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Uma investigação experimental sobre *Luffa operculata* (L.) Cogn. (buchinha-do-norte) como planta abortiva

Sofia Louise Santin Barilli, Sílvia Tonial dos Santos, Tatiana Montanari



Sofia Louise Santin Barilli

Sofia Louise Santin Barilli é enfermeira, graduada pela Universidade Federal do Rio Grande do Sul (UFRGS) em 2007. Durante a carreira acadêmica, dedicou-se à pesquisa científica, tendo publicado um artigo completo e 35 artigos resumidos em anais de congressos ou revistas científicas. Desenvolveu este trabalho como orientada de Iniciação Científica da Profa Dra Tatiana Montanari, coordenadora do Laboratório de Biologia da Reprodução, que tem como linha de pesquisa o “Efeito de plantas medicinais utilizadas como reguladoras da fertilidade sobre a reprodução e o desenvolvimento embrionário e fetal”.

ABSTRACT

OBJETIVES: The concentrated decoct of *Luffa operculata* (buchinha-do-norte) is taken as an abortifacient. This study aims to verify its action on gestation and to identify estrogenic and teratogenic activities.

METHODS: The fruit decoct (4mL/kg/day) was administered orally to mice from the first to the third gestation day – preimplantation period, from the fourth to the sixth gestation day – implantation period, or from the seventh to the ninth gestation day – when organogenesis and placentation start. The females were killed on 18 day gestation. The number of corpora lutea, implantation sites, reabsorptions and fetuses were recorded. The fetuses were weighed and examined to malformations and skeletal anomalies. To evaluate an estrogenic activity by *L. operculata*, sexually immature females received the decoct for three days, and the uteri were collected and weighed.

RESULTS: There was a significant reduction on the birth rate when the administration was from the fourth to the sixth gestation day, suggesting an antiimplantation effect. In this same group, many fetuses presented a retarded bone development. The decoct did not induce uterotrophic effect.

CONCLUSIONS: *L. operculata* might be abortive by an antiimplantation effect, which is not caused by an estrogenic activity. Furthermore, it impaired fetal growth.

KEYWORDS: *Luffa operculata*; Cucurbitaceae; medicinal plant; abortifacient agent; pregnancy; mice

Introduction

Luffa operculata (L.) Cogn., known popularly in Brazil as “buchinha-do-norte”, is used for sinusitis and allergic rhinitis, causing frequently nausea, vomiting and haemorrhage as side effects. Despite the intoxication risk, the concentrated fruit decoct is taken to induce illegal abort^{1,2}. Other species from Cucurbitaceae family are also used for this intent, and the mechanisms of action include contractions of the uterine muscle, decrease of progesterone level, suppression of the decidual reaction, placenta necrosis, and embryotoxic and teratogenic effects³⁻¹³. To verify if *L. operculata* is an abortifacient agent, this investigation was made.

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Material and methods

Preparation of the decoct

Dried *L. operculata* fruits were acquired at the Public Market at Porto Alegre, RS, Brazil. The decoct was made boiling one fruit (weighing 2.165 g) in 350 mL of distilled water during 10 min. It was kept frozen until ready for use. To establish the solid content, 98 mL of decoct were lyophilized: 1 mL of decoct was equivalent to 0.755 mg of dry matter.

Animals

Adult, 2- to 3-month-old, and young, 23- to 24-day-old, *Mus domesticus domesticus* CF1 mice were used. They were obtained from State Foundation of Production and Research in Health (Porto Alegre, RS, Brazil) and kept in the Department of Morphological Sciences of the Federal University of Rio Grande do Sul at 20-25°C and a 12-h light/12-h dark cycle. The animals were fed with Nuvilab Cr-1® rat food (Nuvital, Colombo, PR, Brazil) and water ad libitum.

All procedures were performed in accordance with the ethical principles for animal research adopted by the Brazilian College of Animal Experimentation.

Dose and route of administration

The females of the treated groups received 4 mL/kg per day of decoct, thus 3 mg of dry matter per kg body weight. The control groups received the same dose of distilled water. The administration was oral, using a curved needle and a tuberculin syringe.

Abortive activity

The adult females received the decoct or the distilled water from the first to the third gestation day (gd) (pre-implantation period), from the fourth to the sixth gd (implantation period), or from the seventh to the ninth gd (post-implantation period, when organogenesis and placentation start). The first gd was the day on which the vaginal plug was observed. Each group contained 10 animals. The animals were weighed on the first gd, on the first day of administration, on the day after the last dose, and on the 18th gd, when they were killed by cervical dislocation. The ovaries were collected and weighed. Its corpora lutea (or albicans) were counted under a stereomicroscope. The uterus was opened for counting of live and dead fetuses, degenerated embryos and late reabsorptions. After it was incubated in 10% ammonium sulfide for 10min for counting of implantation sites and early reabsorptions¹⁴. The placentae and the live fetuses were weighed. The fetuses were examined for external malformations and fixed either in Bouin's fluid for posterior analysis of internal malformations¹⁵ or in 95% ethanol for staining with alizarin red S¹⁶ and identification of skeletal anomalies, observation of the skull plates and counting of metacarpals, metatarsals, sternbrae and xiphisternum, ribs, lumbar vertebrae, and sacral and caudal vertebrae.

Estrogenic activity

Sexually immature females received decoct or distilled water for three days (10 animals/group). If the vagina was opened, smears were taken and stained by Shorr's technique¹⁷. The females were weighed on the first day of administration and on the day after the last dose, when they were killed, and uteri were collected and weighed¹⁸.

Statistical analysis

The body, organs and fetus' weight and the number of corpora lutea, implantation sites and live fetuses were expressed as mean \pm standard deviation and analyzed by Student's *t* test. The number of reabsorptions, degenerated embryos and dead fetuses, and the reproductive indices were expressed as median and interquartile range, and analyzed by Mann-Whitney U test. This test was also used to skeletal and teratological data¹⁹⁻²¹. A probability level of less than 5% was considered significant.

Results

Abortive activity

The effect of the *L. operculata* decoct on the body weight can be observed in Tables 1, 2 and 3. The females treated from the fourth to the sixth gd and from the seventh to the ninth

gd did not gain weight during the period of administration, resulting a significant difference between the weight of the group treated from the fourth to the sixth gd and the respective control. The significant difference between the weight of the treated and control groups on the 18th gd in this experiment is due to three treated females without fetuses.

Table 1 - Effect of the *L. operculata* decoct administered from the first to the third gd on body weight.

Group	Bodyweight (g)		
	1st gd	4th gd	18th gd
Treated	30.29 \pm 2.21	31.03 \pm 2.19	50.03 \pm 11.24
Control	30.16 \pm 1.62	30.87 \pm 1.85	55.85 \pm 3.71

Table 2 - Effect of the *L. operculata* decoct administered from the fourth to the sixth gd on body weight.

Group	Bodyweight (g)			
	1st gd	4th gd	7th gd	18th gd
Treated	29.88 \pm 1.23	31.09 \pm 1.21	31.50 \pm 1.24*	43.62 \pm 9.75*
Control	30.87 \pm 1.71	31.53 \pm 1.93	33.35 \pm 2.0	53.49 \pm 4.25

* Significance relative to control: $p < 0.05$ by Student's *t* test

Table 3 - Effect of the *L. operculata* decoct administered from the seventh to the ninth gd on body weight.

Group	Bodyweight (g)			
	1st gd	7th gd	10th gd	18th gd
Treated	37.51 \pm 3.44	39.56 \pm 4.11	39.87 \pm 5.05	57.08 \pm 11.34
Control	37.75 \pm 2.28	39.97 \pm 2.08	41.51 \pm 2.81	58.40 \pm 8.25

The effect of *L. operculata* on the reproductive parameters and rates is shown in Tables 4 and 5. In spite of not being statistically significant there was a decrease in the number of implantation sites and live fetuses in the group treated from the fourth to the sixth gd. It was due to three females without implantation, and it caused a lower implantation rate and a significant decrease in the birth rate. It is noteworthy that a female of this group had 11 reabsorptions, and the four fetuses were in the same uterine horn. In the group with administration from the first to the third gd there was a female with implantation only in a uterine horn. It had four fetuses and two reabsorptions, but its ovaries presented 15 corpora lutea.

Ovary, placenta and fetus' weight are exhibited in Table 6. There was a significant difference between the ovary weight of the groups treated from the seventh to the ninth gd and control, but it probably does not have functional meaning.

About skeletal examination, in the group treated from the fourth to the sixth gd, of 35 analyzed fetuses, 11 presented a retarded bone development, with absence of ossification of the supraoccipital plate and delay in the ossification of the interparietal plate, metacarpals, metatarsals, sternbrae, and sacral and caudal vertebrae. But in the control group, of 55 analyzed fetuses, no one had this retarded growth.

Some malformations were observed in the group treated from the seventh to the ninth gd, the period when the organogenesis begins. An interesting find was a fetus very small, with 433 mg, exhibiting agnathia and exophthalmia. Two fetuses had cleft palate and one fetus had exencephaly, but these malformations were found also in the control groups.

Table 4 - Effect of the *L. operculata* decoct on reproductive parameters.

Group	Corpora lutea	Implantationsites	Reabsorptions	Degenerated/dead fetuses	Livfetuses
Treated					
1st to 3rd gd	15.0±2.49	12.7±5.93	1[0-2]	0[0-0]	10.1±6.66
4th to 6th gd	15.8±2.04	10.0±7.06	0.5[0-3]	0[0-0]	7.3 ± 6.13
7th to 9th gd	18.3±1.49	13.9±5.95	2[0-3]	0[0-0]	11.8±5.35
Control					
1st to 3rd gd	16.7±2.41	15.7 ± 2.0	1[0-1]	0[0-1]	14.2±1.55
4th to 6th gd	15.2±1.32	14.1±2.13	2[1-3]	0[0-0]	11.9±3.48
7th to 9th gd	17.4±1.43	12.6±5.44	1[1-2]	0[0-1]	11.0±5.21

^a Implantation rate = no. of implantation sites/no. of corpora lutea x 100;

^b Reabsorption rate = no. of reabsorptions/no. of implantation sites x 100;

^c Birth rate = no. of live fetuses/no. of corpora lutea x 100;

* Significance relative to control: p = 0.017 by Mann-Whitney U test.

Table 5 - Effect of the *L. operculata* decoct on reproductive rates.

Group	Implantation rate ^a (%)	Reabsorption rate ^b (%)	Birth rate ^c (%)
Treated			
1st to 3rd gd	100[81.8-100]	8.9[0-23.5]	82.7[26.7-93.3]
4th to 6th gd	79.5[0-100]	3[0-18.8]	51.0[0-76.5]*
7th to 9th gd	85.7[76.2-90]	14.2[0-21.4]	73.0[61.1-76.5]
Control			
1st to 3rd gd	97.2[88.9-100]	6.7[6.3-8.3]	86.7[78.9-88.9]
4th to 6th gd	100[93.3-100]	13.3[5.6-30]	84.0[60-94.4]
7th to 9th gd	85.4[56.3-100]	7.7[5.6-17.6]	75.8[43.8-86.7]

^a Implantation rate = no. of implantation sites/no. of corpora lutea x 100;

^b Reabsorption rate = no. of reabsorptions/no. of implantation sites x 100;

^c Birth rate = no. of live fetuses/no. of corpora lutea x 100;

* Significance relative to control: p = 0.017 by Mann-Whitney U test.

Estrogenic activity

The decoct did not induce neither a premature opening of the vagina, nor an uterotrophic effect (Table 7).

Discussion

There are reports about adverse effects by *L. operculata*, such as nausea and vomiting. In this study, toxicity is also suggested because the gain weight was impaired in some animals, principally when treated from the fourth to the sixth gd. In this group of administration, some females had few or no fetus, reducing significantly the birth rate. As implantation occur in this period, *L. operculata* probably affected this process. This result was not due to an estrogenic action because the decoct was not uterotrophic. Various species of Cucurbitaceae possess abortive properties. *Lagenaria breviflora* Robert fruit, used as an abortifacient in Nigeria, demonstrated antiimplantation and oxytocic activities⁵⁹.

The aqueous extract of *Luffa cylindrica* M. Roem promoted also contractions of the rat isolated uterus³, and proteins from its seeds inhibited the biosynthetic activity of implanting embryos and endometrial cells¹¹. The aqueous extract of *Momordica angustisepala* root produced contractions on guinea pig isolated uterus⁶.

β-momorcharin, a glycoprotein found in the seeds of *Momordica charantia* L., injected intraperitoneally to mice on the fourth gd and on the sixth gd, resulted 60% of females without

Table 6 - Effect of the *L. operculata* decoct on ovary, placenta and fetus' weight.

Group	Ovary weight (mg)	Placenta weight (mg)	Fetus weight (mg)
Treated			
1st to 3rd gd	13.35±2.46	98.58±11.75	831.28±65.50
4th to 6th gd	12.55±1.69	103.46±19.33	839.2±156.16
7th to 9th gd	13.15±1.78*	101.17±10.97	859.66±141.12
Control			
1st to 3rd gd	13.55±2.54	100.08±5.10	870.18± 115.75
4th to 6th gd	13.65±2.0	105.32±9.63	862.5±103.81
7th to 9th gd	15.9±2.53	103.57±8.93	882.84±104.39

* Significance relative to control: p = 0.011 by Student's *t* test.

Table 7 - Effect of the *L. operculata* decoct on corporal and uterine weight of immature females.

Group	Initial body weight (g)	Final body weight (g)	Uterine weight (mg)	Relativeuterine weight (mg/100g)
Treated	11.44±3.02	13.83±2.92*	11.30±3.68	81.19±19.34
Control	11.40±3.21	14.41±3.65*	12.30±4.0	84.68±20.45

*Significance relative to initial body weight: p < 0.05 by Student's *t* test.

implants. When injected on day 8 of pregnancy, it had not significant effect. In vitro studies showed that the protein disturbed peri-implantation development by blocking the hatching of embryos from the zona pellucida, decreasing the attachment of the blastocyst, reducing the trophoblast outgrowth, disrupting the development of inner cell mass, and suppressing the decidual response. An interaction of the unfavourable uterine environment with poorly developing embryos might be the cause of the termination of early pregnancy by this substance^{7,8}. These mechanisms might also be responsible by antiimplantation effect of *L. operculata*.

Other important findings were delayed ossification in fetuses which females received the decoct in the implantation period, and the small fetus with facial anomaly and exophthalmia from the group treated in the early organogenic period. Luffin-c, a protein isolated from *Luffa aegyptiaca* seeds, affected the development of mouse embryos in culture. About 40% of the embryos did not have an active yolk sac circulation and most of the abnormal embryos had a shorter body length¹³. Momorcharins were teratogenic to the cultured mouse embryos at the early organogenesis stage. Morphological abnormalities were seen in the head, trunk

and limbs. Growth of the embryos was also retarded. The retarded growth was related to an inhibitory action on protein synthesis in the embryonic tissue and the yolk sac¹⁰.

In conclusion, *L. operculata* might be abortive by an antiimplantation effect, which is not caused by an estrogenic activity. Furthermore, it impaired fetal growth.

Acknowledgments

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Resumo

Objetivos: O decocto concentrado de *Luffa operculata* (buchinha-do-norte) é tomado como um abortivo. Este estudo tem como objetivo verificar seu efeito sobre a gestação e identificar atividades estrogênica e teratogênica.

Material e métodos: O decocto dos frutos (4mL/kg/dia) foi administrado oralmente a camundongas do primeiro ao terceiro dia de gestação – período pré-implantação, do quarto ao sexto dia de gestação – período da implantação, e do sétimo ao nono dia de gestação, quando inicia a placentação e a organogênese. As fêmeas foram sacrificadas no 18º dia de gestação. O número de corpos lúteos, sítios de implantação, reabsorções e fetos foram registrados. Os fetos foram pesados e examinados para malformações e anomalias esqueléticas. Para avaliar uma atividade estrogênica por *L. operculata*, fêmeas imaturas sexualmente receberam o decocto por três dias, e o útero foi coletado e pesado.

Resultados: Houve uma redução significativa no índice de natalidade quando a administração foi do quarto ao sexto dia de gestação, sugerindo um efeito antiimplantação. Neste mesmo grupo, muitos fetos apresentaram um desenvolvimento ósseo retardado. O decocto não teve um efeito uterotrófico.

Conclusões: *L. operculata* pode ser abortiva por um efeito antiimplantação, que não foi causado por atividade estrogênica. Além disso, ela prejudicou o crescimento fetal.

UNITERMOS: *Luffa operculata*; Cucurbitaceae; plant medicinal; agente abortivo; gestação; camundongo.

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