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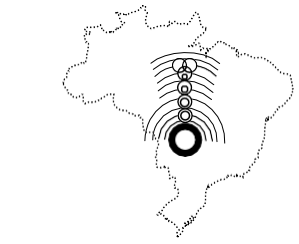

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An experimental investigation on effect of the medicinal plant *Pfaffia glomerata* (Spreng.) Pedersen on gestation

Investigação experimental sobre o efeito da planta medicinal *Pfaffia glomerata* (Spreng.) na gestação

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Abstract

Objective: *Pfaffia glomerata* (*P. glomerata*, Brazilian ginseng) is a medicinal plant used for several therapeutic indications. This study aimed to verify its action on gestation and to identify teratogenic and estrogenic activities. Material and methods: to evaluate its effect on gestation, the hydroalcoholic extract of its roots (1,000 mg/kg/day) was administered orally to mice in the pre-implantation, implantation or early organogenic period. The females were killed on the 18th day of gestation, and *corpora lutea*, implantation sites, reabsorptions and fetuses were counted. The fetuses were examined to malformations and skeletal anomalies. To evaluate an estrogenic activity by *P. glomerata*, sexually immature females received the extract for three days, and the uteri were collected and weighed. Results: the extract did not promote embryonic loss before the implantation nor inhibited this process. However, the females did not gain significant weight during the administration from the first to the third gestation day (gd) and they had more degenerated embryos/dead fetuses and malformed fetuses. The extract did not demonstrate an estrogenic activity by bioassay employing immature mouse uterus. Conclusions: *P. glomerata* extract did not impair the implantation of embryos in mice, but there were more embryonic and fetal death and malformations when administered in the pre-implantation period.

Uniterms: *Pfaffia glomerata*; Plants, medicinal; Pregnancy; Mice.

Resumo

Objetivo: *Pfaffia glomerata* (*P. glomerata*, ginseng brasileiro) é uma planta medicinal empregada para várias indicações terapêuticas. Este trabalho teve como objetivos avaliar sua ação sobre a gestação e identificar atividades teratogênica e estrogênica. Material e métodos: para avaliar o efeito na gestação, o extrato hidroalcoólico das raízes (1.000 mg/kg/dia) foi administrado oralmente a camundongos no período pré-implantação, quando ocorre a implantação dos embriões ou se inicia a organogênese. As fêmeas foram sacrificadas no 18° dia de gestação, e corpos lúteos, sítios de implantação, reabsorções e fetos foram contados. Os fetos foram examinados para malformações e anomalias esqueléticas. Para avaliar uma atividade estrogênica por *P. glomerata*, fêmeas imaturas sexualmente receberam o extrato por três dias, e o útero foi coletado e pesado. Resultados: o extrato não promoveu perda embrionária antes da implantação, nem inibiu este processo. Entretanto, as fêmeas não ganharam peso significativo durante a administração do primeiro ao terceiro dia de gestação e tiveram mais embriões degenerados e fetos malformados ou mortos. O extrato não demonstrou atividade estrogênica empregando-se o bioensaio do útero de camundonga jovem. Conclusões: o extrato de *P. glomerata* não afetou a implantação de embriões em camundongos, mas houve mais morte embrionária e fetal e malformações quando administrado no período pré-implantação.

Unitermos: *Pfaffia glomerata*; Plantas medicinais; Gravidez; Camundongos.

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Introduction

Pfaffia glomerata (Spreng.) Pedersen, Amaranthaceae family, is a Brazilian medicinal plant used popularly to treat gastric disturbances and chronic inflammation[1,2]. It has attracted the attention in recent years as an economically accessible substitute to ginseng (*Panax ginseng* C.A. Meyer). Aiming at its commercialization as an adaptogen and a stimulant, psychopharmacological assessment, studies on the reproductive performance and biological *in vitro* assays were carried out[3-9]. However, the effect on gestation and on the embryo-fetal development had not been investigated. To this aim, the same methodology employed to evaluate abortive plants was used[10-12], the experimental model was the mouse and the administration was restricted to the first half of the gestation, segmented in the periods pre-implantation, implantation and post-implantation.

Material and methods

Vegetal material and preparation of the extract

Pfaffia glomerata (Spreng.) Pedersen roots were obtained from Chemical, Biological and Agricultural Research Center (CPQBA) of the State University of Campinas (Unicamp), at Paulínia, São Paulo, Brazil. The identification was made by Agronomist Engineer Ílio Montanari, and a voucher specimen was deposited in the herbarium of this institute under number 0238.

The extract was provided for doctor Grace Gosmann, from Faculty of Pharmacy of Federal University of Rio Grande do Sul (UFRGS). The roots were reduced to small pieces, dried in circulating air stove (45 °C) and triturated. The powder was extracted with 60% ethanol under reflux (1;10, plant; solvent) during six hours. The ethanol was removed under vacuum, and the resulting residue was lyophilized. The yield was 28% in relation to the dried vegetal material. The lyophilized hydroalcoholic extract was stored frozen from where it was taken when required.

Animals

Adult, 2- to 3-month-old, and young, 23- to 24-day-old, *Mus domesticus domesticus* CF1 mice were used. They were maintained in the Laboratory of Reproduction Biology from the Department of Morphological Sciences of the UFRGS, at 20 to 25 °C, under a natural photoperiod. The animals were fed with Nuvilab Cr-1® rat food (Nuvital Nutrientes S/A, Colombo, PR, Brazil) and water *ad libitum*.

All procedures were performed in accordance with the ethical principles for animal research adopted by the Brazilian College of Animal Experimentation.

Dose and route of administration

The females of the Treated Groups received 1,000 mg/kg per day of the extract suspended in distilled water in a proportion of 250 mg to 1 mL. The Control Groups received only the vehicle (4 mL/kg). The administration was oral, using a curved needle and a tuberculin syringe.

Abortive activity

The females received the extract or the distilled water from the first to the third gestation day (gd, pre-implantation period), from the fourth to the sixth gd (implantation period), or from the seventh to the ninth gd (post-implantation period, when organogenesis and placentation start). The first gd was the day when the vaginal plug was observed. Each group contained 20 animals, which were weighed on the first gd, on the first day of administration, on the day after the last dose and on the 18th gd, when they were killed by cervical dislocation. The ovaries were collected and weighed. Its *corpora lutea* (or albicans) were counted under a stereomicroscope. The uterus was opened for counting of live and dead fetuses, degenerated embryos and late reabsorptions. After it was incubed in 10% ammonium sulfide for ten minutes for counting of implantation sites and early reabsorptions[13]. The placentae and the live fetuses were weighed. The fetuses were examined for external malformations and fixed either in Bouin's fluid for posterior analysis of internal malformations[14] or in 95% ethanol for staining with alizarin red S[15] and identification of skeletal anomalies, observation of the skull plates and counting of metacarpals, metatarsals, sternebrae and xiphisternum, ribs, lumbar vertebrae, and sacral and caudal vertebrae.

Estrogenic activity

Sexually immature females received the extract (n=10) or distilled water (n=10) for three days. If the vagina was opened, smears were taken and stained by Shorr's technique[16]. The females were weighed on the first day of administration and, on the day after the last dose, when they were killed, and uteri were collected and weighed[17].

Statistical analysis

The body, organs and fetus' weight and the number of *corpora lutea*, implantation sites and live fetuses were expressed as mean±standard deviation and analyzed by *t*-Student test. The number of reabsorptions, degenerated embryos and dead fetuses, and the reproductive indices were expressed as median and interquartis range, and analyzed by Mann-Whitney U-test. This

test was used also to skeletal and teratological data[18-20]. A probability level of less than 5% was considered significant.

Results

The females treated with *P. glomerata* extract from the first to the third gd did not have a significant weight gain during the period of administration (controls gained significant weight), but there was no statistically difference to corporal weight between the Treated and Control Groups. In other experiments, both control and treated-females had a significant weight gain during the administration (Tables 1, 2 and 3).

The effect of *P. glomerata* on the reproductive parameters and rates is shown in Tables 4 and 5. The number of implantation sites, reabsorptions and live fetuses was not altered by extract. In spite of not being statistically significant, there was an accentuated number of degenerated embryos and dead fetuses in the group treated from the first to the third gd: 14, while Control and other treated groups had 1 to 4. Out of 20 treated females, eight had degenerated embryos and dead fetuses. The death and birth rates were not significantly different between the Treated and Control Groups.

There was no significant difference between the ovary, placenta and fetus' weight of the Treated and Control Groups (Table 6).

In the group treated from the first to the third gd, there were more cases of malformations: of 133 analyzed fetuses, 12 had cleft palate or exencephaly, while of 119 control fetuses, only three were abnormal. By skeletal analysis, a fetus with misaligned vertebral column was found in this Treated Group. A case of micrognathia was observed in the group treated from the fourth to the sixth gd, and other in the Control Group from the seventh to the ninth gd. Retarded bone development was not found in the Treated Groups.

The extract had no estrogenic activity, because it did not induce neither a premature opening of the vagina nor an uterotrophic effect. The relative uterine weight (mg/100 g) was 107.84±21.94 in the Treated Group and 109.74±19.90 in the Control Group (Table 7).

Table 1 - Effect of the *P. glomerata* extract administered from the first to the third gd on body weight

Group	Body weight (g)		
	1st gd	4th gd	18th gd
Treated	29.94±1.36	30.22±2.05*	54.56±4.60
Control	29.91±1.43	30.57±1.35	52.01±5.73

*No significance relative to initial body weight: p=0.421 by *t*-Student test.

Table 2 - Effect of the *P. glomerata* extract administered from the fourth to the sixth gd on body weight

Group	Body weight (g)			
	1st gd	4th gd	7th gd	18th gd
Treated	30.22±1.84	31.65±1.78	33.42±2.92	49.13±9.43
Control	30.85±2.42	31.94±2.09	33.03±2.13	52.27±7.42

Table 3 - Effect of the *P. glomerata* extract administered from the seventh to the ninth gd on body weight

Group	Body weight (g)			
	1st gd	7th gd	10th gd	18th gd
Treated	34.55±1.33	37.23±1.69	38.86±1.84	55.15±9.18
Control	35.89±2.82	38.0±2.76	39.58±2.98	56.77±7.88

Table 4 - Effect of the *P. glomerata* extract on reproductive parameters

Group	Corpora lutea	Implantation sites	Reabsorptions	Degenerated/ dead fetuses	Live fetuses
Treated					
1st to 3rd gd	15.65±1.63	14.75±2.12	1 [0-2]	0 [0-1.5]	12.6±2.64
4th to 6th gd	14.9±1.55	11.0±6.1	2 [0.5-2]	0 [0-0]	9.15±5.38
7th to 9th gd	17.25±1.94	12.4±6.45	1 [0-2.5]	0 [0-0]	10.5±5.51
Control					
1st to 3rd gd	15.05±1.23	12.85±3.75	1 [0-2]	0 [0-0]	11.1±3.89
4th to 6th gd	15.25±2.05	12.5±4.63	2 [0-2.5]	0 [0-0]	10.5±4.27
7th to 9th gd	17.15±1.57	13.25±5.29	1.5 [1-2.5]	0 [0-0]	11.1±4.69

Table 5 - Effect of the *P. glomerata* extract on reproductive rates

Group	Implantation rate[a] (%)	Reabsorption rate[b] (%)	Death rate[c] (%)	Birth rate[d] (%)
Treated				
1st to 3rd gd	100 [90.0-100]	7.2 [2.7-14.3]	0 [0-9.4]	83.2 [75.1-93.3]
4th to 6th gd	97.1 [62.6-100]	13.3 [3.1-17.2]	0 [0-0]	78.5 [43.1-86.2]
7th to 9th gd	87.0 [54.6 -93.9]	11.2 [0-20.6]	0 [0-0]	69.5 [47.0-82.9]
Control				
1st to 3rd gd	93.7 [86.2-100]	7.1 [3.1-20.9]	0 [0-0]	83.4 [61.3-92.9]
4th to 6th gd	93.8 [80.6-100]	12.9 [0-18.4]	0 [0-0]	80.0 [62.4-86.2]
7th to 9th gd	88.9 [68.3-100]	11.2 [6.2-19.9]	0 [0-0]	75.7 [55.3-80.0]

[a]Implantation rate=(number of implantation sites/number of *corpora lutea*) x 100; [b]reabsorption rate=(number of reabsorptions/number of implantation sites) x 100; [c]death rate=(number of degenerated embryos and dead fetuses/number of implantation sites) x 100; [d]birth rate=(number of live fetuses/number of *corpora lutea*) x 100.

Table 6 - Effect of the *P. glomerata* extract on ovary, placenta and fetus' weight

Group	Ovary weight (mg)	Placenta weight (mg)	Fetus weight (mg)
Treated			
1st to 3rd gd	12.33±1.55	97.08±9.51	919.38±101.43
4th to 6th gd	12.2±1.70	100.01±10.64	969.91±129.30
7th to 9th gd	13.45±1.93	102.49±16.28	890.44±82.58
Control			
1st to 3rd gd	11.75±1.33	100.93±9.60	886.77±97.73
4th to 6th gd	12.73±2.02	100.47±7.71	959.86±75.32
7th to 9th gd	14.73±2.19	103.35±9.34	861.23±92.82

Table 7 - Effect of the *P. glomerata* extract on corporal and uterine weight of immature females

Group	Initial body weight (g)	Final body weight (g)	Uterine weight (mg)	Relative uterine weight (mg/100 g)
Treated	13.45±1.33	16.69±1.30*	18.10±4.56	107.84±21.94
Control	12.21±1.54	15.76±1.68*	17.50±4.79	109.74±19.90

*Significance relative to initial body weight: p=<0.05 by *t*-Student test.

Discussion

The hydroalcoholic extract of *P. glomerata* root did not promote embryonic loss before the implantation, did not inhibit the process of implantation of the embryos, nor increased the incidence of embryonic reabsorptions. The reproductive rates did not differ statistically between the Treated and Control Groups in all the periods of administration. However, the females which received the extract from the first to the third gd had impaired weight gain during the administration. In this Treated Group, many degenerated embryos and dead fetus, and more cases of malformations and skeletal anomalies were found. These results might be due to presence of cytotoxic substances. Evaluating the same type of extract, antiviral, antifungal, antiproliferative and antimicrobial activities were not identified[8,9]. However, in a study to evidence the antioxidant action of *P. glomerata* root, the methanolic extract showed cytotoxicity on mice macrophages, probably due to ginsenosides[21].

Despite of the identification of phytosteroids in *Pfaffia paniculata* (Mart.) O. Kuntze root, which increased the levels of sex hormones in males and females of mice[22], *P. glomerata* extract did not demonstrate estrogenic activity by the applied methodology.

Although this study has been the first approach on the effect of *P. glomerata* on gestation and has been carried through in mice, the findings with the administration in the pre-implantation period, specially the embryonic and fetal death, indicate the necessity of caution in its popular and commercial use for women in reproductive age.

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Referências bibliográficas

1. Freitas CS, Baggio CH, da Silva-Santos JE, Rieck L, de Moraes Santos CA,Júnior CC, et al. Involvement of nitric oxidein the gastroprotective effects of an aqueous extract of Pfaffia glomerata (Spreng.) Pedersen, Amaranthaceae, in rats. Life Sci. 2004;74(9):1167-79.

2. Neto AG, Costa JM, Belati CC,Vinhólis AH, Possebom LS, da Silva Filho AA, et al. Analgesic and anti-inflammatory activity of a crude root extract of Pfaffia glomerata (Spreng.) Pedersen. J Ethnopharmacol. 2005;96(1-2):87-91.

3. de-Paris F, Neves G, Salgueiro JB, Quevedo J, Izquierdo I, Rates SM. Psychopharmacological screening of Pfaffia glomerata Spreng. (Amaranthaceae) in rodents. J Ethnopharmacol. 2000;73(1-2):261-9.

4. Marques LC, Galvão SM, Espínola E, Dias RF, Mattei R, Oliveira MG, et al. Psychopharmacological assessment of Pfaffia glomerata roots (extract BNT-08) in rodents. Phytother Res. 2004;18(7):566-72.

5. Kohguchi M, Toshimori Y, Hanaya T, Arai S, Ikeda M, Kurimoto M. Enhancing effect of Brazilian Pfaffia glomerate on the reproductive ability of male golden hamsters and of male mice. Nat Med. 1998;52:68-73.

6. Kohguchi M, Toshimori Y, Hanaya T, Arai S, Ikeda M, Kurimoto M. Enhancing effect of Brazilian Pfaffia glomerate on the declined reproductive ability of aged male golden hamsters. Nat Med. 1999;53:42-5.

7. Arletti R, Benelli A, Cavazutti E, Scarpetta G, Bertolini A. Stimulating property of Turnera diffusa and Pfaffia paniculata extracts on the sexual behavior of male rats. Psychopharmacology (Berl). 1999;143(1):15-9.

8. Gosmann G, Gattuso S, Gattuso M, Fenner R, Pacheco EF, Ferraz A, et al. Botanical (morphological, micrographic), chemical and pharmacological characteristics of Pfaffia species (Amaranthaceae) native to South Brazil. RBCF. 2003;39:141-7.

9. Vigo CLS, Narita E, Nakamura CV, Marques LC.Avaliação dos efeitos das raízes de Pfaffia glomerata (Spreng.) Pedersen sobre o tempo de sono e crescimento bacteriano. Rev Bras Farmacogn. 2003;13(Supl.2):14-7.

10. Montanari T, Bevilacqua E. Effect of Maytenus ilicifolia Mart. on pregnant mice. Contraception. 2002;65(2):171-5.

11. Freitas TG de, Augusto PM, Montanari T. Effect of Ruta graveolens L. on pregnant mice. Contraception. 2005;71(1):74-7.

12. Barilli SLS, Santos ST dos, Montanari T. An experimental investigation on Luffa operculata (L.) Cogn. (buchinha-do-norte) as abortifacient plant. Reprodução & Climatério. 2007;22(4):165-8.

13. Salewski E. Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Archiv Pathol Pharmakol. 1964;247(4):367.

14. Barrow MV, Taylor WJ. A rapid method for detecting malformations in rat fetuses. J Morph. 1969;127(3):291-306.

15. Dawson AB. A note on the staining of the skeleton of cleared specimens with Alizarin Red S. Stain Techn. 1926;1:123-4.

16. Shorr E.A new technique for staining vaginal smears: III, a differential stain. Science. 1941;94(2449):545-6.

17. Rubin BL, Dorfman AS, Black L, Dorfman RI. Bioassay of estrogens using the mouse uterine response. Endocrinology. 1951;49(4): 429-39.

18. Weil CS. Selection of the valid number of sampling units and a consideration of their combination in toxicological studies involving reproduction, teratogenesis or carcinogenesis. Fd Cosmet Toxicol. 1970;8(2):177-82.

19. McCaughran DA, Arnold DW. Statistical models for numbers of implantation sites and embryonic deaths in mice. Toxicol Appl Pharmacol. 1976;38:325-33.

20. Manson JM, Zenick H, Costlow RD. Teratology test methods for laboratory animals. In: Hayes AW, editor. Principles and methods of Toxicology. New York: Raven Press; 1982. p. 141-84.

21. de Souza DJ, Alves K, da Silva JD, da Silva e Souza P, de Carvalho M, Freire R, et al. Free radical scavenging activity of Pfaffia glomerata Pederson. Indian J Pharmacol. 2005;37(3):174-8.

22. Oshima M, Gu Y. Pfaffia paniculata-induced changes in plasma estradiol-17-beta, progesterone and testosterone levels in mice. J Repr Dev. 2003;49(2):175-80.

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