

Analysis of the abortive and/or infertilizing activity of *Stryphnodendron adstringens* (Mart. Coville)

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Investigação da atividade abortiva e/ou infertilizante de *Stryphnodendron adstringens* (Mart. Coville)

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SUMMARY

Stryphnodendron adstringens (barbatiman) is a tree found from Pará to Mato Grosso do Sul and São Paulo States (Brazil). The aim of this study was to determine the toxicity of the extracts of the broad beans of this species and to verify if they have some effect on the pregnancy of rats. The broad beans were collected in the region of Cuiabá (MT, Brazil) and separated in husks and seeds. The crude hydroalcoholic (2:1) extracts were prepared at room temperature and dried at a maximum of 55°C. Female virgin rats were mated and received the extracts (0.5 ml/100 g weight, 100 g/l) or water in the same proportion (control) by gavage from day 1 to day 7 of gestation. Laparotomies were made at day 7 to count the number of uterine implants and the rats were sacrificed on the 21st day of gestation. The seed extracts reduced the weight of the uterus and the number of live fetuses compared to the control group. The mean lethal dose (LD₅₀) calculated for this extract was 4,992.8 mg/kg and the LD₅₀ of the extract of the husk was higher than 5,000 mg/kg. We may conclude that the extract of *S. adstringens* seeds impaired the gestation of rats, and its ingestion could be harmful to herbivorous animals.

UNITERMS: Infertility; Poisonous plants; *Stryphnodendron adstringens*.

INTRODUCTION

The genus *Stryphnodendron* Mart., *Leguminosae*, subfamily *Mimosoideas*, has 12 species in Brazil, North of Paraguay and other South American countries¹. *Stryphnodendron adstringens* (Mart. Coville) is a tree from the "cerrado", occurring in Brazil from Pará to Mato Grosso do Sul and São Paulo⁷. It is commonly known as "barbatimão" (barbatiman), "barbatimão-verdadeiro", "barbade-timão", "chorãozinho-roxo", and "casca-da-virgindade". This species has the following synonymy: *S. barbatiman* Mart., *Mimosa barbadetiman* Vell., *S. obovatum* (Menth.). The tree is 4-5 m high, has a trunk 20-30 cm in diameter, and has bipinnate leaves, with 5-8 leaflets. The wood is used for construction and the bark has a high level of tannin⁷. The husks and seeds are considered toxic^{7,11}.

The broad beans of this plant are commonly known by cattle breeders and technicians to have an abortive and/or

infertilizing activity. Consequently, the aim of this study was to determine the toxicity of the extracts of the broad beans of this specie and to verify its effect on the pregnancy of rats.

MATERIAL AND METHOD

Extracts

The broad beans of *S. adstringens* were collected in the region of Cuiabá (Mato Grosso, Brazil) and separated into husks and seeds. These portions were maintained in a ventilated oven at a temperature of less than 40°C for drying and stabilization. The material was then pulverized in a Tigre A-3 mill and stored in brown glass bottles.

The crude extracts were prepared with 50 g of seeds or husks (empty) immersed in 500 ml of hydroalcoholic solution (2:1) at room temperature for 24-48 h, with occasional stirring. The mixture was then filtered through Selecta 589³ filter paper

and submitted to a new extraction to obtain adequate drainage of the active principles of the plant. The filtrates were concentrated in a Büchi rotary evaporator at a maximum temperature of 55°C, and left in a ventilated oven at less than 40°C till the weight remained unchanged. The dried residue was scraped and stored in a brown glass bottle under refrigeration.

Abortive and/or infertilizing activity

Twenty-five female virgin rats (90-120 days) were placed separately in cages with males (3 females to 1 male) for mating. The presence of spermatozoa in the vaginal fluid of the female rats was analyzed daily to determine if they had copulated. The day when spermatozoa were found was considered the first day of pregnancy. The pregnant rats were weighed and divided at random into three groups, receiving respectively by gavage the seed extract 1 ml/100 mg of the extract, the husk extract (500 mg/kg live weight), or water (control) from day 1 to day 7 of pregnancy. Body weight and food and water consumption were measured every two days. On the seventh day of pregnancy the animals were anesthetized with ethyl ether and laparotomized to count the number of uterine implants. The rats were sacrificed on the 21st day of pregnancy. The uterus was separated and weighed and the fetuses were removed to evaluate the following parameters: number of live and dead fetuses, macroscopic alterations (presence of normal head, tail and paws), and number of corpora lutea. Comparisons of these parameters between the experimental and control groups were made by one-way analysis of variance and the Duncan test with the aid of the

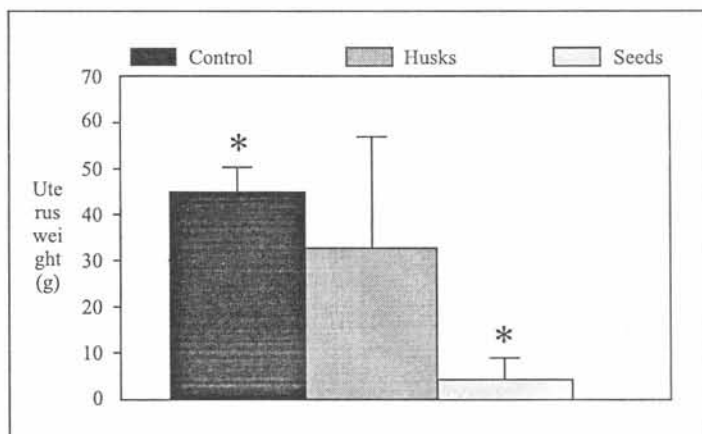


Figure 2

Effect of the extracts of seeds and husks of *S. adstringens* on the uterus weight of female rats.

* Significantly different from the control group ($p < 0.05$).

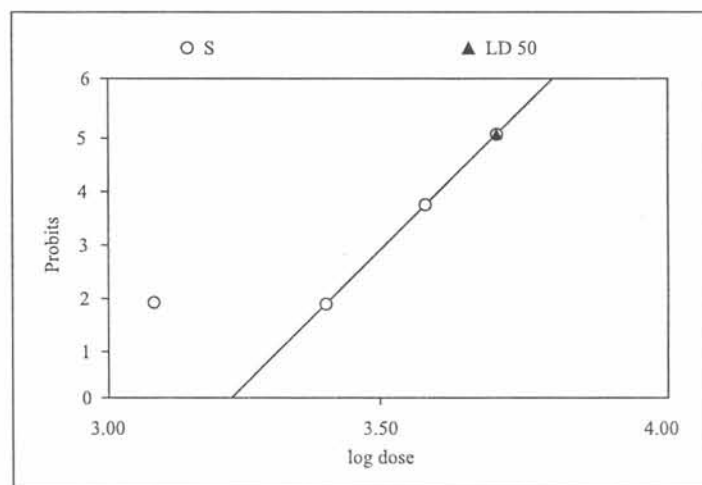


Figure 3

Mortality of mice submitted to oral administration of extracts seeds of *S. adstringens*. Number of animals per group: 10. Confidence interval (4,331.7 - 20,720.4 mg/kg).

SPSS program (version 1986). All values were expressed as mean \pm SE, and the minimum significant level was $p < 0.05$.

Oral acute toxicity (method adapted from Heuvel, 1990)

Fifty adult mice of both sexes (19.7 ± 0.4 g), received by gavage one dose of *S. adstringens* seed or husk extract (1,250; 2,500; 3,750; or 5,000 mg/kg live weight) after a 24 h fast. All doses were calculated on the basis of previous experiments and all animals received 0.1 ml/10 g live weight. The same procedure was applied to the control group, but the animals received water instead of the extracts. After the

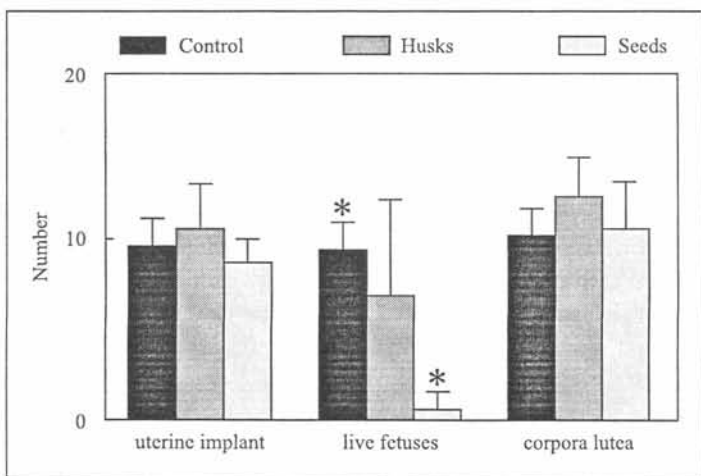


Figure 1

Effect of the extracts of seeds and husks of *S. adstringens* on the number of uterine implants (UI), live fetuses (LF) and corpora lutea (CL) of female rats.

* Significantly different from the control group ($p < 0.05$).

Table 1

Uterine implants, live fetuses, and corpora lutea of control, husks, and seeds (number). (Data as mean \pm S.E.M.)

	control	husks	seeds
uterine implant	9.6(\pm 1.52)	10.56(\pm 2.45)	8.71(\pm 1.25)
live fetuses	9.4(\pm 1.52)	6.89(\pm 5.25)	0.57(\pm 0.98)
corpora lutea	10.2(\pm 1.48)	12.33(\pm 2.12)	10.57(\pm 2.57)

Table 2

Uterus weight of control, husks and seeds(g). (Data as mean \pm S.E.M.)

	control	husks	seeds
Uterus weight	44.94 (\pm 5.12)	32.67 (\pm 24.00)	4.19 (\pm 4.86)

administration the mice were given food and water *ad libitum* at a temperature of 25°C. General toxic signs and effects on locomotion, behavior, and respiration were observed after administration. The mortality rate was observed for 48 hours. The median lethal doses (LD_{50}) and the equations (administered doses \times mortality) for each extract were calculated with the aid of the statistical program "CL₅₀". The logarithms and the probits were determined for all doses according to Carlini².

RESULTS

The administration of the seed extract reduced the number of live fetuses and the weight of the uterus of female rats compared to the control group, but other parameters (body weight, food and water consumption, number of uterine implants and corpora lutea), (Tab. 1 and 2) remained unchanged. The husk extract did not alter any parameter compared to the control group. These results are illustrated at Fig. 1 and 2.

The oral administration of *S. adstringens* seed extracts induced the death of mice at the highest dose. The LD_{50} calculated for this extract was 4,992.8 mg/kg (Fig. 3). However, the LD_{50} of the husk extract was not determined because doses beyond 5,000 mg/kg (PO) are considered unreliable⁶. No alteration in respiration, locomotor activity or general behavior was observed after administration of *S. adstringens* seed or husk extracts.

The following equation was fitted to the seed extract:

$$y = -33.74 + 10.47x \quad (p < 0.05, r^2 = 0.998)$$

where y = probits and x = logarithm of the dose.

DISCUSSION

Administration of the hydroalcoholic extract of *S. adstringens* seeds from the first to the seventh day of pregnancy reduced the number of live rat fetuses. Since embryo implantation occurs during this period in rats⁵, it is possible that this reduction was due to interference of the extract with embryo implantation. The reproduction of rats was also impaired by the ingestion of seeds of *Stryphnodendron polyphyllum* M., which increased the reabsorption of embryos¹², and induced abortion when administered during the implantation period (5th - 6th days of gestation) or just after implantation (7th - 8th day of gestation)⁴. Considering that the extract of *S. adstringens* was administered during this period, the abortive effect of this plant may occur during the implantation period or shortly thereafter. The extract of seeds (72 mg/kg) of another species of this genus, *S. obovatum*, inhibited fetal growth and changed the development of bones and nervous system when administered on days 6, 10, and 16 of pregnancy³.

The ingestion of *S. polyphyllum* seeds reduced ovarian weight; the administration of both the plant and progesterone did not alter the number of reabsorbed embryos, indicating that the embryotoxic effect was not due to a deficit in progesterone level. Consequently, Vitral *et al.*¹² proposed that the effect of the plant was due to an alteration of the basal region of the placenta, which would cause the death of the embryo and atrophy of the corpus luteum. The effect of *S. adstringens* may follow the same mechanism, since its effects are comparable to those of *S. polyphyllum*.

The oral ingestion of the extract of *S. adstringens* seeds caused the death of mice at the highest dose, indicating that this plant is toxic. In addition, these animals also presented

malnutrition, dehydration, edema, leucopenia, lymphopenia, higher levels of TGO (AST), bilirubin, phosphorus, urea, dextrose, and lower levels of serum calcium than control animals. These symptoms were attributed to the high levels of tannins (12%) of this plant^{8,9}. The oral administration of the broad beans (husks and seeds) of *S. coriaceum* to cattle caused irritation of the gastrointestinal tract, hepatic lesions, and

photosensitization, i.e. increased sensitivity of the skin of the animals to light, sometimes inducing drought gangrene, with death of the animals within 3 to 23 days, according to the dose¹⁰. Consequently, it is recommendable to prevent domestic animals from ingesting the beans of this plant, because this would probably cause some deaths, or at least a reduction of productivity.

RESUMO

Stryphnodendron adstringens (barbatimão) é uma árvore natural dos Estados do Pará ao Mato Grosso do Sul e São Paulo (Brasil). O objetivo deste estudo foi determinar a toxicidade dos extratos das vagens desta espécie e verificar efeitos sobre a gestação de ratas. As vagens foram coletadas na região de Cuiabá (MT, Brasil) e separadas em cascas e sementes. Foram feitos extratos brutos hidroalcoólicos (2:1), a frio e depois levados à secura (máximo 55°C). Ratas fêmeas virgens foram acasaladas e receberam extratos (0,5 ml/100 g de peso, 100 g/l) ou água na mesma proporção (controle) por gavagem do 1º ao 7º dia de gestação. No 7º dia, foram feitas laparotomias para contagem do número de implantes uterinos. As ratas foram sacrificadas no 21º dia de gestação. Os extratos das sementes reduziram o peso do útero e o número de fetos vivos em relação ao grupo controle. A dose média letal (DL₅₀) calculada para o extrato das sementes foi de 4.992,8 mg/kg. A DL₅₀ do extrato das vagens foi maior que 5.000 mg/kg. Pode-se concluir que o extrato das sementes de *S. adstringens* interfere na gestação de ratas, sendo que esta ingestão pode prejudicar animais herbívoros.

UNITERMOS: Infertilidade; Plantas tóxicas; *Stryphnodendron adstringens*.

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