Acta Farm. Bonaerense **25** (3): 421-4 (2006)
Recibido el 6 de agosto de 2005
Aceptado el 2 de enero de 2006

The Action of Extract of the Dry Leaves of *Cissus sicyoides* L. in Pregnant Rats

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SUMMARY. The hydroalcohol and alcohol extract of the dry leaves of *Cissus sicyoides*, showed abortive and teratogenic action in pregnant rats given orally at doses 300, 600, and 1000 mg.kg^{-1}.
RESUMEN. "Acción del extracto de hojas secas de *Cissus sicyoides* en ratas grávidas". Los extractos alcohólico e hidroalcólico de la hojas secas de *Cissus sicyoides* mostraron acción abortiva yñ teratogénica en ratas embarazada adminsitrados oralmente en dosis de 300, 600 y 1000 mg.kg^{-1}.

INTRODUCTION

Medicinal plants have been extensively used by the Brazilian people to treat bodily ailments [1]. *Cissus sycioides* L. (CS) is a plant originally from the Dominican Republic [2-5], and locally is known as "insulina vegetal", "cipo-pucá", "bejuco de porra", "bejuco caro", puci" and "anil trepador" [4-8]. It is used in folk medicine as diuretic, anti-inflammatory [3], anti-diabetic and anti-lipemic [9-11]. It has demonstrated a vasoconstrictor effect on guinea-pig aorta rings [12]. This plant also presents antibacterial and oxytocic activity [5,13,14]. CS has been evaluated for its anticonvulsant property in Brazil, where it is used against epilepsy [15]. The tea induced an increase in the amount of chromosomal damage in bone marrow cells without altering the cell division cycle [16] and cytotoxic activity [8].

Phytochemical study showed the presence of the flavonoids kaempferol 3-0-rhamnoside and quecetin 3-0-rhamnoside obtained from aerial plants of CS and coumarin glycoside from *Cissus sicyoides* [17]. The genus *Cissus* contains sterols, quinones and phenolic compounds in its leaves. It also contains anthocyanins, saponins and flavonoids in its fruit [18]. The purpose of the present study was to determine the toxicity of hydroalcohol and alcohol extracts of the dry leaves of *Cissus sicyoides* in pregnant rats.

MATERIALS AND METHODS

Plant material

Aerial parts of CS were collected in the vicinity of Recife - State of Pernambuco - Brazil in January 2004. The plant material was identified botanically and the voucher specimens were deposited in the herbarium Geraldo Mariz UFP under number 29040 of the Botanical Department of the Federal University of Pernambuco.

The leaves were washed, dried at room temperature (approximately 28 °C) in the laboratory for approximately 25 days, then ground in a mill to a grain size of <1 mm. Next, 360 g of the powdered plant material was added to 1000 mL of a alcohol water mixture (70:30). The dry leaf powder yielded 30% of extract. For pharmacological testing, the extract was dissolved in saline plus Tween 80 (0.025%). The preliminary chemical test was performed using three tubes of different pH values: pH 3, pH 8.5 and pH 11, and the color was consigned. The second test was performed with two tubes containing the hydroalcoholic extract, one at pH 1-3 and the other at pH 11, then the tubes were warmed

KEY WORDS: *Cissus sicyoides* L., Embriofetotoxic effect, Pregnant rats.
PALABRAS CLAVE: Cissus sicyoides L., Efecto embriofetotóxico, Ratas embarazadas.

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and the color was consigned. The test with saponin was performed using the hydroalcohol extract, which was evaporated and then reextracted with chloroform. The insoluble part was dissolved in water with strong agitation [19].

Animals

Adult Wistar rats (150-250 g) were kept under standard laboratory conditions of temperature (23° ± 1 °C), approximately 60% relative humidity and 12/12 h light/dark cycle. The animals received a commercial rat diet (Purina®) and tap water *ad libitum*. All the animals were carefully monitored and maintained in accordance with the ethical recommendation of the Brazilian College of Animal Experimentation (COBEA) and the National Institute of Health Guide for Care and use of Laboratory Animals.

Phytochemical screening

The hydroalcoholic plant extract obtained from the leaves of CS has been shown to contain flavones, flavanones, flavononols, leucoanthocianidins and saponins. The presence of yellow or red orange color presumably indicates the presence of the first named compounds. The leucoanthocianidin was detected by the red color in the acid tube. Later, enough saponin was detected [19].

Study of pregnant rats

Female Wistar rats were mated with males of previously confirmed fertility (one male for three females). Vaginal smears were examined each morning for the presence of sperm on day 1 of pregnancy [20, 21]. Inseminated animals were isolated in cages and divided into 12 groups, each group with 06 animals, which were treated with the extract alcohol and hydro-alcohol extracts of *Cissus sicyoides* according to Tables 1 and 2. Each treated group had its corresponding control group. The extracts was administered (orally) 0.1 mL/100g. Each animal was weighed and killed by cervical displacement on day 19 of gestation. A laparotomy was performed and the uterus and ovaries were removed. Resorptions [20] (embryotoxicity/fetotoxity) were counted and viable implants were examined. The number of live/dead fetuses, viability, growth and deformity of new borns and maternal weight gain were recorded.

Statistical evaluation.

The data was submitted to analysis of variance (ANOVA). Posthoc comparison between individual treatments and controls was made using Dunnett's multiple comparison tests or Student's *t*-test depending on the case. The results obtained were considered significant when $P < 0.05$

RESULTS

As observed in Tables 3 and 4, the hydro-alcohol extract in a dose of 300 and 600 $mg.Kg^{-1}$ produced fetotoxicity (Reabsorption index) administered from 1° to day 6° and 7° to day 12°; alcohol extract in a dose of 600 and 1000 $mg.Kg^{-1}$ administered from day 1° to day 6° and 70 to day 120 led to fetal toxicity. Furthermore malformations of the implant and viable fetuses were observed in animals killed on day 19 of pregnancy: absence of tail, organs outside abdominal cavity and syndactyly at doses of 300, 600 and 1000 $mg.Kg^{-1}$ in hydro-alcohol extract and alcoholic extract (Table 5 and 6).

DISCUSSION

The action of *Cissus sicyoides* as hypoglycemic and anti-lipemic [9-11], antiinflammatory [3] antibacterial and oxytocic [5,13,14], and also as hav-

Group	Dose ($mg.kg^{-1}$)	Day of pregnancy	Number of rats
1	300	1-6	6
2	600	1-6	6
3	1000	1-6	6
4	Control	1-6	6
5	300	7-12	6
6	600	7-12	6
7	1000	7-12	6
8	Control	7-12	6

Table 1. Group treated with hydro-alcohol extract of the dry leaves of *Cissus sicyoides*.

Group	Dose ($mg.kg^{-1}$)	Day of pregnancy	Number of rats
1	300	1-6	6
2	600	1-6	6
3	1000	1-6	6
4	Control	1-6	6
5	300	7-12	6
6	600	7-12	6
7	1000	7-12	6
8	Control	7-12	6

Table 2. Group treated with alcohol extract of the dry leaves of *Cissus sicyoides*.

Group	Dose ($mg.kg^{-1}$) and day	Implants	Resorptions	Corporea lutea	Resorption index	Average total body weight
1	300 Day 1-6	9.0±2.0	2	7.0±1.0	3,17	1,48±0,04
2	600 Day 1-6	7.0±2.0	4	8.0±2.0	9,09	1,61±0,13
3	1000 Day 1-6	7.0±1.0	2	7.0±2.0	4,87	1,47±0,02
4	Control Day 1-6	10.0±00	0	9.0±1.0	0,00	1,59±0,01
5	300 Day 7-12	7.0±2.0	2	9.0±2.0	4,35	1,49±0,06
6	600 Day 7-12	9.0±2.0	2	9.0±1.0	3,33	1,46±0,04
7	1000 Day 7-12	8.0±1.0	1	7.0±2.0	1,85	1,57±0,07
8	Control Day 7-12	9.0±3.0	0	8.0±1.0	0,00	1,59±0,01

Table 3. Incidences of resorption in the hydro-alcohol extract and control groups of *Cissus sicyoides*. Values are mean ± SEM for 6 animals; $p< 0.5$ *vs* control group (chi-square test).

Group	Dose	Implants	Resorptions	Corporea lutea	Resorption index	Average total body weight
1	300 day 1-6	11.0±1.0	2	9.0±3.0	3,17	1,485±0,04
2	600 day 1-6	8.0±2.0	4	8.0±1.0	9,09	1,614±0,13
3	1000 day 1-6	8.0±2.0	3	7.0±2.0	4,87	1,474±0,02
4	Control day 1-6	10.0±1.0	0	7.0±1.0	0,00	1,59±0,01
5	300 day 7-12	9.0±2.0	4	9.0±2.0	4,35	1,493±0,06
6	600 day 7-12	8.0±3.0	8	9.0±1.0	3,33	1,468±0,04
7	1000 day 7-12	8.0±2.0	3	8.0±1.0	1,85	1,578±0,07
8	Control Day 7-12	10	0	9.0	0,00	1,59±0,01

Table 4. Incidences of resorption in the alcohol extract and control groups of *Cissus sicyoides*. Values are mean ± SEM for 6 animals; $p< 0.5$ *vs* control group (chi-square test). Reabsorption index = (Number of Reabsorptions / Number of implantations) X 100.

Fetal observation	Dose $mg.kg^{-1}$	Dose $mg.kg^{-1}$	Dose $mg.kg^{-1}$
	300 Day 1-6	600 Day 1-6	1000 Day 7-12
Abdominal organs outside cavity	-	16.6 %	16.6 %
Sindactly	16.2 %	16.6 %	-
Absence of tail	-	-	-

Table 5. Fetal malformation percentages in the viable Implants with hydro-alcohol extract of *Cissus sicyoides*.

Fetal observation	Dose $mg.kg^{-1}$	Dose $mg.kg^{-1}$	Dose $mg.kg^{-1}$
	300 Day 1-6	600 Day 1-6	1000 Day 7-12
Abdominal organs outside cavity	-	16.6 %	16.6 %
Sindactly	16.2 %	16.6 %	-
Absence of tail	-	33.2	-

Table 6. Fetal malformation percentages in the viable Implants with alcohol extract of *Cissus sicyoides*.

ing a vasoconstrictor effect on guinea-pig aorta rings [12] was reported. Research done so far seems to confirm the folklore usage of *Cissus sicyoides* for anti-diabetic and anti-inflammatory treatment. However, *in vitro* studies of cytotoxic activity of *Cissus sicyoides*, in the antimitotic assay, showed complete inhibition of cell division at 24 h of treatment against HEp-2 cells [8]. The assay with the tea induced an increase in the amount of chromosomal damage in bone marrow cells without altering the cell division cycle [16]. The study of pregnant rats treated orally with 600 and 1000 $mg.kg^{-1}$ of both crude (hydroalcohol and alcohol) extracts demonstrated anatomi-

cal alterations in the fetus. Incidents of blastocystotoxic-antizygotic action and few cases of abortive activity were also observed. This indicates that *Cissus sicyoides* probably acts at the beginning of egg division and also during implantation. The physiological mechanism of this effect is the major area of interest for future studies.

Acknowledgements. The authors wish to express their thanks to CNPq (Conselho Nacional de Pesquisa) and to UFPE (Universidade Federal de Pernambuco) for financial assistance.

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