Reproductive alterations in male rats exposed perinatally to *Solanum lycocarpum* fruits

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Abstract

This work employed pregnant rats treated with *Solanum lycocarpum* unripe fruits (10% in diet) from gestation day (GD) 06 to post-natal day (PND) 07, for the evaluation of the sperm number, daily sperm production and epididymal sperm transit time of the male offspring at PND 60 and PND 90. No differences were observed in the daily sperm production (DSP) and sperm number in the testis of the exposed males at PND 60 and PND 90. Also, no alterations were observed in sperm transit time in the caput epididymis of the exposed males at PND 60 and PND 90. However, a reduced sperm transit time was observed in the corpus/cauda epididymis of the experimental males at PND 90. The last data may explain the reduced sperm number observed in the corpus/cauda epididymis of the experimental male rats at PND 90. These data show that the male rats exposed to *S. lycocarpum* fruits during gestation did not present alterations in testis sperm production and number, however the sperm transit time through epididymis was impaired, resulting in a decreased number of spermatozoa in epididymis cauda. We conclude that *S. lycocarpum* may cause imbalance on hypothalamus-pituitary gland axis.

Key words:
Solanum lycocarpum.
Solasodine.
Reproductive function.
Fertility.
Sperm production.
Rat.

Introduction

Compounds derived from plants with hormonal activity are frequently found in nature. *Solanum lycocarpum* St. Hil, Solanaceae, is a shrub commonly found in the Brazilian savanna that presents the glycoalkaloids solamargine and solasonine. The aglycone portion, solasodine, show steroidal structure similar to sexual hormones.

It is known that solasodine can be employed as a key starting compound for the manufacture of steroidal drugs, like contraceptives. It can give origin to the compound 3-B-acetoxypregna-5-16-dien-one, key starting source for progesterone production[1] and the compound 16-dehydroepiandrosterone, key starting source for androgen production[2]. Previous studies demonstrated that solasodine promotes anti-spermatogenic activity when administered to dogs (20 mg/kg for 30 days)[3] and to monkeys (150 mg/kg for 150 days)[4]. Treated dogs showed testicular degeneration and lack of epididymal sperm while treated monkeys showed reduced testicular and epididymal sperm count. Also, an in vitro test performed in human and bovine showed that solasodine reduces sperm motility in both species.[5] In this way, *S. lycocarpum*, as a natural source of solasodine and of the steroidal glycoalkaloids solasonine and solamargine, may disrupt the

reproductive endocrine system if sufficient amounts are ingested at a sufficient period of time.

The ingestion of *S. lycocarpum* fruits 10% in diet by pregnant rats and during the onset of lactation promotes impaired sexual behavior of the adult female offspring but no alteration in the male sexual behavior[6]. However, adult females present reversible alteration while males present important and irreversible testicular degeneration. In addition, the female and male adult offspring maintain fertility capability and normal sexual hormones levels[7], suggesting that the alterations observed may not be able to produce reproductive impairment.

The present study was designed as a complement to previous data and aimed to evaluate the possible effects of *S. lycocarpum* 10% in diet given to pregnant rats on sperm count, daily sperm production and the period of epididymal sperm transit of the adult exposed male offspring. The purpose was to possible explain the lack of alterations previously observed in the fertility capability of the male offspring even in the presence of an important testicular degeneration.

Material and Method

The *S. lycocarpum* unripe fruits were collected in Itajubá (Minas Gerais - Brazil) and authenticated by Prof. Dr. João Rubens Pirani from Institute of Botany - São Paulo University. The voucher specimens were kept in the HSF Herbarium (registration: Aline Schwarz, # 01).

Female Wistar rats (180-240 g) were acclimatized in standard conditions (room temperature at 20 ± 3º C and light from 6:00 to 18:00 h) and mated. Daily vaginal smears were then carried out within the first day of pregnancy considered to be the morning in which spermatozoa were found. The pregnant dams were randomly distributed into control (n=10) and experimental (n=10) groups and individually housed in polypropylene cages (40 x 50 x 20 cm). The treated group received chow mixed with 10% of dry milled unripe fruits from gestation day (GD) 06 to post-natal day (PND) 07. On PND 01 all the litters were examined externally and sexed. Each dam was maintained with 8 newborns (4 males and 4 females) until weaning. On PND 21 the offspring was weaned. Male pups from the control and experimental groups were identified and isolated from the females and housed in collective polypropylene cages (40 x 50 x 20) with bedding of wood shavings, 5 animals per cage.

The animals used in this study were maintained in accordance to the Ethical Principles in Animal Research adopted by Brazilian College of Animal Experimentation and approved by the Ethical Committee for Animal Research from the Faculty of Pharmaceutical Sciences - USP.

At birth (PND 01) one male rat per litter was weighted and the body length (cm) and anogenital distance (mm) were measured with the use of a pachymeter. The litter was considered the unit of analysis. The ratio between the anogenital distance and the cube root of the body weight was adopted as the appropriate method for measuring anogenital distance[8]. Also, the testis descent day detected by scrotum palpation was recorded by daily observations beginning on post-natal day 16.

At 30, 60 and 90 days of age, 10 male rats per group (one per litter) were weighed and euthanized by inhalation of carbon monoxide. The male sexual organs were removed through an incision in the ventro-pubic region and weighted. Data were normalized and expressed as a ratio of organ weight/body weight.

At PND 60 and PND 90, 8 males per group, from different litters, were weighted and then euthanized by carbon monoxide inhalation. Promptly, the right testis and epididymis were trimmed of fat and weighted. Testicular tunica albuginea was also removed. Testicular parenchyma and the epididymal corpus/cauda and caput were homogenized in saline-triton (NaCl + Triton 100-0,5mL/L) for two minutes. For homogenization, 5mL of the saline-triton solution was added to testicular parenchyma,

1mL to 100mg of epididymal caput and 1mL to 200mg of epididymal corpus/cauda. Homogenization-resistant testicular spermatids as well as sperm in the epididymal corpus/cauda and caput were then counted with the use of a Newbauer chamber. The methodology of Robb, Amann e Killian[9] was employed. Daily sperm production (DSP) was derived by dividing the mena of the total number of homogenization-resistant spermatids per testis by 6.1 days (the number of days of the seminiferous cycle in which these mature spermatids are present). The period of sperm transit through the epididymal caput or corpus/cauda was calculated by dividing the mean of the total number of homogenization-resistant sperm within each of these regions by DSP.

For the statistical analysis the Student's t-test (parametric data) and Mann-Whitney test (non-parametric data) were employed when necessary, and the results were considered significant for $p<0.05$. In all cases, the data obtained from experimental animals were compared with those obtained from control animals within the same age.

Results

At PND 01 the experimental male pups presented reduced body weight ($t=2.911$, $p<0.05$) and reduced body length ($t=2.457$, $p<0.05$) when compared to control ones. However, no alteration of anogenital distance to cube root of body weight ratio and testis descent day were observed between experimental and control male pups (Table 1).

No variation between the groups was observed in the wet weight of the sexual organs/body weight ratio of male pups at PND 30, PND 60 and PND 90 as presented in table 2.

Table 1 - Body weight, body length, testis descent day and anogenital distance ratio of control and perinatally (GD 06-PND07) exposed male pups

Parameters	Control	Experimental
Body weight (g)	5.71 ± 0.12	5.36 ± 0.17*
Body length (cm)	5.03 ± 0.05	4.91 ± 0.04 *
Anogenital distance (mm)	2.04 ± 0.05	1.97 ± 0.06
Testis descent day appearance	20.60 ± 0.14	20.57 ± 0.10

Means ± S.E.M.; * p<0.05 (Student's t test)

Table 2 - Testicular, seminal vesicle and epididymal wet weight ratio of sexual organs of control and perinatally (GD 06-PND 07) exposed male rats at PND 30, PND 60 and PND 90

PND	Testis	Seminal vesicle	Epididymis
30 control	3.93±0.16	0.17 ±0,01	1.33±0.17
experimental	3.91±0.10	0.17±0.01	1.28±0.06
60 control	5.54±0.12	1.66±0.15	2.42±0.12
experimental	5.46±0.09	1.74±0.10	2.36±0.09
90 control	4.75±0.17	2.15±0.15	3.27±0.10
experimental	4.47±0.33	2.04±0.16	3.14±0.14

Means ± S.E.M. (Student's t test)

In addition, no differences between the groups were observed in the number of mature spermatids found in the testis and the number of spermatozoa found in epididymal caput and corpus/cauda in relation to DSP and period of sperm transit time at PND 60 or PND 90. However, there was a significant diminished sperm count in the epididymal corpus/cauda of the experimental males at PND 90 when compared to the control group, as presented in table 3.

Discussion and Conclusions

Previous study reported that *S. lycocarpum* ingested by pregnant rats at 10% in diet promoted weak maternal toxicity and light fetotoxic effects[6]. In addition, fetotoxicity was not able to impair gestation or intra-uterine fetuses' growth of the exposed adult offspring[7]. In the former study[6], it was suggested that *S. lycocarpum* may act by long-term effects of estrogens, promoting impaired female sexual behavior and impaired testicular development of the male adult rats exposed perinatally to the fruit. A subsequent study[7]demonstrated that no alterations in the fertility capability occur in perinatally exposed adults. However, impaired female sexual behavior was detected suggesting that *S. lycocarpum* acts as phytohormones, probably through estrogenic effect.

With the purpose to contribute with previous studies and to investigate the possible estrogenic activity of the fruits, directly sperm evaluation of the male offspring was performed in the present research. Our results suggest that the treatment did not impair gestation but reduced intrauterine fetuses growth since body weight and body length of the perinatally exposed males were inferior to control pups at PND 01. However, no alteration was observed in anogenital distance at PND 01 and testis descent day, as stated elsewhere[6].

It is possible that the decreased sperm count observed in the corpus/cauda epididymis of exposed male of the reduced period of sperm transit (in days) within this epididymal portion during the same moment of observation. However, if the testis presents normal sperm production and the epididymis shows both decreased corpus/

Table 3 - Sperm production and epididymal period of sperm transit of control and perinatally (GD 06-PND 07) exposed male rats at PND 60 and PND 90

	PND 60		PND 90	
	Control	Experimental	Control	Experimental
Spermatid number (x10^6/testis)	92.99±3.52	89.96±4.25	155.35±3.81	147.62±6.58
DSP (x10^6/testis/day)	15.24±0.58	14.75±0.70	25.47±.63	24.20±1.08
Corpus/cauda ESC (x10^6/organ)	48.26±3.84	44.30±6.99	192.83±6.09	142.54±5.73***
Caput ESC (x10^6/organ)	57.08±3.38	50.97±1.95	89.05±4.71	95.50±4.77
Period of epididymal sperm transit (days)				
Corpus/cauda	3.17±0.24	3.07±0.49	7.57±0.17	5.97±0.37*
Caput	3.77±0.23	3.56±0.28	3.50±0.19	3.96±0.18

Mean ± S.E.M.; *p<.05, ***p<.001 (Student's t test).
Differences are between control and experimental data in animals within the same moment of observation

cauda sperm count and delay in sperm transit, we can hypothesize that the sperm quality may be impaired as well, due to alteration at the epididymal sperm maturation. It is known that alterations in the gonadotropin-releasing hormone (GnRH) pituitary gland axis may alter spermatogenesis and/or spermatozoa transit through epididymis, since testosterone is responsible not only for testicular spermatogenesis but also for transportation of spermatozoa through epididymis, possible explaining the data obtained. No such results were previously observed in monkeys and dogs treated with solasodine. However, solasodine concentration in the unripe fruits employed in this experiment was lower than the dose of isolated solasodine administered in the referred studies, explaining the lack of alterations in testicular sperm count.

Finally, the normal testicular spermatid count and the daily sperm production observed here possibly explain the maintainence of fertility capability previously reported[6] even in the presence of testicular degeneration. We assume that the delay in epididymal corpus/cauda sperm transit is the major consequence of the decreased epididymal sperm count observed in this work. It is possible that the exposition of experimental males to this amount of the unripe fruits at gestation and beginning of lactation may disrupt the hypothalamus-pituitary gland axis by increasing epididymal contraction and consequently resulting in a reduced period of epididymal sperm transit, without impairment on testicular sperm production. It is known that the epididymal contraction is promoted by testosterone and that this androgen release is mediated by GnRH. The latter hormone, at hypothalamus, induces follicular stimulant hormone (FSH) and intersticial cell stimulant hormone (ICSH) synthesis at hypophysis. ICSH act at the Leydig intersticials cells inducing testosterone synthesis. This androgen contribute with sperm maturation and induce sperm transit through epididymis, being GnRH dependent.

It is important to emphasize that the reduction in sperm count and transit time is not a direct measure of fertility, unless a very drastic effect had been induced[10]. It is known that in some strains of rats and mice, sperm production can be reduced by 90% without compromising fertility. However, less severe alteration can have dramatic consequence for men whose function nears the threshold for reproductive competence in terms of sperm count[11,12].

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Alterações reprodutivas em ratos machos expostos perinatalmente aos frutos da *Solanum lycocarpum*

Resumo

Ratas prenhes foram tratadas do dia 06 da gestação (GD 06) ao dia 07 pós-natal (PND 07) com frutos verdes secos e moídos da *Solanum lycocarpum* (10% na ração). Após nascimento das ninhadas, foi avaliado na prole masculina adulta aos 60 e 90 dias de vida, o número de espermátides e a produção espermática diária nos testículos e o tempo de trânsito espermático no epidídimo. A exposição não foi capaz de promover alterações na produção espermática diária (DSP) e no

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número de espermátides produzidas pelo testículo dos ratos expostos aos frutos verdes da *S. lycocarpum* durante a gestação e início da lactação. Não foram observadas alterações no tempo de trânsito espermático na cabeça do epidídimo, porém, foi constatado menor número de espermatozóides no corpo/cauda do epidídimo nos machos experimentais com 90 dias de vida, provavelmente resultante do menor tempo de trânsito espermático observado no corpo/cauda do epidídimo aos PND 90. Estes dados sugerem que a exposição de ratos aos frutos verdes da *S. lycocarpum* durante a gestação e início da lactação, não foi suficiente para promover alterações na produção mas sim no trânsito espermático, indicando possível alteração no eixo hormônio liberador das gonadotrofinas hipotálamo-hipófise-gônada.

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