Toxicology evaluation of *Pfaffia glomerata* (Spreng.) Pedersen root extract in rats

Luis Carlos Marques[1*], Suzana Maria Galvão Cavalcanti[2], Elisaldo de Araújo Carlini[3,†]

[1]*Fitoscience Ensino (MEI), Rua Barão do Triunfo 763, 04602-003, São Paulo, Brazil*
[2]*Center for Health Sciences, Universidade Estadual do Piauí, Brazil*
[3,†]*Federal University of São Paulo, Brazil - In memorian*
**Corresponding author: luis.marques1957@gmail.com*

ABSTRACT

Pfaffia glomerata (Spreng.) Pedersen (PG) is one of the species referred to as 'Brazilian ginseng', widely commercialized in Brazil. Acute and repeated dose toxicological evaluations of PG lyophilized root extract was conducted in young male and female Wistar rats. The acute test did not result in mortality or alter the weight evolution of the animals, but it led to a decrease in heart weight in animals orally treated with doses of 4.3 and 5.8 g/kg. In the repeated dose test, one death occurred in the control group of females; organ weights were equivalent in all groups, and anatomopathological analysis did not show treatment-related changes. Hematological data showed significant increases in red blood cell count, hematocrit, and hemoglobin content in females, which may express a possible hematopoietic stimulating effect of the extract; in biochemical data, an increase in CPK values in treated males and a decrease in values of renal function tests, such as uric acid, urea, and creatinine, especially in males, were highlighted, among several other significant data. Overall, at the doses employed, low toxicity is attributed to the PG lyophilized root extract in male and female rats.

Keywords: *Pfaffia glomerata*; suma; Brazilian ginseng; toxicity studies

INTRODUCTION

Pfaffia glomerata (PG) is one of the species known as Brazilian ginseng, para-tudo, or fafia, and its roots and aerial parts are popularly used as a general tonic, analgesic, rejuvenator, and aphrodisiac, among other uses, being prepared by infusion, decoction or tincture according to the region of the country (Prudente et al. 2024). Chemically, they mainly contain about 13% triterpene saponins and ecdysteroids (Vigo et al. 2003), with substances such as β-ecdysone, 20-hydroxyecdysone rubrosterone, pfameric acid, glomeric acid, among others, being prominent (Nishimoto et al. 1990; Nishimoto 1992; Franco et al. 2024).

Pharmacological studies have shown positive effects in learning and memory models after chronic administration (Marques et al. 2004), as well as gastroprotective (Freitas et al. 2004), analgesic, and anti-inflammatory actions (Neto et al. 2005), neuroprotective effects (Franco 2021), adaptogenic properties (Mendes 2011), among others.

In terms of safety, positive genotoxicity was reported by Rivera et al. (1994) in aqueous extracts, and Souza-Daniel et al. (2005) found cytotoxicity of butanolic extract from PG roots in peritoneal macrophages of mice; however, Almeida et al. (2017) demonstrated antimutagenic activity of PG. Marques et al. (2004) conducted an acute toxicological test in mice with a 50% hydroalcoholic extract of PG roots at an oral dose of 3 g/kg and observed for 14 days, with no mortality or other signs of alterations occurring.

In the use of PG during pregnancy, Toledo et al. (2004) and Barilli and Montanari (2008) assessed effects on pregnant rats and mice, finding indicative alterations of risks in this condition. Regarding the effects of PG on males, Matta (2012), Dias (2018), Dias et al. (2020) and Matta et al. (2020) observed negative changes in spermatogenesis, reduction in daily sperm production volume, contradictory effects on testosterone, among other effects. It was confirmed that prolonged use of PG can cause deleterious effects on spermatogenesis.

Received: December 30, 2023
Accepted after revision: August 1, 2024
Published on line: August 5, 2024
ISSN 1983-084X

Focusing on studies about PG and the liver, Silva (2021) and Dias et al. (2023) found alterations such as decreased glutathione *S*-transferase, increased malondialdehyde, nitric oxide and IL-10, sinusoidal congestion, reduction in liver weight and number of viable hepatocytes, and contradictory effects on levels of aspartate aminotransferase and alanine aminotransferase. Taken together, the results indicate that treatment with PG may induce changes in oxidative stress markers and lead to liver injury.

However, there are no pre-clinical toxicological studies in the regulatory standards of a specific extract and standardization. Therefore, it was decided to conduct the present study with a lyophilized 50% hydroalcoholic extract of PG roots, aiming at the development of an herbal medicine for the pharmaceutical market.

MATERIAL AND METHODS

This study was conducted at the Psychobiology Department of the Federal University of São Paulo in collaboration with the Pathological Anatomy Department of the same institution.

Plant material

The roots of PG were collected from Porto Rico city, state of Parana, Brazil. The taxonomic identity was confirmed by the botanist Josafá Siqueira, curator of the Herbarium Friburguense (FCAB), where a voucher specimen has been deposited (number 5426).

In relation to SISGEN (National System for the Management of Genetic Heritage and Associated Traditional Knowledge) register, the collection carried out for the present study took place in 1995, therefore before the publication of Provisional Measure 2186-16 of 2001 and Law 13123 of 2015.

Extract preparation and chemical quantification

The dry plant roots were powdered, and the extract was prepared with 50% ethanol (v/v) by turbolysis for 30 min with temperature control and subsequently filtered, concentrated, lyophilized, and placed in a vacuum freeze dryer. Just before administration, the lyophilized extract was weighed and made soluble in water.

The vegetable drug and the lyophilized extract were analyzed in relation to the marker β-ecdysone (Sigma) using a high-performance liquid chromatographer, Hewlett Packard model 1050, Merck Lichrosphere 100 (RP-18) 5µm column sized 250x4 mm. The conditions used were: a temperature of 27 °C, a running time of 18 min, a fluid rate of 1 ml per minute, and a mobile phase of methanol-water (40:60, v/v). The detection was performed at a wavelength of 235 nm.

Animals

The study involved 3 to 4-month-old male and female Wistar rats, weighing between 360-400 g. All animals were housed in separate rooms with a 12-h light/dark cycle and maintained at a constant temperature of 22±1 °C. They had free access to water and food. In regard to animal ethics, the protocol (nº 112/95) was submitted to the Animal Ethics Committee of the Federal University of Sao Paulo, having been approved without restrictions.

Single dose toxicological evaluation

Male rats were weighed, divided into groups of 5 animals each, housed in identified polypropylene boxes, and deprived of food for a 14-h period. In the oral test, four groups of animals received gavage administration of water (control group) or lyophilized extract at doses of 3.5, 4.3, and 5.8 g/kg. In the intraperitoneal (i.p.) test, the control group plus three other groups of animals treated with lyophilized extract at doses of 0.6, 0.9, and 1.5 g/kg were used. After administration, the animals were observed for a few hours, and then daily for 14 days. Their weight evolution was monitored, and at the end of the period, all animals were euthanized, and their kidneys, heart, and liver were removed, weighed, and evaluated for macroscopic abnormalities (Andrade et al. 2002).

Repeated dose toxicological evaluation

Groups of young male and female rats aged 3-4 months were weighed and separated into groups of five animals. Oral administration was carried out for 90 days, with a control group receiving water, and groups receiving lyophilized extract at doses of 10 and 100 mg/kg. Throughout this period, all animals were periodically weighed to monitor their weight evolution.

At the end of the administration period, the animals were fasted for approximately fifteen hours and then euthanized by decapitation (Andrade et al. 2002; AVMA 2020), with blood immediately collected for hematological (type H3 - Technicon device, Bayer) and biochemical (Technicon device - RA XT by Bayer) testing.

For anatomopathological examination, the animals' organs (kidneys, liver, and heart) were removed, weighed, and placed in 10% formaldehyde solution. Subsequently, they were embedded in paraffin blocks, cut using a microtome, stained using the hematoxylin-eosin technique, and evaluated under a binocular microscope.

Statistical analysis

The data were analyzed by means of a one-way Analysis of Variance (ANOVA), followed by later comparisons by Duncan's Multiple Comparison Test, for the evaluation of the animals' biochemical and hematological data. Two-way ANOVA followed by later comparisons also by Duncan's Test were performed for the ponderal evolution evaluation during sub-chronic treatment. Qui-square (X2) Test was applied for the evaluation of the sub-chronically treated rat's mortality rate, and Fisher's exact test (one-dimensional) was applied for the evaluation of the anatomopathological results of the animals' organs. A $p \leq 0.05$ level of significance was considered for the statistical analyses.

RESULT AND DISCUSSION

Chemical quantification of the drug and lyophilized extract

The chemical quantification of the drug and the lyophilized by HPLC in terms of β-ecdysone yielded values of 0.8% for the dry roots and 1.1% for the lyophilized extract (Retention times: 12.084 for the standard, and 12.305 and 11.796 min for the drug and the extract, respectively). The chromatograms of the vegetable drug and the lyophilized extract are presented in figures 1S and 2S (Supplementary Material).

Single dose toxicological evaluation

The acute testing in rats did not result in mortality during the 14 days of observation for either administration route used. Thus, it is not possible to determine the LD_{50}, which could be found with doses higher than those used in the present experiment (5.8 g/kg orally and 1.5 g/kg i.p.). These findings align with those reported by Marques et al. (2004) for PG roots, indicating a low acute toxicological profile. No behavioral changes were observed during the observation period, and the weight evolution of the animals was consistent across all groups (see Table 1).

There were no evident macroscopic changes in the organs of the animals. However, a significant decrease in the weight of the hearts was observed in rats orally treated with the extract at doses of 4.3 and 5.8 g/kg ($p = 0.003$ and 0.017, respectively). In the intraperitoneal test, there was also a tendency towards decreased heart weight in rats treated with the higher dose of 1.5 g/kg ($p = 0.055$). The weights of the other organs (liver and kidneys) were equivalent to those of the control group. This effect is likely dose-dependent, evident at doses well above those commonly used, approximately 2 g or 30 mg/kg of powdered roots per day (Pronat 1994; Brasmédica).

Table 1. Ponderal evolution and weight (in grams ± s.d.) of the organs of the male rats treated acutely with water or lyophilized extract of PG (N= 5 animals per group).

			PG lyophilized doses	
Oral route	**Control**	**3.5 g/kg**	**4.3 g/kg**	**5.8 g/kg**
Day 0	368.8 ± 34.5	369.9 ± 36.0	363.9 ± 14.6	381.5 ± 46.2
Day 7	388.2 ± 32.7	381.4 ± 42.6	377.2 ± 4.6	402.9 ± 31.6
Day 14	397.7 ± 33.5	381.6 ± 17.6	380.3 ± 14.5	405.8 ± 36.5
Heart	2.02 ± 0.29	1.82 ± 0.28	1.46 ± 0.19 *	1.59 ± 0.19 *
Kidneys	2.89 ± 0.17	2.98 ± 0.31	3.08 ± 0.18	3.07 ± 0.50
Liver	17.69 ± 1.96	18.68 ± 4.02	18.38 ± 1.28	18.33 ± 2.10
			PG lyophilized doses	
I.p. route	**Control**	**0.6 g/kg**	**0.9 g/kg**	**1.5 g/kg**
Day 0	349.2 ± 18.2	343.2 ± 9.5	341.2 ± 24.4	363.9 ± 17.2
Day 7	368.5 ± 19.6	363.3 ± 11.7	375.6 ± 46.6	379.9 ± 22.6
Day 14	373.5 ± 18.0	364.6 ± 10.5	380.5 ± 47.2	378.6 ± 20.5
Heart	1.63 ± 0.20	1.52 ± 0.29	1.50 ± 0.19	1.32 ±0.17 #
Kidneys	2.69 ± 0.19	2.66 ± 0.21	2.80 ± 0.33	2.77 ± 0.24
Liver	17.34 ± 1.74	17.08 ± 0.85	18.32 ± 2.90	17.39 ± 1.16

* Statistically different from the control (ANOVA, $p \leq 0.05$ for Duncan's Test)

Tendency to significance – $p = 0.055$

Repeated dose toxicological evaluation

The data regarding the weight evolution of male and female rats are presented in Figure 1, illustrating consistent weight gain across all groups without any discrepancies observed at any of the evaluation time points. Approximately 60 days into the administration period, there was one mortality case in a female rat belonging to the control group, with no mortality cases in the experimental groups.

Hematological data are provided in Table 2. The most notable findings include significant increases in red blood cell count, hemoglobin content, and hematocrit in the blood of females treated with both doses of the extract. These results appear to demonstrate a hematopoietic stimulatory effect on the red blood cell series, which, although not indicative of toxicological risk, may be related to the popular use of PG in cases of anemia (Sigrist 2012). In a similar way, Gao et al. (1992) found that *Panax ginseng* extract can promote the proliferation of normal hematopoietic progenitor cells in healthy individuals and patients with aplastic anemia, while Wilkes et al. (2021) also observed this effect with ginsenoside Rb1 from this same species.

Similar results to those of this study were obtained by Rao et al. (2001) with guggul gum resin oil from *Commiphora mukul* Engl., whose steroid actives could be affecting androgen levels and stimulating hematopoiesis. Thus, PG extract could be acting in a similar manner by stimulating testosterone, although stimulating data on the production of this hormone in females are evident in *P. paniculata* (Oshima and Gu 2003), but are contradictory in studies with males treated with PG, with an increase in serum values at a dose of 400 mg/kg (Mata 2012), a reduction in these values at doses above 200 mg/kg (Dias 2018), or no changes after treatment (Fernandes et al. 2015). Further investigations are necessary for a better understanding of this effect on the red blood cell series in females.

Regarding the white series, eosinophil values are elevated in all groups compared to literature parameters (Ward et al. 2018), and this increase may be due, among other factors, to a possible infestation scenario that can occur in laboratory animals (Andrade et al. 2002). In the statistical evaluation, a decrease in values was found in females treated with both doses of the PG lyophilized extract compared to the control group, although this difference was not significant in male data. Its reduction in both treated groups may be related to some modulating effect of the innate immune system or a possible anti-protozoan activity, already verified in PG and other species of the genus, such as *P. townsendii* and *P. tuberosa*, concerning parasites such as *Trypanosoma cruzi* and *Leishmania* (L.) *amazonensis* (Neto et al. 2004; Corrêa 2014). However, since this reduction was not observed in the male group, further investigation is needed for a comprehensive understanding of this data.

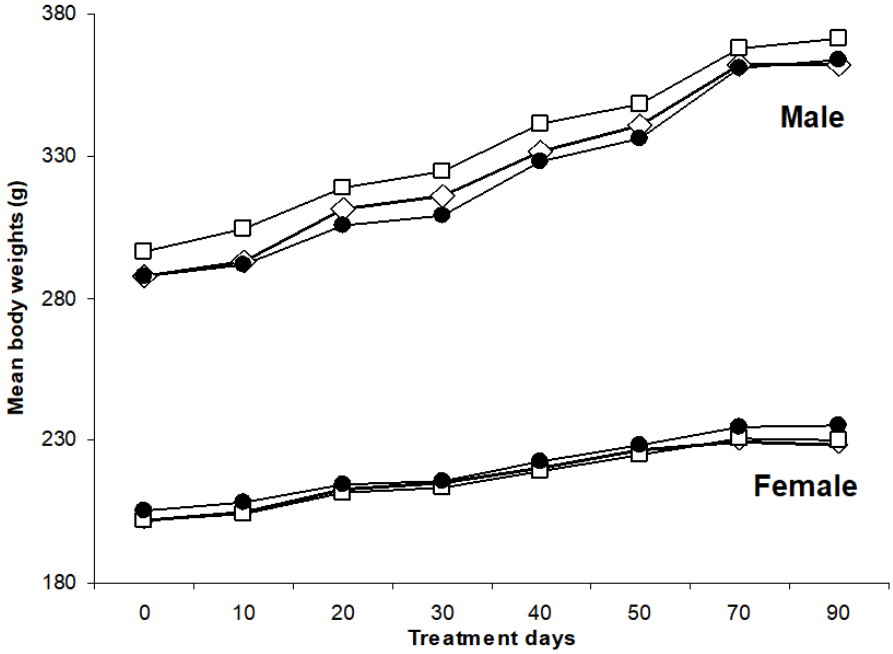

Figure 1. Ponderal evolution of young male and female rats treated with water (control) and with PG lyophilized extract at 10 mg/kg and 100 mg/kg (N= 17 for each group), orally (gavage) for 90 days. The figures are expressed by average. Two-way ANOVA not significant (control ◊ ; PG 10 mg/kg □; PG 100 mg/kg •).

Table 2. Hematological parameters of male and female rats treated chronically with water or PG lyophilized extract (N= 17 per group; mean ± s.d.).

Parameters	Control	PG 10 mg/kg	PG 100 mg/kg	Normal values (Ward et al. 2018)
Males				
Red blood cells (millions/mm³)	7.5 ± 0.6	7.6 ± 0.6	7.7 ± 0.4	5,8-10
Hemoglobin (g/dl)	12.5 ± 1.0	12.8 ± 1.1	12.8 ± 0.7	13-23
Hematocrit (%)	40.7 ± 2.3	41.4 ± 2.3	40.8 ± 1.9	39-69
MCV (u³)	54.2 ± 2.4	54.3 ± 2.1	52.8 ± 2.0	58-77
MCH (uuG)	16.6 ± 0.5	16.7 ± 0.7	16.6 ± 0.5	17-25
MCHC (%)	30.7 ± 1.1	30.9 ± 1.2	31.3 ± 1.1	27-38
Platelets (mil/mm³)	500 ± 141	513 ± 212	468 ± 143	800-2,600
White blood cell (mil/mm³)	8.6 ± 2.1	8.7 ± 1.4	10.0 ± 2.4	4,8-18
Neutrophils (%)	43.9 ± 10.2	43.8 ± 12.7	38.9 ± 6.2	16-54
Eosinophils (%)	6.0 ± 3.1	6.3 ± 2.9	8.2 ± 2.7	0,1-4,3
Basophils (%)	0.4 ± 0.3	0.4 ± 0.1	0.4 ± 0.2	0-1,7
Lymphocytes (%)	38.2 ± 9.3	36.3 ± 12.8	40.9 ± 7.3	38-79
Monocytes (%)	7.8 ± 1.1	9.4 ± 2.4*	7.9 ± 1.4	3-11
Females				
Red blood cells (millions mm³)	7.0 ± 0.6	7.4 ± 0.4*	7.4 ± 0.5*	5,8-10
Hemoglobin (g/dl)	12.2 ± 1.0	13.0 ± 0.7*	13.1 ± 0.8*	13-23
Hematocrit (%)	39.4 ± 2.3	41.4 ± 2.5*	42.5 ± 1.8*	39-69
MCV (u³)	56.5 ± 2.8	56.0 ± 3.1	57.4 ± 4.0	58-77
MCH (uuG)	17.4 ± 0.7	17.7 ± 0.8	17.6 ± 1.0	17-25
MCHC (%)	30.9 ± 1.3	31.6 ± 1.3	30.8 ± 2.0	27-38
Platelets (mil/mm³)	577 ± 163	575 ± 209	681 ± 131	800-2,600
White blood cell (mil/mm³)	6.7 ± 1.3	6.8 ± 1.3	7.4 ± 1.5	4,8-18
Neutrophils (%)	38.4 ± 13.3	41.1 ± 11.6	31.1 ± 10.2	16-54
Eosinophils (%)	9.5 ± 9.3	4.2 ± 2.4*	5.0 ± 4.0*	0,1-4,3
Basophils (%)	0.5 ± 0.2	0.5 ± 0.6	0.4 ± 0.2	0-1,7
Lymphocytes (%)	38.7 ± 16.7	42.2 ± 10.8	48.4 ± 8.1	38-79
Monocytes (%)	8.6 ± 3.0	7.8 ± 2.4	9.9 ± 5.4	3-11

* Statistically different from the control - ANOVA, ($p \leq 0.05$ - Duncan's Test)

As for the increase in monocytes, detected in males only at the dose of 10 mg/kg PG, although statistically significant, it falls within the limits mentioned in the literature (Ward et al. 2018).

The biochemical findings are presented in Table 3, demonstrating several statistical changes of the treated groups, as compared to the control group. Several of them fall within the normal ranges reported in the literature, such as bilirubin and glucose (Giknis and Clifford 2008); others occurred in only one sex (amylase and alkaline phosphatase only in males; LDH and glucose only in females); some occurred only at the lower dose (amylase, alkaline phosphatase) without changes at the higher dose; and two of them show significant reduction in parameters as a positive sign (reduction in total cholesterol in males; reduction in LDH in females). Thus, they do not appear to express toxicological risks.

The reduction in albumin levels in male rats treated with both doses may represent a negative effect of PG lyophilized on the animals due to possible interference in hepatic synthesis. Previously, Silva (2021) and Dias et al. (2023) reported several negative aspects of PG on the liver, such as decreased glutathione S-transferase, sinusoidal congestion, among others, which may be related to the reduction in albumin found in treated males. However, this effect did not replicate

Table 3. Biochemical parameters of male and female rats treated chronically with water or PG lyophilized extract (N= 17 per group; mean ± s.d.).

Parameters	Control	**PG 10 mg/kg**	**PG 100 mg/kg**	**Normal values** (Gyknis and Clliford 2008)
Males				
Albumin (g/100 ml)	3.3 ± 0.8	2.9 ± 0.1*	2.8 ± 0.1*	3,4-4,8
Alkaline phosphatase (U/l)	350.1 ± 163.8	448.9 ± 60.7*	360.1 ± 66.6	62-230
Amylase (U/l)	811 ± 412	1160 ± 200*	1012 ± 341	absent
CPK (U/l)	5630 ± 4665	12466 ± 6852*	17722 ± 5277*	162-1184
Creatinine (mg/dl)	0.47 ± 0.12	0.37 ± 0.06*	0.36 ± 0.05*	0,2-0,5
Direct bilirubin (mg/dl)	0.03 ± 0.009	0.04 ± 0.009*	0.04 ± 0.012*	0,03-0,05
Glucose (mg/dl)	114.8 ± 12.8	112.4 ± 7.4	119.7 ± 9.3	70-208
Indirect bilirubin (mg/dl)	0.01 ± 0.009	0.02 ± 0.009*	0.03 ± 0.012*	0,01-0,12
LDH (U/l)	4193 ± 844	3832 ± 1094	3920 ± 1283	absent
SGOT/AST (U/l)	344.0 ± 107.9	339.9 ± 79.2	339.6 ± 79.1	74-143
SGPT/ALT (U/L)	108.2 ± 38.7	121.5 ± 17.5	113.8 ± 17.6	18-45
Total bilirubin (mg/dl)	0.04 ± 0.011	0.06 ± 0.012*	0.07 ± 0.014*	0,05-0,15
Total cholesterol (mg/dl)	68.2 ± 13.4	65.2 ± 8.7	53.6 ± 20.3*	37-85
Triglycerides (mg/dl)	90.7 ± 29.3	90.4 ± 29.7	83.9 ± 23.4	20-114
Urea (mg/dl)	52.1 ± 4.2	50.3 ± 4.6	45.2 ± 4.3*	12,3-24,6
Uric acid (mg/dl)	2.7 ± 1.6	1.3 ± 0.2*	1.3 ± 0.2*	absent
Female				
Albumin (g/100 ml)	3.3 ± 0.4	3.3 ± 0.2	3.5 ± 0.2	3,6-5,5
Alkaline phosphatase (U/l)	217.4 ± 63.7	207.0 ± 60.5	225.8 ± 37.4	26-147
Amylase (U/l)	554 ± 128	487 ± 56	529 ± 119	absent
CPK (U/l)	12261 ± 3114	12316 ± 3467	11635 ± 4586	163-1085
Creatinine (mg/dl)	0.49 ± 0.08	0.49 ± 0.07	0.51 ± 0.03	0,2-0,6
Direct bilirubin (mg/dl)	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01*	0,03-0,06
Glucose (mg/dl)	84.0 ± 10.8	83.2 ± 5.0	96.7 ± 8.4*	76-175
Indirect bilirubin (mg/dl)	0.06 ± 0.02	0.06 ± 0.02	0.08 ± 0.02*	0,03-0,15
LDH (U/l)	3980 ± 775	3527 ± 794	3165 ± 773*	absent
SGOT/AST (U/l)	311.1 ± 72.5	278.3 ± 48.1	259.9 ± 69.2	65-203
SGPT/ALT (U/l)	112.3 ± 51.4	94.7 ± 33.1	86.7 ± 19.5	16-48
Total bilirubin (mg/dl)	0.12 ± 0.03	0.12 ± 0.02	0.15 ± 0.04*	0,05-0,15
Total cholesterol (mg/dl)	65.3 ± 6.7	63.3 ± 8.8	64.3 ± 5.3	24-73
Triglycerides (mg/dl)	61.6 ± 9.9	52.2 ± 11.7	58.2 ± 15.6	14-46
Urea (mg/dl)	44.7 ± 5.3	43.7 ± 9.1	49.5 ± 12.4	13,2-27,1
Uric acid (mg/dl)	1.7 ± 0.3	1.6 ± 0.3	1.3 ± 0.2*	absent

* Statistically different from the control - ANOVA, ($p \leq 0.05$ - Duncan's Test)

in females nor was it expressed in other markers of liver function (AST and ALT) or in an increase in anatomopathological alterations (Table 5), but should be highlighted as a warning regarding high doses and chronic use of PG extracts.

Particularly notable are the significant and dose-dependent increases in CPK values in males; in females, although not significant compared to the control, the values were also well above the normal values indicated in the literature (Giknis and Clifford 2008). This increase is likely related to the euthanasia procedure (Meltzer et al. 1971) combined with laboratory stress conditions (Matte and Seifert 1978), with this elevation always higher in males (Amelink et al. 1988). The absence of anatomopathological changes, particularly in the cardiac muscle, also supports the concept that this increase is not associated with toxic effects of the extract, however, further studies on this parameter would be appropriate

Another noteworthy alteration concerns the decrease in renal function test values. Uric acid concentrations in treated males were reduced with the two extract doses (-37% and -51.8%, respectively), and in females only at the dose of 100 mg/kg (-23.5%); urea values decreased in males at the higher dose, and creatinine decreased in males, with both doses although it did not decrease in females, collectively indicating an apparent improvement in renal function. Popular and commercial use refers to PG's ability to "reduce uric acid and be useful in cases of gout" (Pronat 1994), and the literature also mentions these effects for several species rich in saponins, either by increasing renal excretion or by inhibiting enzyme activity related to uric acid synthesis (Cheng-yuan and Jian-gang 2023).

The organ weighing results in the animals did not show significance in any of the comparisons among the three groups, both in males and females (see Table 4). In this long-term evaluation, the changes detected in heart weight in the acute testing were not replicated.

The anatomopathological evaluation of the organs revealed some changes of rare, non-widespread incidence. Significant cases included a lower incidence of tubular-interstitial nephritis in females of the 100 mg/kg PG group, and also a lower incidence of centrilobular congestion in the liver of males treated with the extract at the 10 mg/kg dose. Other incidences were sporadic and localized, with no further evidence of significant anatomopathological findings related to the assessed extract (Table 5).

Previous studies with various extracts and doses have shown indications of hepatic risks with the use of PG roots, including pulmonary atrophy due to hepatomegaly and increased liver weight (Toledo et al. 2004), as well as sinusoidal congestion and other effects (Silva 2021). However, Fernandes et al. (2015) did not find changes in organ weight, including the livers of treated animals. The data from the present research, however, did not show such risks because there was no reduction in liver weight in the acute test even with high doses, nor in prolonged treatment. Similarly, there were no biochemical changes indicative of hepatotoxicity in liver function tests, except for a reduction in albumin levels in male rats. This set of negative evidence is complemented by the lack of anatomopathological changes indicative of hepatic risk, differences that may be related to the type of extract and the doses used in the studies.

Therefore, it can be concluded that the lyophilized extract obtained through 50% ethanol from the roots of *Pfaffia glomerata*, originating from the northwest region of Paraná, and standardized to 1.1% in β-ecdysone, shows low toxicity in Wistar rats treated acutely and for 90 days, even when doses approximately 10 times higher than the therapeutic dose used in humans are employed.

Table 4. Weight of the organs (in grams) of the male and female rats treated chronically with water or PG lyophilized extract (N= 17).

	Groups	Weights of the organs (mean ± s.d.)		
		Heart	Liver	Kidneys
Males	Control	1.3 ± 0.2	13.6 ± 1.6	2.8 ± 0.3
	PG 10 mg/kg	1.3 ± 0.2	13.7 ± 1.3	2.8 ± 0.2
	PG 100 mg/kg	1.2 ± 0.1	13.1 ± 1.3	2.7 ± 0.2
Females	Control	0.84 ± 0.09	6.4 ± 0.7	1.9 ± 0.2
	PG 10 mg/kg	0.86 ± 0.08	6.1 ± 0.5	1.9 ± 0.2
	PG 100 mg/kg	0.85 ± 0.08	6.2 ± 0.4	1.8 ± 0.1

ANOVA not significant

Table 5. Histopathological findings (frequency) of kidneys, liver, and heart of male and female rats treated chronically with water or PG lyophilized extract (N= 15-17).

Organs	Histopathological Findings	Groups					
		Male			Female		
		Control	PG 10 mg/kg	PG 100 mg/kg	Con-trol	PG 10 mg/kg	PG 100 mg/kg
Kidneys	Normal	9	6	7	6	13	13
	Tubular-intersticial nephritis	7	7	4	8	4	3*
	Inflammatory infiltrate in pelvis	1	3	6	1	0	0
Liver	Normal	11	15	13	15	16	15
	Centrilobular congestion	4	0*	2	0	1	0
	Subcapsular congestion	2	2	0	0	0	0
	Centrilobular steatosis	0	0	1	0	0	1
Heart	Normal	6	9	9	13	14	14
	Congestion	8	2	5	0	3	1
	Inflammation	2	4	3	2	0	0
	Miocyte degenerations	0	2	0	0	0	1
	Pericarditis	1	0	0	0	0	0

*Statistically different from the control group ($p \leq 0.05$ - Fisher's Test., one-dimensional)

ACKNOWLEDGEMENTS

We would like to express our gratitude to the professors and staff of the Psychobiology and Pathological Anatomy departments at the Federal University of São Paulo and a special thanks to professors Dr. Oswaldo Gianotti and Dr. Francy Reis Silva Patrício for the anatomopathological evaluation of the organs of the animals tested. Additionally, we extend our appreciation to the team at Sanrisil S/A for conducting the chromatographic measurements.

AUTHORS' CONTRIBUTIONS

This work constitutes a part of LCM's doctoral thesis, under the supervision of ELAC. LCM and SMPG were involved in methodology, investigation, data acquisition and analysis, manuscript preparation, editing, and review. ELAC contributed to conceptualization, methodology, funding acquisition, project administration, supervision, and the acquisition and analysis of raw data (*in memorian*).

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

Almeida IV, Düsman E, Mattge GI, Toledo F, Reusing AF, Vicentini VEP (2017) *In vivo* antimutagenic activity of the medicinal plants *Pfaffia glomerata* (Brazilian ginseng) and *Ginkgo biloba*. GMR 16(3). https://doi.org/10.4238/gmr16039785

Amelink GJ, Kamp HH, Barr PR (1988) Creatine kinase isoenzyme profiles after exercise in the rat: sex-linked differences in leakage of CK-MM. Pflugers Arc 412:417-421. https://doi.org/10.1007/BF01907561

Andrade A, Pinto SC, Oliveira RS (2002) Animais de Laboratório: criação e experimentação [online]. Rio de Janeiro: Fiocruz. 388p.

AVMA - American Veterinary Medical Association (2020) Guidelines for the Euthanasia of Animals: 2020 edition. Schaumburg, IL. 121p.

Barilli SLS, Montanari T (2008) An experimental investigation on effect of the medicinal plant *Pfaffia glomerata* (Spreng.) Pedersen on gestation. Reprod Clim 23(2):72-6.

Brasmédica S/A Indústrias Farmacêutica (s.data) Folheto do medicamento Tai Ginseng do Brasil. São Paulo.

Cheng-yuan W, Jian-gang D (2023) Research progress on the prevention and treatment of hyperuricemia by medicinal and edible plants and its bioactive components. Front Nutr 10. https://doi.org/10.3389/fnut.2023.1186161

Corrêa WL (2014) Prospecção de substâncias bioativas em *Pfaffia townsendii* e *Pfaffia tuberosa* (Gomphreneae, Amaranthaceae). 168p. Tese (doutorado em Ciências), Universidade Estadual de Campinas, Campinas, Brazil.

Dias FCR, Cupertino MC, Silva PG, Oliveira EL, Ladeira LCM, Matta SLP, Otoni WC, Gomes MLM (2023) Exposure to *Pfaffia glomerata* causes oxidative stress and triggers hepatic changes. Braz J Biol 83:e271425. https://doi.org/10.1590/1519-6984.271425

Dias FCR, Gomes MLM, Melo FCSA, Menezes TP, Martins AL, Cupertino MC, Otoni WC, Matta SLP (2020) *Pfaffia glomerata* hydroalcoholic extract stimulates penile tissue in adult Swiss mice. J Ethnopharmacol 261: 113182. https://doi.org/10.1016/j.jep.2020.113182

Dias FCR (2018) Extrato hidroalcóolico da raiz de *Pfaffia glomerata* (Spreng.) Pedersen interfere em parâmetros reprodutivos e fertilidade de camundongos machos adultos. 157p. Tese (doutorado em Biologia Celular), Universidade Federal de Viçosa, Viçosa, Brazil.

Fernandes NF, Martino-Andrade AJ, Lourenço ACS, Muller JC, Spercoski KM, Nihi F, Miguel MD, Oliveira VB, Dalsenter PR, Morais RN (2015) Supplementation with *Pfaffia glomerata* (Sprengel) Pedersen does not affect androgenic–anabolic parameters in male rats. J Ethnopharmacol 161: 46–52. https://doi.org/10.1016/j.jep.2014.11.049

Franco RR (2021) Ação neuroprotetora do ginseng brasileiro *Pfaffia glomerata* (Spreng.) Pedersen. 125 p. Tese (doutorado em Genética e Bioquímica), Universidade Federal de Uberlândia, Uberlândia, Brazil.

Franco RR, Franco RM, Justino AB, Borges ALS, Bittar VP, Saito N, Saraiva AL, Júnior NN, Otoni WC, Espindola FS (2024) Phytochemical composition of aerial parts and roots of *Pfaffia glomerata* (Spreng.) Pedersen and anticholinesterase, antioxidant, and antiglycation activities. Protoplasma 10. https://doi.org/10.1007/s00709-023-01916-9

Freitas CS, Baggio CH, Silva-Santos JE, Rieck L, Santos CAM, Corrêa Júnior C, Ming LC, Marques MCA (2004) Involvement of nitric oxide in the gastroprotective effects of an aqueous extract of *Pfaffia glomerata* (Spreng) Pedersen, Amaranthaceae, in rats. Life Sci 74(9):1167-79. https://doi.org/10.1016/j.lfs.2003.08.003

Gao RL, Xu CL, Jin JM (1992) [Effect of total saponins of *Panax ginseng* on hematopoietic progenitor cells in normal human and aplastic anemia patients]. Zhongguo Zhong Xi Yi Jie He Za Zhi 12(5):285-7, 261-2.

Ginknis MLA, Clifford CB (2008) Clinical laboratory parameters for Crl:WI (Han). Wilmington, MA: Charles River Laboratories International. 17p. Obtido de https://www.criver.com/sites/default/files/Technical%20Resources/Clinical%20Laboratory%20Parameters%20for%20Crl-WI(Han)%20Rats%20-%20March%202008.pdf.

Marques LC, Galvão SMP, Espínola E, Dias RF, Mattei R, Oliveira MGM, Carlini ELA (2004) Psychopharmacological assessment of *Pfaffia glomerata* roots (extract BNT-08) in rodents. Phytother Res 18(7): 566-572. https://doi.org/10.1002/ptr.1500

Matta APLF, Leite JPV, Gomes MLM, Morais DB, Carvalho FAR, Otoni WC, Matta SLP (2020) Deleterious effects of *Pfaffia glomerata* (Spreng.) Pedersen hydroalcoholic extract on the seminiferous epithelium of adult Balb/c mice. Int J Exp Path 101:183–191. https://doi.org/10.1111/iep.12363

Matta APLF (2012) Efeitos do extrato hidroalcóolico da raiz de *Pfaffia glomerata* (Spreng.) Pedersen sobre testículo e pênis de camundongos adultos.105 p. Tese (doutorado em Biologia Celular e Estrutural), Universidade Federal de Viçosa, Viçosa, Brazil.

Matte AC, Seifert R. (1978) Activity of creatine kinase in mice under various stress conditions. Aggress Behav 4:19-29. https://doi.org/10.1002/1098-2337(1978)4:1<19::AID-AB2480040103>3.0.CO;2-F

Meltzer H, Guschwan A, Wexler BC (1971) Effect of decapitation on blood levels of cretine phosphokinase: letters to editor. Am Heart J 82(1):138. https://doi.org/10.1016/0002-8703(71)90176-1

Mendes FR (2011) Tonic, fortifier and aphrodisiac: adaptogens in the Brazilian folk medicine. Rev Bras Farmacogn 21(4):754-763. https://doi.org/10.1590/S0102-695X2011005000097

Neto AG, Costa JM, Belati CC, Vinhólis AH, Possebom LS, Da Silva Filho AA, Cunha WR, Carvalho JC, Bastos JK, Silva ML (2005) Analgesic and anti-inflammatory activity of a crude root extract of *Pfaffia glomerata* (Spreng) Pedersen. J Ethnopharmacol 96(1-2):87-91. https://doi.org/10.1016/j.jep.2004.08.035

Neto AG, Silva Filho AA, Vinholis AHC, Souza GHB, Cunha WR, Silva MLAE, Albuquerque S, Bastos JK (2004) Evaluation of the trypanocidal and leishmanicidal *in vitro* activity of the crude hydroalcoholic extract of *Pfaffia glomerata* (Amarathanceae) roots. Phytomedicine 11(7-8):662-665. https://doi.org/10.1016/j.phymed.2003.06.005

Nishimoto N, Shiobara Y, Inoue S, Takemoto T, Akisue G, Oliveira F, Akisue MK, Hashimoto G (1990) Ecdisteroides de *Pfaffia glomerata*. In: Simpósio de Plantas Medicinais do Brasil, 11º. Resumos. UFPB, João Pessoa, Brazil.

Nishimoto N (1992) The constituents of brazilian ginsengs. In: Simpósio de Plantas Medicinais do Brasil, 12º. Resumos. UFPR, Curitiba, Brazil.

Oshima M, Gu Y (2003) *Pfaffia paniculata*-induced changes in plasma estradiol-17beta, progesterone and testosterone levels in mice. J Reprod Dev 49(2):175-80. https://doi.org/10.1262/jrd.49.175

Pronat - Laboratório de Produtos Naturais Ltda (1994) Bula do medicamento fitoterápico *Pfaffia paniculata*.

Prudente CM, Ramos CS, Rodrigues DS, Oliveira Júnior CJF (2024) *Pfaffia glomerata* (Spreng.) Pedersen: ginseng brasileiro, potencial da sociobiodiversidade brasileira. DELOS: Desarrollo Local Sostenible 17(53):01-19.

Rao RM, Khan ZA, Shah AH (2001) Toxicity studies in mice of *Commiphora molmol* oleo-gum-resin. J Ethnopharmacol 76(2):151-154. https://doi.org/10.1016/s0378-8741(01)00189-1

Rivera IG, Martins MT, Sanches PS, Satos MIZ, Coelho MCL, Akisue M, Akisue G (1994) Genotoxicity assessment through the Ames test of medicinal plants commonly used in Brazil. ET&C 9(2):87-93. https://doi.org/10.1002/tox.2530090203

Sigrist S (2012) Ginseng brasileiro. Available at: https://www.ppmac.org/content/ginseng-brasileiro. Accessed on 29 March 2024.

Silva PG (2021) Avaliação hepática de camundongos adultos submetidos à ingestão do extrato hidroalcóolico da raiz de *Pfaffia glomerata* (Spreng.) Pedersen. 72p. Dissertação (Mestrado em Biologia Animal), Universidade Federal de Viçosa, Viçosa, Brazil.

Souza-Daniel JF, Alves KZ, Jacques DS, Souza PVS, Carvalho MG, Freire RB, Ferreira DT, Freire MFI (2005) Free radical scavenging activity of *Pfaffia glomerata* Pederson (Amaranthaceae). Indian J Pharmacol 37(3):174-178.

Toledo MRS, Silva CCA, Antonello D, Vieira KRPMC, Ramos MBM, Heredia NA, Scalon SPQ, Bazzano TSC (2004) Extratos aquosos de *Pfaffia glomerata* Spreng e seu efeito tóxico em ratas prenhes. Hortic Bras 22(2):493.

Vigo CLS, Narita E, Marques LC (2003) Validação da metodologia de quantificação espectrofotométrica das saponinas de *Pfaffia glomerata* (Spreng.) Pedersen – Amaranthaceae. Rev Bras Farmacogn 14 (supl.):46-49.

Wang Cheng-yuan, Dai Jian-gang (2023) Research progress on the prevention and treatment of hyperuricemia by medicinal and edible plants and its bioactive components. Front Nutr 10:1186161. https://doi.org/10.3389/fnut.2023.1186161

Ward JM, Cherian S, Linden MA (2018) 19 - Hematopoietic and lymphoid tissues. In: Piper M. Treuting PM, Dintzis SM, Montine KS. Comparative Anatomy and Histology. A Mouse, Rat, and Human Atlas. 2nd. ed. Massachusetts: Academic Press. 365-401. https://doi.org/10.1016/B978-0-12-802900-8.00019-1

Wilkes MC, Jung K, Lee BE, Saxena M, Sathianathen RS, Mercado JD, Perez C, Flygare J, Narla A, Glader B, Sakamoto KM (2021) The active component of ginseng, ginsenoside Rb1, improves erythropoiesis in models of Diamond-Blackfan anemia by targeting Nemo-like kinase. J Biol Chem 297(3):100988. https://doi.org/10.1016/j.jbc.2021.100988