Effects of High Diluted Solutions of *Palicourea marcgravii* St Hill in Rats Poisoned by Aqueous Extracts of This Plant

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ABSTRACT

Palicourea marcgravii, a shrub causing sudden death in cattle, is a major cause of economic loss to breeders in Brazil. The aim of the present study was to evaluate the effects of high diluted solutions 6cH and 30cH of *P. marcgravii*, on the development of tolerance to the toxic effects of this plant. 14 adult Wistar rats were divided into 3 test groups. AE (aqueous extract) group was composed of 4 rats receiving aqueous extract of *P. marcgravii* intragastrically at a dose of 2g/kg. Groups HD₆AE and HD₃₀AE comprised 5 rats each. Animals in these groups received 1 mL of 6cH and 30cH solutions of *P. marcgravii* respectively by oral route 3 times a day, for 8 days. At the end of this period, they were intragastrically intoxicated with 2g/kg of aqueous extract of *P. marcgravii*, receiving the corresponding high diluted preparation hourly until death. Main symptoms were nervous excitability and convulsions. Even though the times for onset of the first clinical signs, convulsions and death was slightly longer in the animals in group HD₃₀AE when compared to group AE, no evidence indicating that the highly diluted preparations increase tolerance to intoxication by *P. marcgravii* was found.

Keywords: *Palicourea marcgravii*; Intoxication; Isopathy; High dilutions; Wistar rats.

Introduction

Livestock production in Brazil has annual losses of around 1,000,000 adult bovines due to vegetal poisoning. Approximately half of these are caused by ingestion of *Palicourea marcgravii* St. Hill (erva-de-rato), associated to sudden death [1].

Pharmacological studies aiming at a therapeutic solution for the recovery of animals poisoned by *P. marcgravii* have not been fully successful [1-3], even though the active ingredient, or one of the major toxic components of this plant, is known to be monofluoroacetic acid [4-7]. Homeopathic therapeutics has been recommended for organic livestock production [8]. However, experimental studies using high dilution preparations have yielded controversial results partially due to possible placebo effect [9-14]. For this reason, more research in this area is needed.

This study aimed to evaluate the effects of high diluted preparations of extract of *P. marcgravii* on the development of tolerance to the toxic effects of this plant in rats.

Material and Methods

Palicourea marcgravii St. Hill (Figure 1) was collected in the city of Valença, Rio de Janeiro, Brazil. It was identified and cataloged as RG: 22,971 in the Herbarium of the Department of Botany at Federal Rural University of Rio de Janeiro (UFRRJ).

Extraction of aqueous extracts from the leaves of *P. marcgravii* (*Pm*) in flowering and fruiting stages as well as the preparation of diluted and agitated solutions following Hahnemannian centesimal scale in dilutions 6cH and 30cH (*Pm*6cH and *Pm*30cH, respectively) were performed at the School-Pharmacy of the Hahnemannian Institute of Brazil,

Rio de Janeiro, following the Brazilian Homeopathic Pharmacopoeia [15].

Figure 1: *Palicourea marcgravii* during flowering and fruiting.

14 healthy female Wistar rats between 6 and 7 weeks old and weighing 90 to 160 grams were used. Animals were kept under light during the day and in the dark at night, at temperature 22 - 24°C, with water and feed *ad libitum* during the whole experimental period.

All animals were kept under observation for one week before the onset of the experiment to allow for environmental adaptation and evaluation of the health status. They were deprived of water and food 24 hours before the administration of aqueous extract of *Pm*. Rats were randomly divided into the following groups: AE (aqueous extract), HD₆AE (high dilution 6cH + aqueous extract), and HD₃₀AE (high dilution 30cH + aqueous extract).

Group AE was composed of 4 animals that received a single dose of 2g/kg body weight *P. marcgravii* aqueous extract by intragastric route. Groups HD₆AE and HD₃₀AE comprised 5 animals each; these received 1ml of *Pm*6cH and *Pm*30cH solutions respectively by oral route, 3 times a day for 8 days.

On the 8th day, one hour after the administration of the high diluted solutions, animals received *P. marcgravii* aqueous extract in a single dose of 2g/kg body weight by intragastric route. 30 minutes after

administering the aqueous extract, animals were given 1ml of the respective high diluted preparation at hourly intervals for 2 to 9 hours.

Clinical evaluation of the animals took the following parameters into account: weight, food ingestion, motor activity, piloerection, cyanosis of extremities, hypothermia, type of breathing, shivering, pruritus, neurological depression, convulsions and death.

All animals that died were necropsied at the end of the experimental period and macroscopic alterations were recorded. Samples of brain, heart, lungs, liver, kidneys, stomach and intestine were subjected to histological analysis. The animals that did not die were euthanized in a chamber saturated with the inhalational general anesthetic Halothane®.

This work was approved by Ethics Committee of the Veterinary Institute at UFRRJ.

Results

None of the animals in groups HD₆AE and HD₃₀AE showed any clinical alteration and they all exhibited normal behavior during the 7 days of administration of the respective high diluted solution. The first clinical signs of poisoning after administration of *P. marcgravii* aqueous extract appeared within 15 to 45 minutes in group AE, 30 to 37 minutes in group HD₆AE and 50 to 70 minutes in HD₃₀AE. Animals in all groups exhibited reduction in mobility, piloerection, dyspnea, cyanosis of the extremities, hypothermia, shivering and hyperexcitability (Table 1).

Clinical manifestations of convulsive crisis, characterized by tonic and clonic spasms, occurred in all the animals that received *P. marcgravii* aqueous extract but for one rat in group AE and 2 rats in group HD₃₀AE. The time for onset of convulsions was longer for animals in groups HD₆AE and HD₃₀AE as shown in Table 2. On the other hand, handling of these animals for repeated administration of the high diluted solutions resulted, in some cases, in convulsive crisis and, sometimes, death.

The administration of *P. marcgravii* aqueous extract caused death always in a hyperacute manner, as observed in 1 of the 4 animals in group AE group, 3 of the 5 rats in group HD₆AE group, and 2 of the 5 components of group HD₃₀AE. However, the animals that had received the high diluted solutions survived a little longer (Table 2).

Table 1: Experimental poisoning of rats in groups AE, HD₆AE and HD₃₀AE, which received *Palicourea marcgravii* aqueous extract.

Animal	Group	Time to onset of clinical signs	Evolution (time)		Clinical signs of poisoning (a)						
					Reduced mobility	Piloerection	Dyspnea	Hypothermia	Shivering	Neurological depression	Convulsions
R1	AE	~45min	Euthanasia after 24h		+	+	+	+	+	+	+
R2	AE	~35min	1h15min	1h50min	+	+	+	+	+	+	+
R3	AE	~15min	Euthanasia after 24h		-	+	+	-	+	+	-
R4	AE	~35min	Euthanasia after 24h		-	+	+	-	+	+	+
R40	HD ₆ AE	~30min	Euthanasia after 24h		+	+	+	+	+	+	+
R41	HD ₆ AE	~30min	2h05min	2h35min	+	+	+	+	-	+	+
R42	HD ₆ AE	~32min	Euthanasia after 24h		+	+	+	+	+	+	+
R43	HD ₆ AE	~34min	1h30min	2h04min	+	+	+	+	-	+	+
R44	HD ₆ AE	~37min	3h41min	4h18min	+	+	+	+	+	+	+
R45	HD ₃₀ AE	~58min	10h15min	11h13min	+	+	+	+	+	+	+
R46	HD ₃₀ AE	~59min	Euthanasia after 24h		+	+	+	+	+	+	+
R47	HD ₃₀ AE	~63min	Euthanasia after 24h		+	+	+	+	+	+	-
R48	HD ₃₀ AE	~58min	3h35min	4h33min	+	+	+	+	-	+	+
R49	HD ₃₀ AE	~70min	Euthanasia after 24h		+	+	+	+	+	+	-

(a) + manifested clinical sign, - clinical sign absent

Table 2: Clinical parameters of convulsions in rats poisoned by *Palicourea marcgravii* aqueous extract.

Convulsion parameters					
Animal	Group	Number of convulsions	Time for onset of convulsions	Interval between convulsions (time)	Convulsion score (0 to 3)
R1	AE	4	55 min	10min, 30min and 2h45min	3
R2	AE	3	40 min	15 and 30 min	3
R3	AE	0	-	-	0
R4	AE	1	59 min	-	3
R40	HD ₆ EA	1	3h48 min	-	2
R41	HD ₆ EA	1	2h33min	-	3
R42	HD ₆ EA	1	2h45min	-	3
R43	HD ₆ EA	4	1h33min	10 to 15 min	3
R44	HD ₆ EA	2	1h45min	2h27min	3
R45	HD ₃₀ EA	3	4h09min	6h3min and 45 min	2
R46	HD ₃₀ EA	1	3h50min	-	2
R47	HD ₃₀ EA	0	-	-	0
R48	HD ₃₀ EA	4	3h19min	30, 11 and 7 min	3
R49	HD ₃₀ EA	0	-	-	0

The main pathological finding in animals in groups AE, HD₆AE and HD₃₀AE was hepatic congestion, although some animals also showed signs of cardiac dilatation. Histological analysis revealed various degrees of congestion in the liver, lungs and spleen. 7 animals exhibited the most characteristic and conspicuous lesion, hydropic-vacuolar degeneration of renal distal convoluted tubules (Figure 2). Significant histological alterations were not observed in other organs.

Discussion

In this study, the clinical manifestations of poisoning and hyperacute death of rats were similar to those found in rats and Guinea pigs experimentally poisoned by *P. marcgravii* [16], and in other animals poisoned by sodium monofluoroacetate [1,5,7,17]. In these cases, hyperacute death occurs due to interruption of the aerobic metabolism, which results in accumulation

of large amounts of citrate in the tissues and inhibition of adenosine triphosphate production [6,18]. This indicates that the cellular dysfunction caused by poisoning by *P. marcgravii* is irreversible, thus complicating therapeutic recovery as verified in the literature [3].

In this experiment, the animals that had previously received a high diluted solution of *P. marcgravii* showed the same clinical picture of hyperacute death observed in the animals in group AE. Longer times for onset of the first clinical signs of poisoning, convulsions and death were observed in the animals in these groups, especially in those that received *Pm30cH* solution.

Drugs [3] or plant extracts [19,20] are considered effective when they promote an increase in the time for onset of convulsions. However, in this experiment, the number of animals employed was considered too small to establish whether high

diluted solutions of *P. marcgravii* have indeed anticonvulsant activity. On the other hand, it may be possible that manipulation related to repeated oral administration of the high diluted solutions contributed to worsen the effects of poisoning as data from literature indicate that exercise worsens the clinical picture of convulsions and can precipitate the death of animals [1,21].

Figure 2: Hydropic-vacuolar degeneration of renal distal convoluted tubules in a rat poisoned by *Palicourea marcgravii* aqueous extract. Obj. 25x.

Conclusion

In this study, no evidence indicating that high diluted solutions of *P. marcgravii* increase resistance to poisoning by the aqueous extract of this plant was found. However, it is not possible to discard the possibility that ingestion of these solutions prior to poisoning exerts an anticonvulsant effect.

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