

Early development of the rat embryo in mothers treated with *Solanum lycocarpum* St. Hil.

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RESUMO: Desenvolvimento precoce de embriões de ratas tratadas com *Solanum lycocarpum*

St. Hil. O fruto da lobeira (*Solanum lycocarpum*, St. Hil.- Solanaceae) é usado por pacientes diabéticos no estado de Minas Gerais (Brasil) como hipoglicemiante. Ele contém um alcalóide com configuração estereoespecífica para a síntese de esteróides e solamargina e solasonina das quais se obtém a 3 β -acetoxipregna-5,16-dien-20-ona. Como agente hipoglicemiante ou com fonte de hormônios esteroidianos, o polvilho de lobeira provavelmente possa alterar o transporte do embrião pelo oviduto ou os nutrientes necessários à sua sobrevivência, já que cada fase do desenvolvimento necessita de nutrientes específicos, produzidos pela secreção tubária, e um hipoglicemiante pode alterar a constituição bioquímica de tal secreção. Para examinar tal hipótese, ratas inseminadas receberam suspensão aquosa de polvilho de lobeira (100 mg /Kg de peso corporal), por gavagem, nos dias 1, 2, 3, e 4 da prenhez. O grupo controle recebeu 5 ml de água destilada/Kg de peso corporal, no mesmo esquema. As mães foram pesadas no início do tratamento e no dia do sacrifício (quinto dia da prenhez) e foram examinadas, diariamente, com critérios clínicos para avaliar a toxicidade do tratamento. Os ovidutos e cornos uterinos foram removidos e lavados com solução salina para contar os embriões em fase precoce do desenvolvimento. A suspensão aquosa de lobeira não causou toxicidade materna, nem alterou o transporte ou o desenvolvimento do pré-embrião, podendo-se concluir que na dose administrada não foi tóxico para mãe ou para o pré-embrião.

Palavras-chave : Alcalóides de solanaceos, ratos wistar, desenvolvimento fetal, hipoglicêmicos, testes de toxicidade.

ABSTRACT: The lobeira fruit (*Solanum lycocarpum*, St. Hil- Solanaceae) is under current use by diabetic patients in Minas Gerais state (Brazil) as a hypoglycemic agent. It contains an alkaloid with stereospecific configuration for the synthesis of steroid hormones and solamargine and solasonine from which a 3 β -acetoxipregna-5,16-dien-20-one is obtained. As a hypoglycemic agent or as a potential source of steroid hormone, lobeira powder can alter embryo transport through the oviduct or the nutritional requirement for their survival since each phase of development needs specific nutritional requirements, and a hypoglycemic agent has the potential capacity to change the biochemical constitution of the oviduct secretions. To examine this hypothesis, inseminated rats received an aqueous suspension of 100 mg of the lobeira powder /Kg of body weight, by oral gavage, on days 1, 2, 3, and 4 of pregnancy. A control group received 5 ml of distilled water/ Kg of body weight in the same schedule. Pregnant rats were weighed at the beginning of treatment and on sacrifice day (on 5th day of pregnancy), and were examined daily by clinic criteria to evaluate toxicity of the treatment. The oviducts and uterine horns were removed and flushed with a saline solution buffer for counting the pre-embryos. The aqueous suspension of lobeira did not cause maternal toxicity, alteration of the pre-embryo transport, reduction of the number of expanded blastocysts, or increase of the number of degenerated pre-embryos. Considering the results it was concluded that the lobeira powder in the dose used is not toxic to mothers or to pre-embryos.

Key words : Solonaceous alkaloids, rats wistar, fetal development, hypoglycemic agents, toxicity tests

INTRODUCTION

On the first four days of the rat embryonic development, the zygote undergoes successive clivages and structural alterations which convey it to the blastocyst stage when it then implants (Kanjapothi *et al.*, 1981; Singh *et al.*, 1993). For each of this development stage, nutrients and several growth factors, important for the conceptus survival and normal development, are necessary (Brown, 1994; Paria & Dey, 1990; Harvey & Kaye,

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1992; Kano *et al.*, 1998).

As the embryonic development stage advances, the conceptus is progressively transported from the oviduct to the uterus where it implants. This transport is dependent on the mother's hormonal conditions. In the rats, estrogens accelerate and progesterone retards the conceptus oviductal transport (Forcelledo & Croxatto, 1988; Vinijsumun *et al.*, 1990; Croxatto *et al.*, 1991).

Several agents may interfere with the embryo's early development. They usually cause the embryo's death which may not be noticed and can be mistaken for an infertility diagnosis. However, some of these agents can reduce the cleavage rate and retard the development (Mason & Kang, 1994; Brocas *et al.*, 1997) and others can produce genetic alterations which cause malformations.

Many medicinal plants have been used without a previous study concerning their safety for human use during gestation. Among these plants, the lobeira or jurubebão fruit (*Solanum lycocarpum*, St. Hil - Solanaceae) is highly used in Minas Gerais state as a hypoglycemic agent. The plant is a 2 to 3.5 m – high – tortuously-stemmed branched, arboreous or shrub-like perennial plant. Its leaves are hard and thorny and the globous berry-like fruits of 8 – 12 cm in diameter are edible and have been reported to possess sedative, diuretic, antiepileptic and antispasmodic activity (Cruz, 1982; Lorenti, 1991; Kissmann & Doris, 1995).

A pharmacognostic analysis of the lobeira fruit showed two glycoalkaloids – solamargine and solasonine (Motidome *et al.*, 1970) from which a 3 β -acetoxipregna-5,16-dien-20-ona is obtained (Haraguchi *et al.*, 1978) as well as an alkaloid with stereospecific configuration to the synthesis of steroid hormones (Kerber, 1993). Therefore, acting as hypoglycemic agent or as a possible source of steroid hormones, the lobeira powder could alter the early phases of the embryonic development, altering its transport or the biochemical composition of the oviductal and uterine secretions.

The embryotoxic potential of the lobeira fruit (*Solanum grandiflora*) has been investigated previously in Wistar rats. The data obtained showed that the administration of an aqueous extract of lobeira fruit did not affect the process of blastocyst implantation (Peters *et al.*, 1997) nor the organogenesis of the rat (Guerra *et al.*, 1997). Toxic effects in the mother as well as her ability to carry out pregnancy have not been detected either (Guerra *et al.*, 1997; Peters *et al.*, 1997). However, the phase which precedes implantation has not been studied yet, being this the objective of the present work.

MATERIAL AND METHOD

The plant used in this study was collected in Caratinga (MG) and authenticated by Dr. Fatima S. Pires in the Herbarium Leopoldo Krieger - Universidade Federal de Juiz de Fora (UFJF) (Juiz de Fora, MG, Brazil), where voucher specimens are kept (vaucher number 30114). The plant was identified as *Solanum lycocarpum* St. Hil, family Solanaceae.

The powder of this plant was supplied by Dr. João Evangelista de Paula Reis, from the Faculdade de Farmácia - UFJF.

Pregnant Wistar rats were obtained from the Centro de Biologia da Reprodução (CBR) vivarium of UFJF on the 1st day of pregnancy (day 1 = spermatozoa in the vaginal smear) and randomly distributed in the following experiments:

Treatment on 1st day of pregnancy
Treatment on 2nd day of pregnancy
Treatment on 3rd day of pregnancy
Treatment on the 4th day of pregnancy

Each experiment has a control and a treated group of at least 10 animals each.

The control group received 5 mL of distilled water/ kg of body weight and the treated group received an aqueous suspension of 100 mg of lobeira powder /kg of body weight by oral gavage.

The lobeira suspension dose is ten - fold greater than that used in humans (10 mg/ kg of body weight – personal communication by Dr. J.E.P.Reis). Treatment with water or lobeira suspension was always done at 10 am.

Pregnant rats were weighed before the first administration of the aqueous suspension of lobeira and on sacrifice day. During this interval, maternal toxicity was evaluated by clinic criteria (death, weight loss, piloerection and locomotion activity) (Manson & Kang, 1994).

Females of all groups were killed by cervical dislocation on 5th day of pregnancy. The genital tract was removed and oviducts and uterine horns were flushed separately with a saline solution buffer. All flushing were screened with a dissecting microscope at 25 X magnification to count the number of expanded blastocysts and other phases of the pre-embryos' development (Forcelledo *et al.*, 1981; Ortiz *et al.*, 1989). Pre-embryos grossly underdeveloped or exhibiting signs of cytolysis were designated as degenerated. The apparently normal pre-embryos were classified as two-cells, morula, early blastocyst or expanded blastocyst (Ortiz *et al.* 1979; Roblero *et al.*, 1987).

For statistical purposes, Student's t-test was used to compare the mean maternal weight on treatment and sacrifice days (paired t - test) and the number of expanded blastocysts (unpaired t - test). The chi square test was used to compare the proportion of blastocyst (expanded and early) with respect to the total of pre-embryos collected. The significance of the tests was $\alpha = 0.05$.

The experimental protocol was submitted to and approved by the ethical committee of the CBR/ UFJF.

RESULT

The administration of lobeira to pregnant rats has not showed any significant clinical signs of toxicity since there was no death, no alteration of locomotion activity, no piloerection and all animals gained weight between the treatment and sacrifice days (Table 1).

The mean number of expanded blastocysts obtained in the flushing of uterine cornua was similar in all experimental groups (Table 2)

TABLE 1. Body weight of control and *Solanum lycocarpum* treated rats on days 1,2,3,or 4 of pregnancy . Sacrifice on 5th day.

Day of pregnancy	Body Weight (g)*	
	Control	Lobeira
1	169.0 ± 10.6 (9)	170.3 ± 13.4 (10)
5	176.6 ± 10.9 (9)	177.8 ± 15.1 (10)
2	174.4 ± 15.3 (10)	176.3 ± 14.9 (10)
5	181.8 ± 15.1 (10)	181.9 ± 16.4 (10)
3	182.4 ± 14.8 (11)	171.3 ± 17.4 (11)
5	187.5 ± 15.4 (11)	180.5 ± 13.6 (11)
4	182.2 ± 13.5 (11)	173.3 ± 19.8 (11)
5	184.1 ± 13.2 (11)	176.7 ± 18.5 (11)

* Mean ± Standard Deviation. n= number of mothers.

All mothers gained weight between the treatment and sacrifice days ($p < 0.05$), except the animals treated with lobeira on day 4 that had no weight alteration .

TABLE 2. Mean of expanded blastocyst/uterus of control and *Solanum lycocarpum* (10 mg/ 100 g of body weight) treated rats on days 1,2,3, and 4 of pregnancy. Sacrifice on 5th day.

TREATMENT ON DAYS	EXPANDED BLASTOCYST ON 5 th DAY	
	Control	Lobeira
1	8 ± 3.2 (11)	8 ± 2.2 (9)
2	9 ± 1.6 (9)	8 ± 2.6 (10)
3	8 ± 2.4 (11)	8 ± 1.2 (11)
4	8 ± 2.4 (11)	9 ± 3.1 (11)

* Mean ± Standard deviation. n= number of mothers. There were no significant differences among the experimental groups

The proportion of blastocysts with respect to morula and degenerated struture was high in all experimental groups (Table 3) and the number of degenerated pre-embryos was negligible.

TABLE 3. Proportion of blastocysts with respect to the total number of pre-embryos in control and *Solanum lycocarpum* (10 mg/ 100 g of body weight) treated rats on days 1,2,3, and 4 of pregnancy. Sacrifice on 5th day.

TREATMENT ON DAYS	EXPANDED BLASTOCYST ON 5 th DAY	
	Control	Lobeira
1	8 ± 3.2 (11)	8 ± 2.2 (9)
2	9 ± 1.6 (9)	8 ± 2.6 (10)
3	8 ± 2.4 (11)	8 ± 1.2 (11)
4	8 ± 2.4 (11)	9 ± 3.1 (11)

*(Blastocysts/ total number of pre-embryos) n = number of mothers in the group
There were no significant differences among the experimental groups

DISCUSSION AND CONCLUSION

In order to observe toxic effects upon pre-embryos, it is necessary to distinguish maternal toxicity from pre-embryo toxicity. Some clinical criteria were adopted, according to Mason & Kang (1994), to observe maternal toxicity. Considering the used dose, it is possible to assume that the lobeira aqueous suspension was not toxic to the mothers, since there were no clinical signs of discomfort in the animals (alteration of locomotion activity or piloerection) and no weight loss.

During the first four days of pregnancy, a complex set of factors are critical to the development such as transforming growth factor, retinoic acid and proteins (Brown, 1994). Growth factors such as TGF- α , TGF- β and EGF promote growth of the 8-cell phase to blastocyst in mice (Paria & Dey, 1990). "In vitro" studies have shown that hyaluronic acid added to the culture medium increases the number of zygote that reaches the blastocyst phase (Kano, *et al.*, 1998). In addition to that, Harvey & Kaye (1992) reported that 8-cell mice pre-embryos have insulin receptors that stimulate their metabolism and growth and also that the presence of IGF-1 stimulates cellular divisions in the embryonic pole. This finding suggests that glucose must play a role in the early embryonic development.

On the basis of this information it is possible to assume that if there were some hypoglycemic factor in the lobeira aqueous suspension, and could this factor be able to decrease the glucose level in the maternal blood or in the oviductal environment, some degree of growth delay of the pre-embryo would be observed. Since the mean number of expanded blastocysts/ mother and the proportion of total blastocysts collected was similar in the treated and control groups of all experiments, it is pertinent to speculate that there was no factor in the lobeira aqueous suspension that has interfered with the glucose needed to the conceptus development.

During the first day of gestation the fertilization process is completed; on the second day there are two blastomeres; on the third day there are four and eight blastomeres; on the fourth day there is morula and in the afternoon of this day early blastocysts can be found (Singh *et al.*, 1993; Souza, 1997). The administration of the lobeira powder in each of these day of gestation allowed the embryo's early development stages to be followed, showing that it did not affect the development in any of these specific stages.

Another important factor concerning pre-embryo viability is its oviductal transport, as there is a strict relationship between the developing phase of the conceptus and the maternal hormonal phase. In rats, estrogens accelerate and progesterone retards the conceptus oviductal

transport (Forcelledo & Croxatto, 1988; Vinijsamun *et al.*, 1990; Croxatto *et al.*, 1991). Based on this information, it is possible to assume that in the used dose there was no hormonal factor in the lobeira aqueous suspension able to alter the embryo transport, at least under the experimental conditions of the present experiment.

In conclusion, the administration of the lobeira aqueous suspension (100 mg / Kg of body weight/day) is not toxic to rat and to pre-embryos.

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