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Absence of Interceptive Effect in Female Wistar Rats Exposed to *Jacaranda decurrens* During Organogenesis Period

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ABSTRACT

Jacaranda decurrens subsp. *symmetrifoliolata* Farias and Proença (Bignoniaceae) popularly known as "carobinha", "carobinha-do-campo" or "caroba" is used in folk medicine as a blood cleanser, wound healing and to treat uterus and ovary inflammations. In order to evaluate the reproductive performance and embryotoxic effects of the *Jacaranda decurrens* during organogenic period, the pregnant *Wistar* rats received, via gavage, 27.5 mg/Kg/day of aqueous extract. The control group received only distillate water. No clinical signs of maternal toxicity were observed and the term fetuses did not present malformations or anomalies. Our results suggest that *Jacaranda decurrens* aqueous extract, in these experimental conditions, seems not to cause embryo deaths, growth retardation, and/or malformations in Wistar rats.

INTRODUCTION

Jacaranda decurrens subsp. *symmetrifoliolata* Farias and Proença (Bignoniaceae) popularly known as "carobinha", "carobinha-do-campo" or "caroba" is an endemic species of the Brazilian Cerrado widely used as folk medicine in the southwestern and southeast of the Brazil [1, 2]. *Jacaranda decurrens* leaves and/or roots has been employed as a blood cleanser, wound healing and to treat uterus and ovary inflammations [1, 3]. Phytochemical analyses of the leaves of *Jacaranda decurrens* indicated the presence of triterpenes, ursolic and oleanolic acids and glycosylated flavonoids providing support to identify the antioxidant activity of this plant [4]. A recent study showed that *Jacaranda decurrens* hydroethanolic extract also has anti-inflammatory properties in rats without causing acute toxicity [5]. Additionally, some *in vitro* studies revealed that different species of *Jacaranda*, as *Jacaranda copaia* and *Jacaranda obtusifolia* have demonstrated cytostatic and cytotoxic activity, respectively, against tumor cell lines [6].

Worldwide there are virtually thousands of herbal medicine products in use. In general, pregnant women use herbal medicine products, particularly as such remedies are often perceived as being "natural and therefore free of risks" [7]. However, there are herbal medicine products associated with risks to pregnant women and their babies [8]. It is important to realize that any negative intervention into the normal course of pregnancy, either on genetic or non-genetic basis, inevitably leads to a sequence of subsequent changes resulting in the development of congenital developmental disorders [9]. Then, the aim of the present investigation was to evaluate the impact of the *Jacaranda decurrens* aqueous extract on female Wistar rats

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exposed during the organogenic period.

Material and Methods

Preparation of the extracts

The roots of *Jacaranda decurrens* subsp. *symmetrifoliolata* were collected in the Medicinal Plant Garden of the Federal University of Grande Dourados, Mato Grosso do Sul State, Brazil. A voucher specimen was identified by Dr. Rosana Farias Singer and deposited (register: W. G. Garcia14.008) in the Herbarium of the Department of Botany at Biology Institute of the State University of Campinas. *Jacaranda decurrens* roots were washed with water dried and prepared by adding 500 ml of distilled water to 500g of plant material at 95°C and left to macerate for 24 h in room temperature. After this period, the sample was dried in a desiccator. The yield of the crude extract was 19.64%. During the treatment, the extract was dissolved in distilled water.

Animals

Twenty pregnant *Wistar* rats were used in the present investigation: ten for the control and ten for the *Jacaranda decurrens* exposed group. The females were mated with males and the gestational day 0 (GD0) was determined if there were spermatozoa in the vagina. These animals were housed in a standard animal facility under controlled temperature (22°C) and photoperiod (12 h light, 12 h dark) with access to water and rodent food *ad libitum*. All procedures and protocols followed approved guidelines for the ethical treatment of animals, according to the Ethics Committee in Animal Experimentation from the Federal University of Mato Grosso do Sul (Protocol # 107/2006).

Experimental procedure

The females of the *Jacaranda decurrens* treated group received 27.5 mg/Kg/day of the extract suspended in 0.5 mL in distilled water, via gavage, during organogenic period, from 6th to 15th day of pregnancy (GD6 to GD15). This window of treatment was intended to evaluate possible embryotoxic effects of the plant since it is a period of greater sensibility to teratogens in rats [10]. The dose regimens were based on previous studies [11-13]. The control group received only the vehicle (0.5 mL/kg).

The pregnant animals were weighed on the first day of treatment (GD6), on the last day of administration (GD15) and on the 20th day of pregnancy (GD20), when they were sacrificed, laparotomy was performed and uterine horns were removed. The number of implants, reabsorptions, and dead and live fetuses was then recorded. The ovaries were also observed and the corpora lutea were counted under a stereomicroscope. The reproductive indexes were then calculated. The rate of preimplantation loss was calculated as: no. of corpora lutea- no. of implantations x 100/no. of corpora lutea. Postimplantation loss rate was calculated as: no. of implantation – no. of live fetuses x 100/no. of implantations, the Reabsorption's rate was calculated as: no. of reabsorptions x 100/no. of implantations. Maternal kidney, spleen and liver were also weighted.

The offspring group of control and *Jacaranda decurrens* exposed animals was divided randomly into three subgroups. The first was fixed in Bodian´s solution for visceral examination which was performed using thorax, abdomen and head incisions/microdissection [14-17]. The classification of visceral alterations was based in classical studies at the literature [10, 17- 19]. The second subgroup was reserved for skeletal examination according to the alizarin red technique [17, 20]. In brief, the fetuses were fixed in acetone (PA) for at least 7 days. They were placed in 50 ml of KOH solution (0.8%) plus 4 drops of saturated Alizarin. This solution was replaced every 24 hours for 4 consecutive days. After this time the fetuses were placed in bleaching solution (500 mL benzyl alcohol, 1000 mL glycerin and 1000 mL ethanol), and this was changed every 24 hours for 7 consecutive days. The classification of skeletal alterations was based in classical studies at the literature [17-19, 21]. All the analyses were performed on magnifying stereomicroscope with a 4-fold increase. The third subgroup was reserved for histological analysis. In brief, representative fetuses' fragments were excited and fixed in Bouin's solution. Once fixed, the tissue fragments were dehydrated, cleared and embedded in paraffin wax. The samples were cut into 6µm thick sections and stained with hematoxylin-eosin for histological analyses.

Statistical analysis

Values are expressed as mean±SEM and data were analyzed using *t* test in Graphpad Prism (version 5; GraphPad Software Inc., San Diego, CA, USA). The significance level was set at $p<0.05$.

Results

The maternal exposure to *Jacaranda decurrens* during the organogenic period (from 6th to 15th day of pregnancy) did not show clinical signs of toxicity. There was no significant weight gain or loss during the experiment, since no alterations were observed in the body weight of *Jacaranda decurrens* exposed animals in comparison with control group (Table-1). Also, no clinical symptoms of maternal toxicity-head flicking, piloerection, tremors and diarrhea- were observed for the administration of *Jacaranda decurrens* throughout the gestational period (data not shown).

Table-2 presents the data concerning absolute weight of maternal liver, spleen, kidney, ovary, placenta and uterine;

Table 1: Body weight of control and *Jacaranda decurrens* treated rats from 6th to 15th day of pregnancy.

Day of pregnancy	Body weight (g)	
	Control (n=10)	*J. decurrens* (n=10)
6	192.8 ± 3	215 ± 7
15	218.4 ± 4	240.1 ± 9
20	243.6 ± 6	279.8 ± 12

Results expressed in mean ± standard error. P>0.05.

number of corpora lutea, implantations and reabsorptions. No significant alteration was found for these variables (P>0.05). The reproductive indices followed the same trend and the implantation, reabsorption, pre and postimplantation loss and birth rate (fetal viability) were similar (P>0.05) between controls and *Jacaranda decurrens* exposed animals (Table 3).

In relation to fetuses' parameters, significant increases were observed in fetuses' size and weight in rats that were treated in utero with *Jacaranda decurrens*, compared to the control group (Table 4). However, the number of live and dead fetuses was similar in both groups analyzed. Furthermore, no significant visceral (Table 5) and skeletal (Table 6) malformations and variations were observed in *Jacaranda decurrens* exposed animals, in comparison with control group.

Table 2: Maternal parameters of control and *Jacaranda decurrens* exposed rats from 6 to 15 days of pregnancy.

Parameters	Control (n=10)	*J. decurrens* (n=10)
Liver weight (g)	11.1 ± 0.4	11.8 ± 0.5
Spleen weight (g)	0.5 ± 0.01	0.6 ± 0.05
Kidney weight* (g)	0.76 ± 0.02	0.78 ± 0.03
Ovary weight** (mg)	88.1 ± 5.7	83± 2.6
Placenta weight (g)	4.7 ± 0.3	4.3 ± 0.3
Uterine weight (g)	3.8 ± 0.1	3.7 ± 0.1
Corpora lutea (no)	11.6 ± 0.6	10.5 ± 0.2
Implantation sites (no)	10.5 ± 0.6	9.6 ± 0.3
Reabsorptions (no)	0.8 ± 0.2	0.6 ± 0.2

*Results expressed in mean ± standard error. P>0.05. * Left and right kidney average weight. ** Left and right ovary average weight*

Table 3: Effect of *Jacaranda decurrens* exposure on reproductive rates (%)

Parameters (%)	Control (n=10)	*J. decurrens* (n=10)
Implantation	91 ± 4.2	91.7 ± 2.8
Reabsorption	8 ± 2	6 ± 2
Preimplantation loss	9 ± 4.2	8.3 ± 2.8
Postimplantation loss	8.1 ± 2.6	9.5 ± 4.1
Birth rate	91.8 ± 2.6	90.5 ± 4.1

Results expressed in mean ± standard error. P>0.05.

Table 4: Effect of *Jacaranda decurrens* exposure on fetuses parameters

Parameters	Control (n=10)	*J. decurrens* (n=10)
Live fetuses (no)	9.6 ± 0.6	8.7 ± 0.5
Dead fetuses (no)	2 ± 0.05	1.8 ± 0.05
Fetuses size (cm)	3.3 ± 0.03	3.5 ± 0.3*
Fetuses weight (g)	2.6 ± 0.02	2.74 ± 0.004*

Results expressed in mean ± standard error. P>0.05.

Table 5: Visceral abnormalities and variations found in the offspring of the control and *Jacaranda decurrens* experimental groups.

Parameters	Control	*J. decurrens*
Number of offspring analyzed	32	24
Hydrocephalus	30	17
%MF	93.33±4.44	68.33±12.78
Hydronephrosis	0	0
%MF	0.00	0.00
Renal agenesis	0	0
%MF	0.00	0.00
Gonadal agenesis	0	0
%MF	0.00	0.00

%MF, mean value of malformationpercentage ± standard error. P>0.05.

Discussion

Due to the popular use of *Jacaranda decurrens* to treat prostate, uterus and ovary inflammations [1, 3] and that it is one of the ten folk medicine most consumed to the people in the Southwestern of the Brazil [3], it would be possible to suppose that substances capable of exerting some estrogenic effect would be present among the constituents of this plant's extract. Additionally, previous phytochemical analyses showed the presence of triterpenes and glycosylated flavonoids in the leaves of *Jacaranda decurrens* [4] and these compounds are known to present estrogen-like effects [22, 23]. So it seems important to screening the toxic effect on embryo after administration of *Jacaranda decurrens* to rats as a part of the studies on the toxicology of this aqueous extract.

Then, the primary aim of the present studies was to evaluate whether *Jacaranda decurrens* maternal exposure was able to interfere in the female reproductive performance and in the embryofetal development. As part of this evaluation, a more general aim was to investigate if *Jacaranda decurrens*, to the same dose based on the average consumption by the population, could have a direct interference in organogenesis and could act as a teratogenic compound to the pups that were exposed in utero.

In the present study, no clinical signs of maternal toxicity were

Table 6: Skeletal abnormalities and variations found in the offspring of the control and *Jacaranda decurrens* experimental groups.

Parameters	Control	*J. decurrens*
Number of offspring analyzed	33	35
Sternum	4	13
%MF	14.9±7.98	34.5±9.1
Parietal	2	4
%MF	6.67±6.67	15.00±8.03
Interparietal	4	13
%MF	11.67±4.84	35.83±9.55
Supraoccipital	5	5
%MF	14.99±5,09	14.17±4.82
Frontal	0	0
%MF	0.00	0.00
Nasal	0	0
%MF	0.00	0.00

%MF, mean value of malformationpercentage ± standard error. P>0.05.

observed. It is known that monitoring body weight provides information on the general health level of animals [24]. The body weight of pregnant rats, at the dose given, was not altered due to *Jacaranda decurrens* exposure. Our study is in agreement with previous studies confirming earlier findings that *Jacaranda decurrens* did not produce toxic symptoms in rats in doses up to 2000mg/kg, which indicated a low toxicity extract [5, 13].

The reproductive performance of mothers can be considered normal since the implantation index, the mean of implants and corpora lutea were similar in all experimental groups [25].
Since reabsorption is an *in situ* autolyse of embryos or fetuses [26] it is possible to assume that *Jacaranda decurrens* is not toxic to the embryos during the early organogenic period. Corroborating this finding, the rate of postimplantation loss was similar between controls and *Jacaranda decurrens* exposed animals, which indicates that this window of treatment (from 6th to 15th day of pregnancy) did not cause embryolethality [27]. Regarding the significant increase of the weight and size of fetuses exposed to *Jacaranda decurrens*, these data might be explained, for example, for the difference of the number of live fetuses, which had a tendency to be decreased in *Jacaranda decurrens* animals. Moreover, fetal body weight is not a conclusive parameter in development studies because it may vary depending on litter size [28]. Based on the finding that the fetal viability (birth rate) was similar in both groups analyzed, we can also assume that the *Jacaranda decurrens* aqueous extract did not interrupt the progress of the embryofetal development.

A recent study showed that *Jacaranda decurrens* interfered with the initial development of the male reproductive system, but without lasting effects on sperm production in adulthood, although there was no evidence that the aqueous extract of *Jacaranda decurrens* acted as an endocrine disrupter during reproductive development, since the weights of reproductive organs and the sperm count were found to be normal in adult animals [13]. Our fetuses' analyses confirming these findings since there were no visceral malformations and no gonadal agenesis were observed in *Jacaranda decurrens* exposed animals. It is well established in the literature that endocrine disrupter may contribute to human male reproductive disorders that manifest at birth (cryptorchidism, hypospadias) or in young adulthood (impaired semen quality or testicular germ cell tumours). These disorders share risk factors and are hypothesized to comprise a testicular dysgenesis syndrome with a common fetal origin, perhaps involving mild deficiencies in androgen production/action during fetal masculinization [29, 30]. The skeletal abnormalities and variations found in the offspring of the control and *Jacaranda decurrens* exposed animals were similar. As the determination of fetal ossification centers is used as an additional parameter to evaluate fetal development delay [28], we can infer that the progress and organogenesis of the embryos/fetuses exposed to *Jacaranda decurrens*, in utero, was similar to the control group.

In conclusion, to our knowledge, there are no published data which evaluates the effects of *Jacaranda decurrens* in female rats during organogenesis period. Our results suggest that *Jacaranda decurrens* aqueous extract seems not to cause embryo deaths, growth retardation, and/or malformations, when administered to Wistar rats at a dose of 27.5 mg/Kg/day, from 6th to 15th day of pregnancy. However, it is necessary to further evaluate the possibility of higher doses be teratogenic before the clinical use in humans.

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