

Full Length Research Paper

Embriofetotoxic effect and offspring postnatal development exposed to hydroalcoholic fraction extract of *Cissus sicyoides* L. during wistar rats pregnancy

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Cissus sicyoides L (Verbaseae) is popularly known by its antidiabetic action. The hydroalcoholic extract effect in pregnant rats and its offspring was evaluated through the following parameters: gestation duration, number of younglings for hatch, maternal weight gain, postural reflex, and day of eye's opening and day of adult gait. The results suggest that the CS does not exert any effect on the fertility index. However, all doses (300, 600 and 1000 mg/ kg, i.p.) had promoted alteration in the postural reflex, but did not have alteration over the eye opening parameter, as well as, in the adult gait, which is an important phase of neurological development for offspring. CS did not modify the maternal body's temperature and animals' ambulation had no alteration in all periods. The CS promoted malformations in foetuses such as abdominal organs outside cavity, sindactly and absence of tail.

Key words: *Cissus sicyoides* L, embryotoxicity, offspring, wistar rats.

INTRODUCTION

The *Cissus sicyoides* (CS) is a plant originated from Dominican Republic (Cano and Volpato, 2004; Garcia, 2000), and locally, it is known as "insulina vegetal", "cipo-pucá", "bejuco de porra", "bejuco caro", "puci", "anil trepador" (Sáenz et al., 2005). The plant is used in popular medicine as diuretic, anti-inflammatory (Garcia, 2000), hypoglycemic and anti-lipemic (Pepato et al., 2003; Viana and Medeiros, 2004). It has demonstrated a vasoconstrictor effect on guinea-pig aorta rings (Garcia et al., 1997). This plant also presents an antibacterial and oxytocic activity (Garcia et al., 1999). CS has been evaluated for its anticonvulsant property in Brazil, where it is used to prevent epilepsy (Elizabestsky, 1988). The tea treatment induces a chromosomal damage increase in bone marrow cells without altering the cell division cycle (Vicentini, 2001) and cytotoxic activity (Feng, 1964). Was demonstrated the methanol extract action on HepG2 cells lineage (Opohu et al., 2000) and a central analgesic

action was demonstrated (Almeida et al., 2006). The phytochemical study showed flavonoids kaempferol 3-O-rhamnoside and quecetin 3-O-rhamnoside obtained from CS aerial plants and Coumarin glycoside from CS (Beltrame et al., 2002; Beltrame et al., 2001). The genus *Cissus* leaves contains sterols, quinones and phenolic compounds. It also contains anthocyanins, saponins and flavonoids in its fruit (21). The proposal of the present study was to determine the *Cissus sicyoides* dry leaves hydroalcoholic extract action in the gestational age of pregnant rats and its effect on the offspring, using the doses of 300, 600, and 1000 mg/ kg by intraperitoneal route.

MATERIALS AND METHODS

Plant material

CS aerial parts were collected near the Department of Antibiotics of Pernambuco Federal University - Recife - State of Pernambuco - Brazil in January 2005. The plant material was botanically identified and the voucher specimens were deposited in the herbarium Geraldo Mariz UFP of the Botanical Department of the Federal

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University of Pernambuco, under number 29040. Nowadays *Cissus sicyoides* presents as synonymous *Cissus verticillata* (Mikich and Silva, 2001)

Preparation of the extract

Leaves were washed and dried at room temperature (approximately 28°C) during approximately 25 days, then ground in a mill to a grain size of <1 mm. Next, 360 g of powdered plant material was added to 1000 mL of alcohol and water (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: pH 3, pH 8.5 and pH 11, and then the colors were observed. The second test was performed with two tubes containing the hydroalcoholic extract, one at pH 1-3 and the other at pH 11. The colors were observed when the tubes were warmed. The test with saponin was performed using the hydroalcoholic extract. It was evaporated and extracted with chloroform. The insoluble part was dissolved in water with strong agitation (Harbone, 1991).

Animals

Adult female Wistar rats (150-250 g), with two months old at the beginning of the experiment, were used. The animals were housed in groups of eight per cage (group), with a light/dark period of 12 h (6:00 am to 6:00 pm). They received food and water ad libitum. All experiments were conducted between 10:00 am and 4:00 pm. Female rats were tested monitoring the oestrus cycle. All the animals were carefully monitored and maintained in accordance with the ethical recommendation of the Colégio Brasileiro de Experimentação Animal (COBEA) and the National Institute of Health Guide for care and use of Laboratory Animals and were approved by the Ethical Committee of the Federal University of Pernambuco (UFPE) under protocol number 008199/2005-29.

Effect of the administration of the CS in the pregnant rats

Female Wistar rats, from the Antibiotics Department vivarium, with about 220 g, were used. These animals were assigned into four groups of eight (control group, which received saline (0.9%; 0.1 mL/100 g), and the three others groups that received CS at doses of 300, 600, and 1000 mg/ kg. On the day 19^o, the animals were sacrificed by cervical displacement and the uterus was removed allowing examination of the fetuses. The resorption number, placenta and luteal body weight were observed. The fetuses' weight, size, and morphological examination (external) were also observed (Almeida et al., 2000., Freitas et al., 2005).

Fetuses' external examination

With the help of a magnifying glass, the palate cleft presence or absence was observed, as well as the eye position and ear implantations; the tail (form and length) and termites were examined for abnormalities such as polydactyl or syndactyl (Sertie et al., 2005; Freitas et al., 2005).

CS administration effect in the offspring development

The four remaining females in each group were treated until birth of the hatch. CS possible effects on the hatch behavioral development were assessed by considering the following aspects:

Hatch's average weight: This measurement was done at birth and on day 7, 14 and 21^o after birth. Thus, comparison between the

measurements obtained in control and experimental of the hatch would be detected.

Postural reflex: This measurement was performed at the offspring birthday and on the subsequent day 3 and 7^o. For this purpose, each animal was supine placed on a straight surface and the reflex to return to the upright position was measured in seconds.

Initial eye opening: From the day 7^o after birth, the eyes were observed by the partial displacement of the lid fissure.

Interaction of adult gait: Gait was considered as 'gait on' when the young animal gaited without dragging its hind legs and without touching the ground with its belly.

Ambulation: This measurement was performed on day 15^o after birth. A square arena (30 x 30 cm) with the floor divided in squares of 10 x 10 cm was employed. Only one youngling at a time was placed at the center of the arena; the squares invaded during 1 min were then counted. The measurements described above, except the first, were used to verify possible offspring behavioral changes and/or neurological development. The hatches were standardized by the number of animals per hatch: only four animals per hatch were left for subsequent observation. These animals consisted of 2 male and 2 female (Tufik et al., 1994).

Statistical evaluation

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

RESULTS

Treatment effect with CS hydroalcoholic extract on rat's fertility

Maternal weight's gain, pregnancy duration and number of embryos for hatch during the period of 19 days, where the rats had been displayed to the application of the hydroalcoholic extract of CS data are shown in Table 1. They presented significant values ($P < 0.05$) when compared to the values in the control group. This can indicate that the extract action does not exert some interference that harms the maternal fertility maternal. Although promoted a reduction in the gain of maternal weight.

Effect in the offspring after administration of CS

The evaluation of CS action on the after-birth development can show that delay in the physical maturation did not occur, when comparing the eyes opening parameters and also the adult gait day. Significant alterations ($P < 0.05$) has led to an increase in the postural recovery time in the dosage of 300, 600 mg/ kg during evaluation day 1 and 7^o, as well as resulted in higher significant values ($P < 0.01$) in the dosages of 600 and 1000 mg/ kg, and also in first and in the last evaluation day (Table 2).

Table 1. Effect of the treatment with the hydroalcoholic extract of CS on rat's fertility.

Evaluated parameter	Groups			
	Control	300 mg/kg	600 mg/kg	1000 mg/kg
Duration of the gestation days)	22.5 ± 0.22	22.5 ± 0.22	22.66 ± 0.333	23.0 ± 0.26
Gain of maternal weight (g).	79.83 ± 6.31	67.33 ± 4.12	65.66 ± 5.24*	60.66 ± 1.84*
Nº of embryos per rat.	8.3.66 ± 1.84	8.16 ± 0.60	9.5 ± 1.28	8.33 ± 0.49

Values are expressed on average ± S.D; *P < 0.05; versus control; ANOVA of one way followed of the test of multiple comparisons of Dunnett's.

Table 2. Effect in the offspring after the administration of CS.

Evaluated parameter	Control	300 mg/kg	600 mg/kg	1000 mg/kg
Postural reflex (s.)	-	-	-	-
1° day	10,42 ± 1,106	28,81 ± 6,318*	35,26 ± 5,151**	38,66 ± 2.421**
3° day	10,44 ± 3,749	20,5 ± 4,408	21,69 ± 6,154	10,92 ± 1,72
7° day	0,67 ± 0,096	1,32 ± 0,218*	1,37 ± 0,099**	1,40 ± 0,139**
Eye's opening (day)	14,97 ± 0,208	14,71 ± 0,147	15,16 ± 0,115	14,56 ± 0,178
Adult gait (day)	14,74 ± 0,057	14,74 ± 0,093	14,65 ± 0,142	14,84 ± 0,187

Values are expressed on average ± S.D; *P < 0.05, **P < 0.01; versus control; ANOVA of one way followed of the test of multiple comparison of Dunnett's.

Table 3. Effect of the maternal exposition after treatment with CS on the weight of younglings.

Evaluated parameter	Groups			
	Control	300 mg/kg	600 mg/kg	1000 mg/kg
gain of fetal weight (g)	-	-	-	-
1° day	5,44 ± 0,162	5,65 ± 0,140	5,25 ± 0,112	5,73 ± 0,156
7° day	14,10 ± 0,734	12,07 ± 0,588	9,76 ± 0,606**	10,55 ± 0,591**
14° day	21,49 ± 0,872	21,31 ± 0,858	20,95 ± 2,8	19,08 ± 1,123
21° day	32,2 ± 1,066	34,60 ± 1,259	34,34 ± 4,162	32,37 ± 1,556

Values are expressed on average ± S.D, **P < 0.01; versus control; ANOVA of one way followed of the test of multiple comparison of Dunnett's.

Effect of the maternal exposition after treatment with CS on the weight of younglings

The maternal exposition effect to the CS hydroalcoholic extract, showed to account for the highly significant youngling's weight reduction (P < 0.01) that had received dosages from to 600 and 1000 mg/ kg to day 7^o evaluation. However, such result does not seem to have intervened with these animals development in day 14 and 21^o, when a gradual increase of its weights occurred (Table 3).

External examination of the fetuses

The embryos examination was made after the sacrifice of the animals in day 19^o of the pregnancy. The data are shown in Table 4.

DISCUSSION

The teratogenic effect evaluation of a plant extract or chemical compound, through assays in animals, can be executed in a complex protocol. In the extract effects studies over the offspring development after birth was evaluated. In this stage, were evaluated offspring somatic, neuromotor and sensorial development. Therefore, the maternal exposition to some xenobiotic during the gestation can still cause neurotoxicity in the development and/or abnormality in the behavior of the newborn ones that can persist during all its life, through the action on the Central Nervous System (Castro and Palermo-Neto, 19888; Suite et al., 2005). The corporal mass maternal gain during pregnancy occurred only in the dose of 1000 mg/ kg, and the CS did not show alteration in the rat's fertility level (Table 1). Younglings' weight reduction can

Table 4. Fetal malformations percentages in the viable implants with *Cissus sicyoides*

Fetal observation	Dose mg/kg	Dose mg/kg	Dose mg/kg
	300 Day 1-6	600 Day 1-6	1000 day 7-12
Abdominal organs outside cavity	-	16.6%	16.6%
Sindactily	16.2%	16.6%	-
Absence of tail	-	33.2%	-

can be observed in table 3 at the doses of 600 and 1000 mg/ kg. The toxicity of the development would include four abnormal development manifestations due a birth exposition: alterations of the growth (development delay, fetal corporal mass reduction), death, malformations (table 4), and motor deficit (Jelínek, 2005). In the present study can be observed that younglings' indirect exposition to the extract did not intervene with the opening of the eyes and specifically in adult gait (table 2). Although interferences in postural reflex at doses of 300, 600 and 1000 mg/ kg had occurred, being able to infer in such a way, that the extract promoted an alteration in the medullar reflex (table 2). On basis of the results, had not occurred alterations in the gestation time, and number of younglings for hatch. However, reduction in the maternal weight occurred in such a way, being possible to suggest that CS extract does not exert significant effect over maternal fertility (Table1.). However, it can also be observed that during the youngling's growth phase the weight was reduced during evaluation interval, and that the postural reflex was compromised in all doses, in the intervals through the first to the seventh day (Table 2). However, the animal's ambulation did not have alteration in all the periods. These data have a great interest for not modifying the physiological parameters as eye's opening and also in the adult gait that represents the neuromotor development important phase of animals (Winneke, 1992).

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