the animals. A reduction of body mass or a decrease in weight gain may show varied responses, including anorexia or systemic treatment-induced toxicity.^{24,28,29} PH-HAE did not alter the ponderal evolution of treated animals, nor did it affect food consumption, suggesting lack of systemic toxic effect. In addition, no clinical

signs of toxicity and deaths were observed during the experimental procedure.

Toxic substances or their biotransformation products can cross the testicular barrier and interfere with spermatogenesis. The normal weight of the testicle does not vary much within a species and this low variability indicates that the

Figure 1 (a) Body weight gain of PH-HAE treated and control rats during 63 days. Symbols and vertical bars represent mean and SEM, respectively. ANOVA “one-way” followed by Bonferroni test ($n = 15$ rats). (b) Average daily food consumption of PH-HAE treated and control groups during 63 days. Symbols and vertical bars represent mean and SEM, respectively. ANOVA “two-way” followed by Bonferroni test ($n = 5$ cages). (c) Average daily water intake of PH-HAE treated and control groups during 63 days. Symbols and vertical bars represent mean and SEM, respectively. ANOVA “two-way” followed by Bonferroni test ($n = 5$ cages). (A color version of this figure is available in the online journal.)

Table 1 Absolute and relative weights of reproductive organs and accessory glands of PH-HAE-treated and control animals during 63 days

Organs	Control	PH-HAE 1.22 mg/kg	PH-HAE 6.1 mg/kg	PH-HAE 30.5 mg/kg
Body weight (g)	398.1 ± 5.62	374.7 ± 9.48	368.1 ± 10.06	353.4 ± 8.30
Absolute weight (g)				
Right testicle	1.65 ± 0.05	1.65 ± 0.04	1.59 ± 0.05	1.58 ± 0.04
Left testicle	1.66 ± 0.05	1.65 ± 0.04	1.61 ± 0.04	1.60 ± 0.04
Seminal vesicle	1.39 ± 0.10	1.45 ± 0.07	1.26 ± 0.07	1.36 ± 0.08
Right epididymis	0.63 ± 0.02	0.59 ± 0.03	0.57 ± 0.02	0.54 ± 0.02
Prostate	0.44 ± 0.02	0.44 ± 0.01	0.40 ± 0.02	0.40 ± 0.02
Relative weight (%)				
Right testicle	0.42 ± 0.01	0.44 ± 0.01	0.43 ± 0.02	0.45 ± 0.01
Left testicle	0.42 ± 0.01	0.44 ± 0.02	0.44 ± 0.02	0.45 ± 0.01
Seminal vesicle	0.35 ± 0.03	0.39 ± 0.02	0.35 ± 0.02	0.39 ± 0.02
Right epididymis	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.06	0.15 ± 0.01
Prostate	0.11 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01

Values expressed as mean ± S.E.M., respectively ($n = 15$ rats). ANOVA “one-way” followed by Bonferroni test.

absolute weight of testis is a precise parameter in the evaluation of gonadal injury.^{30,31,35} After exposure to PH-HAE, no significant change in the weights of testes, epididymis, prostate, and seminal vesicles of rats was observed, indicating that the PH-HAE did not affect the functions of the reproductive organs and accessory sex glands, a fact corroborated by the absence of macroscopic changes in these structures. In toxicity tests on the male reproductive

system, it is fundamental to evaluate the concentration and quality of sperm, once reductions in daily production or increase in the concentration of abnormal gametes and changes in sperm morphology reflect germ cells mutagenicity.^{18,26}

The duration of spermatogenesis comprises the time required for the spermatogonia to divide and form spermatozoa, taking around 4.5 cycles of the seminiferous epithelium. In the Wistar rat, each spermatogenic cycle lasts 12.9 days, and the duration of spermatogenesis is approximately 52 days (four cycles of the seminiferous epithelium).

In this study, the duration of treatment was 63 days, which corresponds to the time for the completion of spermatogenesis and passage through the epididymis.^{20,29,30} When spermatozoa reach the epididymis cauda, they are motile and fertilization-competent cells.^{26,31} Therefore, alterations that occur during epididymal transit play an important role in the functional maturation steps, such as motility and the sperm ability to fertilize the oocyte.³²⁻³⁴ The production of gametes and their morphology were not modified by treatment with PH-HAE, as there was no significant difference between the number of normal and abnormal sperm in the control and treated groups. The absence of changes in mass and morphology of the reproductive organs reinforces the idea that the PH-HAE does not have contraceptive action in rats, since reductions

Figure 2 Sperm concentration in the secretion of the left epididymis tail of PH-HAE treated and control rats during 63 days. The columns and vertical bars represent mean $\pm$ S.E.M., respectively. ANOVA "one-way" followed by Bonferroni test ($n = 15$ rats)

Table 2 Proportion of normal and abnormal rat sperm of PH-HAE treated and control groups during 63 days

Sperm	Sperm concentration (10 ⁻⁶ /mL)			
	Control	PH-HAE 1.22 mg/kg	PH-HAE 6.1 mg/kg	PH-HAE 30.5 mg/kg
Normal	96.03 $\pm$ 0.22	95.92 $\pm$ 0.34	96.02 $\pm$ 0.32	95.45 $\pm$ 0.34
Only head	2.17 $\pm$ 0.08	2.36 $\pm$ 0.17	2.56 $\pm$ 0.17	2.68 $\pm$ 0.29
Only tail	1.17 $\pm$ 0.14	1.15 $\pm$ 0.19	0.88 $\pm$ 0.10	1.09 $\pm$ 0.11
Short tail	0.23 $\pm$ 0.04	0.27 $\pm$ 0.007	0.20 $\pm$ 0.04	0.25 $\pm$ 0.04
Long tail	0.39 $\pm$ 0.04	0.34 $\pm$ 0.04	0.55 $\pm$ 0.08	0.59 $\pm$ 0.07

Values expressed as mean $\pm$ S.E.M., respectively ($n = 15$ rats). ANOVA "one-way" followed by Bonferroni test.

Table 3 Reproductive variables in rats (with pregnancy interrupted on day 15 of gestation) mated with males from PH-HAE treated and control groups during 63 days

Variables	Control	PH-HAE 1.22 mg/kg	PH-HAE 6.1 mg/kg	PH-HAE 30.5 mg/kg
Number of mated females	15	15	15	15
Number of inseminated females	15	15	15	15
Number of females with uterine implants	13	12	12	15
Mating index	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)
Gestation index	86.7% (13/15)	80.0% (12/15)	80.0% (12/15)	100% (15/15)
Number of corpora lutea	185	194	197	194
Number of uterine implants	140	131	121	126
Number of resorptions	3	3	7	5
Preimplantation loss	24.3% (45/185)	32.5% (63/194)	38.6%* (76/197)	35.1%* (68/194)
Implantation rate	75.7% (140/185)	67.5% (131/194)	61.4%* (121/197)	64.9%* (126/194)

*Significant difference compared to control ($n = 15$ rats). Fisher's exact test ($p < 0.05$).

Table 4 Males and females born and weaned of females mated with males from PH-HAE treated and control groups during 63 days

Variables	Control	PH-HAE 1.22 mg/kg	PH-HAE 6.1 mg/kg	PH-HAE 30.5 mg/kg
Number of males born	65	70	63	63
Number of females born	52	62	76	68
Total number of births	117	132	139	131
Total number of pups born alive	117	132	139	128
Number of males weaned	65	67	57	59
Number of females weaned	51	60	73	64
Total number of animals weaned	116	127	130	123

Values were analyzed by chi-square test or Fisher's exact test.

Table 5 Reproductive variables in rats mated with rats from PH-HAE treated and control groups during 63 days

Variables	Control	PH-HAE 1.22 mg/kg	PH-HAE 6.1 mg/kg	PH-HAE 30.5 mg/kg
Birth rate	100% (117/117)	100% (132/132)	100% (139/139)	97.7% (128/131)
Viability index	100% (117/117)	100% (132/132)	100% (139/139)	100% (128/128)
Weaning index	99.1% (116/117)	96.2% (127/132)	93.5%* (130/139)	96.1% (123/128)
Rate of postimplantation loss	1.7% (2/119)	7.0% (10/142)	7.3%* (11/150)	9.9%* (14/142)
No. of litters	15	15	15	15
No. of litters with postnatal loss	1	2	4	2
No. of postnatal loss	1	5	9	5

*Significant difference compared to control ($n = 15$). Fisher's exact test ($p < 0.05$).

Birth rate: (No. of pups born alive/number of pups born) $\times$ 100.

Viability index: (No. of pups alive on day 4 of postnatal/number of pups live births) $\times$ 100.

Weaning index: (No. of pups alive at weaning/number of pups born alive) $\times$ 100.

Rate of postimplantation loss: (No. of implantations – number of pups alive/number of implants) $\times$ 100.

of masses in testis and epididymis of rats are indicators of impaired fertility or contraceptive activity.^{36,37} However, despite the absence of these effects, the occurrence of significant changes in other more sensitive variables used in reproductive toxicity testing cannot be neglected, such as the assessment of sperm motility, which represents the percentage of motile spermatozoa and the duration of forward movement (intensity of motility) and the dominant lethality performed to detect mutagenic effects of certain substances in males or females by assessing interference of germ cells during spermatogenesis or oogenesis, with the potential to be lethal to the offspring.^{18,38,39} Fertility studies through analysis of parameters, such as reproductive indexes, evaluate the result of mating after the pretreatment of at least one sex. The assessment of fertility and pregnancy provides important information about the functional consequences of an agent on the reproductive system.¹⁸ The mating index correlates the number of pregnant females (mated and carried pregnancy to term) with the number of mated females (vaginal smear positive for the presence of sperm).¹⁸ There was no significant difference in the mating index of females mated with PH-HAE-treated males and the treatment with PH-HAE did not result in fewer vaginal plugs formed, indicating that the ability to mate was not impaired. Moreover, it was observed that the number of inseminated females and the number of implants did not decrease due to the treatment of male rats.

Male exposure to a particular toxicant may cause adverse effects on their offspring.^{40–42} The passage of

toxicants from the father to the mother may occur through the seminal transfer and male exposure before conception can result in infertility, pre- and postimplantation losses, embryonic death, abnormalities, as well as reduced birth weight, decreased offspring size, and delayed postnatal development.^{41,43,44} The evaluation of the presence of PH-HAE in the seminal fluid was not in the scope of this investigation, but could become part of future studies in order to contribute to the analysis of the observed alterations.

Implantation is the process by which the embryo reaches the intimate physiological and physical contact with the maternal endometrium for the establishment of pregnancy.⁴⁵ The time and integrity of transport of gametes and zygote, important to fertilization and embryo survival, are quite susceptible to changes caused by chemical compounds. They contribute to reduce the rate of fertilization and increase early embryonic loss identified as preimplantation loss.¹⁸

The implantation index correlates with the number of corpora lutea and is considered an indicator of successful implantation of the blastocyst in the endometrium.^{46,47} In contrast to the implantation index, the presence of resorptions indicates a failure in the embryonic development, while postimplantation losses represent the total reproductive loss during pregnancy.⁴⁸ No significant differences were observed in the number of corpora lutea, implantations, resorptions, and the pregnancy index of mated females rats exposed to PH-HAE. However, exposure to PH-HAE significantly increased the levels of preimplantation losses

at 6.1 and 30.5 mg/kg. This result corroborates previous findings in which the PH-HAE compromised the reproductive capacity of female Wistar rats treated during the embryonic preimplantation period, as evidenced by the decreased number of uterine implants and the occurrence of significant preimplantation losses in these animals.¹⁵ The PH-HAE also increased the postimplantation loss index and reduced the implantation index of females mated with male rats that received 6.1 and 30.5 mg/kg PH-HAE. These results indicate that PH-HAE may impair the rat fertility, suggesting the possibility of mutagenic effects.

The birth index reflects the relationship between the number of pups born alive and the total number of born pups. Exposure of male rats to all dose levels of PH-HAE did not interfere with this parameter, probably because the losses occurred in the pre- and postimplantation periods. A decrease in the weaning index in the group of females mated with males treated with 6.1 mg/kg can be attributed to occurrence of deaths of pups evidenced during lactation. This is a fundamental step for proper growth and survival of newborns as it is the only source of nutrition in the early period of life in most mammals.⁴⁹ Thus, considering that these females had the highest number of pups born alive ($n = 139$), this fact may have caused difficulty in feeding the offspring, thereby reducing the number of pups alive at weaning. Other factors that can also alter the reproductive process include disruption of maternal hormonal balance that may interfere with the development and maintenance of pregnancy, affecting the viability of embryos and fetuses, and the occurrence of teratogenic effects.^{50,51}

The evaluation of teratogenicity in rats exposed during organogenesis, and born to males or females exposed during the premating, mating, and gestational periods, is based on the assumption that the gametes may undergo changes prior to fertilization, resulting in the appearance of adverse effects in the subsequent phases.^{50,51} In the present study, morphological abnormalities were detected in two of the three stillborn pups of female rats mated with males exposed to the dose of 30 mg/kg of PH-HAE. In fact, the number of pups exhibiting structural anomalies as well as the number of stillborn may be even higher than the actual reported numbers due to the possible occurrence of maternal cannibalism. In addition, an umbilical hernia was detected in one pup also born to a female mated with male rats exposed to the dose of 30 mg/kg. The observed morphological anomalies are relevant events and indicate developmental toxicity; however, more specific protocols to detect fetal malformation should be undertaken for a more precise evaluation of the teratogenic effects.

In conclusion, although there was no impairment of the organs of the male rat reproductive system or the quantity or quality of sperm, increased pre- and postimplantation losses were observed, suggesting a potential toxicity of the hydroalcoholic extract of *P. huberi* on the reproductive system of rats.

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AOBR, JCLRP, ALX, CLM, KCJ, NAO conducted the experiments, AANM supplied critical reagents; ECSS, RCSS, MFFMD wrote the manuscript.

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DECLARATION OF CONFLICTING INTEREST

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