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Evaluation of reproductive toxicity of aqueous extract of the fruits from *Caesalpinia ferrea* Mart. in rats

[Evaluación de la toxicidad reproductiva de extracto acuoso de los frutos de *Caesalpinia ferrea* Mart. en ratas]

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Abstract

Caesalpinia ferrea Mart. (Caesalpinaceae) presents therapeutic potential as antifungal, antiulcer, antiinflammatory, analgesic, and antineoplastic. In the present paper, the toxicity of *C. ferrea* administered to female rats during the period of blastocyst implantation (5th to 7th day of pregnancy) was evaluated. Thirty inseminated Wistar rats were randomly distributed into Control and Treated groups, which received, by gavage, 0.5 mL of distilled water and 300 mg/kg body weight of *C. ferrea,* diluted in 0.5 mL of distilled water, respectively. Maternal toxicity was indirectly assessed through: body weight, food and water intake, locomotor activity, piloerection, diarrhea, vaginal bleeding, liver and kidneys weight, and deaths. Rats were killed on the 15th day of pregnancy, when fetuses and placentae were removed and weighed. The number of corpora lutea, implantations, resorptions, and living and dead fetuses were recorded. The index of implantation and the proportion of resorptions were calculated. None of the variables analyzed showed significant differences. In the dose administered in the experimental model used, *C. ferrea* does not seem to be toxic to the mother nor interfere with blastocyst implantation.

Keywords: *Caesalpinea ferrea Mart., Embriotoxicity, Implantation, Blastocyst, Rats.*

Resumen

Caesalpinia ferrea Mart. (Caesalpinaceae) presenta un potencial terapéutico como anti-fúngico, anti-úlcera, antiinflamatorio, analgésico, y anti-neoplásico. En el presente artículo, la toxicidad de *C. ferrea* fue evaluada después de la administración a las ratas durante el período de implantación del blastocisto (5 a 7 día de embarazo). Treinta ratas Wistar inseminadas fueron aleatoriamente distribuídas en dos grupos: control y tratado y fueron alimentadas a través de una sonda con 0.5 mL de agua destilada y 300 mg/kg de extracto de *C. ferrea*, diluído en 0.5 mL de agua destilada, respectivamente. La toxicidad maternal se evaluó indirectamente a través de: el peso del cuerpo, consumo de comida e ingestión de agua, actividad locomotora, piloerección, diarrea, sangramiento vaginal, peso de los hígados y riñones y muertes. En el décimo quinto día de embarazo las ratas fueron sacrificadas, cuando los fetos y placentas fueron removidos y pesados. El número de los cuerpos luteos, implantaciones, la reabsorción, y fetos muertos y vivos fueron anotados. Se calculó el índice de implantación y la proporción de reabsorción. Ninguna de las variables analizadas mostró diferencias significativas. En la dosis administrada en el modelo experimental usado, *C. ferrea* no parece ser tóxico a la madre ni interferir con la implantación del blastocisto.

Palabras clave*: Caesalpinea ferrea Mart., Embriotoxicidad, Implantación, Blastocistos, Ratas.*

INTRODUCTION

Caesalpinia ferrea Mart. (Caesalpinaceae) is a large tree distributed in the northern and northeastern regions of Brazil, specially Pernambuco and Ceará states, where it is commonly known as "Jucá or Pau-ferro" (Alzugaray, 1983). The folkloric use of this plant led various researchers to investigate its properties. It has been reported that "Jucá" might be able to relieve diabetic complications (Ueda et al., 2004). It also showed antifungal (Lima et al., 1997), antiulcer (Bacchi, 1986; Bacchi and Sertie, 1994; Bacchi et al., 1995), as well as antiinflammatory and analgesic properties in a crude aqueous extract of its fruits (Almeida, 1993; Carvalho et al., 1996). Treatment of infected and tumor-bearing mice with *C. ferrea* aqueous extract significantly stimulated myelopoiesis, which is an important factor to mediate the early immunological response (Queiroz et al., 2001). It was also demonstrated that gallic acid and methyl gallate, isolated from fruits of *C. ferrea*, significantly decreased the average number of papillomas per mouse in an

experiment on skin tumor formation in mice initiated with 7,12-dimethylbenz[α]anthracene (DMBA) (Nakamura et al., 2002).

Regarding its toxicity, Bacchi et al., (1995) demonstrated that there was no difference between control and 'Juca' treated rats, when it was administered during 30 days (subchronic toxicity), and assessed through body weight variation, water and food consumption and spleen, lung, kidney and liver relative weights. However, there are no studies on its reproductive toxicity. In the present study we aim to assess the toxicity of the crude extract of *C. ferrea* administered to pregnant Wistar rats in the period of blastocyst implantation, in order to contribute to the studies about toxicological risks of human use.

MATERIALS AND METHODS

Plant material

The fruits were collected from May to June 1990 in the University of São Paulo (USP), Campus Ribeirão Preto. A voucher specimen (number 3221) collected by Dr. Antonio Barioni Guzman has been deposited in the herbarium (SPFR) of the Biology Department of the University of São Paulo, Campus Ribeirão Preto, São Paulo, Brazil. The lyophilized crude extract of *C. ferrea* was obtained from the Biopharmacy Laboratory of the University of Alfenas (Alfenas, Minas Gerais, Brazil).

The air-dried fruits were ground and 100 g of the powder was macerated with water at 60 ± 10°C for 2 h. After filtration, the extract should be lyophilized to yield 27.2 g of crude aqueous extract, and it should be standardized by the method suggested by Carvalho et al. (1996).

Animals

Thirty female Wistar rats, 3 months old and weighing 160-190 g, were obtained on the first day after insemination from the vivarium of the Biology of Reproduction Center (Federal University of Juiz de Fora, MG, Brazil). Animals were housed in individual cages, and were maintained on 12 h dark/light cycle in a room with controlled temperature, humidity and ventilation, according to the rules of the International Council for Laboratory Animals Science (ICLAS). Rats were mated with male of proven fertility and the presence of spermatozoa in the vaginal smear was considered the first day *post coitum*.

Test protocol

Pregnant rats were randomly divided into two groups: control (n = 15) and treated (n = 15). Animals from control group received 0.5 mL of distilled water and the treated rats received 300 mg/kg body weight of an aqueous crude extract of *C. ferrea* (corresponding to the dose used to assess its anti-inflammatory effect (Carvalho et al., 1996)). The extract was administered orally, during days five, six and seven of pregnancy, which corresponds to the period of blastocyst implantation.

To observe maternal toxicity, the following clinical criteria were adopted (Manson and Kang, 1994): body weight, food and water intake, alteration in locomotor activity, piloerection, diarrhea, vaginal bleeding and deaths.

Body weight was recorded on the 1st, 5th, 7th and 15th days post coitum. On the 15th day it was performed before and after removal of the reproductive tract.

During the experimental procedure, daily food intake was estimated by the weight difference between the pellet chow placed in the morning of first day and what was left in the morning of the following day. Water intake was calculated in the same way as described above.

Euthanasia was carried out by exsanguination under anesthesia (ketamine + xylazine) on the 15th day of pregnancy. After laparotomy, the maternal reproductive tract was removed. The ovaries were dissected out, weighed and the number of *corpora lutea* was recorded. The number of fetuses and resorptions in the uterine cornua was also recorded. The pre implantation loss [total corpora lutea – total implants/ total corpora lutea] x 100 and post implantation loss [total implants – total live fetuses / total implants] x 100 were calculated. Liver and kidneys were also weighed and fixed in buffered formalin for later histopathological analysis.

The fetuses and placentae were weighed and the former was observed for detection of external malformations (face and limb buds).

The test protocol was approved by the Ethical Committee of Federal University of Juiz de Fora (Protocol number 38/2003 - CEA), which is in accordance with the internationally accepted principles for laboratory animal use and care.

Statistical analysis

The results were expressed as mean ± standard deviation. Statistical analysis between treated and control groups were performed by Student's t-test for

independent samples or Mann Whitney test ($\alpha = 0.05$). Results with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Signs of indirect maternal toxicity such as alterations of locomotor activity, vaginal bleeding, diarrhea, piloerection, and deaths were not registered, and these results were similar to control group.

As outlined in Fig. 1, maternal body weight did not differ between the experimental groups. Food (Fig. 2) and water intake (data not shown) were similar between control and treated animals.

Body weight and the weight of maternal ovaries, kidneys and liver, did not differ between the experimental groups (Table 1).

The number of *corpora lutea*, live fetuses, resorptions, implantations, pre and post implantation losses in control and treated rats did not differ significantly (Table 2). The mean weight of fetuses (g) (C = 0.163 ± 0.014; T = 0.167 ± 0.001) and placentae (g) (C = 0.158 ± 0.020; T = 0.154 ± 0.030) were also not significantly different.

In embryotoxicity studies, it is necessary to separate direct toxicity on the fetus from secondary effects derived from maternal toxicity, since the latter alone is able to cause alterations in embryo morphogenesis (Khera, 1984). Signs commonly used to assess indirect maternal toxicity are: body weight decrease, food and water consumption, locomotor activity, piloerection, diarrhea, vaginal bleeding, weight of organs and deaths (Chahoud et al., 1999). None of those signs were significantly altered in control or *C. ferrea* treated rats, indicating that this drug does not seem to be toxic to mothers.

Number of *corpora lutea*, living fetuses, resorptions, implantations, as well as the percentage of pre and post implantation losses, were similar in control and treated rats. These data suggest that animals from all the experimental groups had a similar number of ovulations, similar pattern of *corpora lutea* growing, and similar hormonal and biochemical profile, allowing embryo to develop until implantation in the uterine cornua.

Fetal toxicity is mainly suggested by body weight reduction and an increase in the number of resorptions (Khera, 1984). As significant differences in placenta and fetal body weight were not observed, it can be assumed that there was no embryo toxicity.

Table 1. Maternal body and organ weights of control and *C. ferrea* extract treated rats.

Variables	Groups	
	Control	**Treated**
Body weight (BW) 1	169.08 ± 9.60	169.09 ± 9.00
BW 5	178.70 ± 9.30	176.28 ± 10.50
BW 7	179.86 ± 8.10	180.38 ± 9.20
BW 15[a]	197.35 ± 9.40	198.23 ± 8.60
Weight gain[b]	28.27 ± 14.60	29.15 ± 10.80
Right ovary	0.041 ± 0.010	0.043 ± 0.011
Left ovary	0.037 ± 0.010	0.040 ± 0.010
Right kidney		
Absolute weight	0.765 ± 0.070	0.784 ± 0.072
Relative weight	0.387 ± 0.026	0.395 ± 0.030
Left kidney		
Absolute weight	0.757 ± 0.095	0.780 ± 0.072
Relative weight	0.383 ± 0.043	0.394 ± 0.027
Liver		
Absolute weight	8.802 ± 0.881	9.210 ± 0.818
Relative weight	4.458 ± 0.377	4.647 ± 0.374

Weight is expressed in g (except the relative weight mg) as mean ± standard deviation. [a]Maternal body weight after removal of uterine horns and ovaries. [b]Difference between BW15 – BW1. $p > 0.05$ (n = 15).

Table 2. Number of corpora lutea, live fetuses, resorptions, implantations, pre and post implantation losses in control and treated rats.

Variables	Groups	
	Control	**Treated**
Corpora lutea		
Right ovary	6.20 ± 1.57	6.40 ± 1.40
Left ovary	5.60 ± 1.84	5.47 ± 1.06
Total	11.93 ± 0.92	11.73 ± 1.83
Live fetuses		
Right cornua	5.47 ± 1.73	5.80 ± 1.74
Left cornua	4.87 ± 1.76	4.73 ± 1.10
Total	10.36 ± 1.74	10.47 ± 1.99
Resorptions		
Right cornua	0.33 ± 0.72	0.53 ± 0.74
Left cornua	0.27 ± 0.46	0.20 ± 0.41
Total	0.60 ± 1.05	0.67 ± 0.90
Mean of implantations	10.93 ± 1.38	11.20 ± 1.47
Pre implantation loss %[a]	9.26 ± 10.36	7.16 ± 4.58
Post implantation loss %[b]	5.23 ± 10.33	7.01 ± 8.77

[a]One treated and six control animals with no pre implantation loss. [b]Eight treated and ten control animals with no post implantation loss. Data are expressed as mean ± standard deviation (n = 15).

Figure 1. Maternal body weight (g) of control (C) and *C. ferrea* treated (T) female rats body weight after removal of reproductive tract..

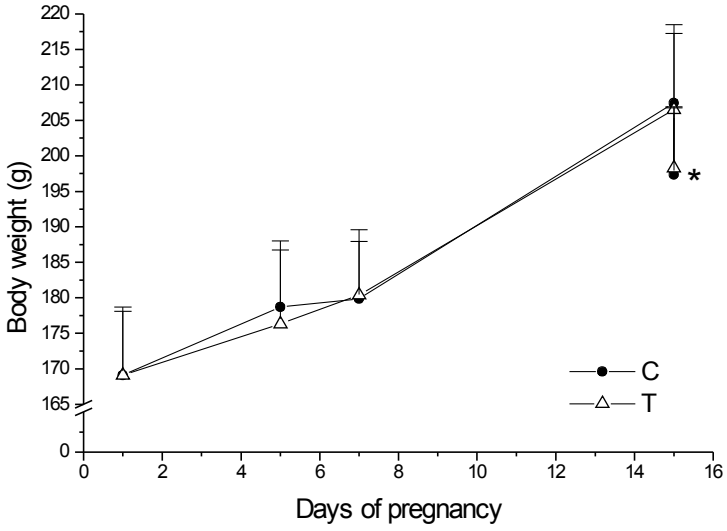
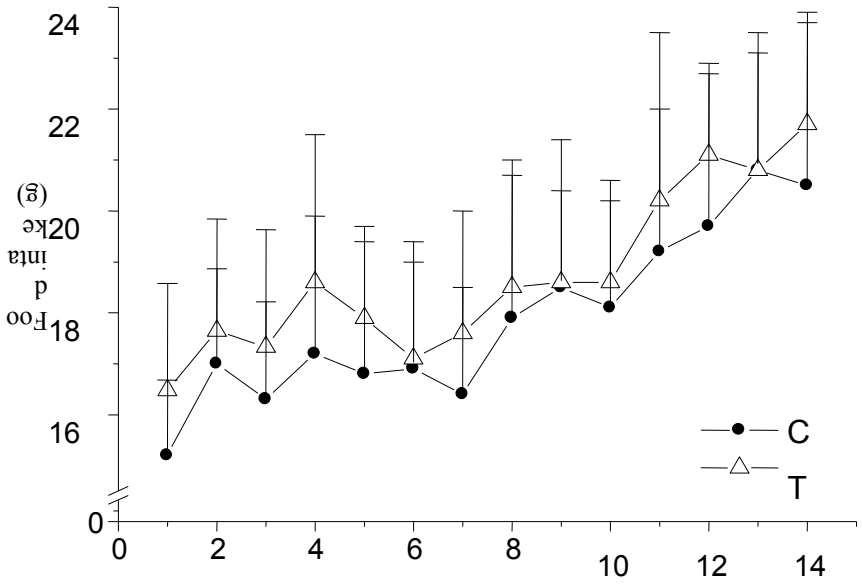


Figure 2. Daily food intake (g) of control (C) and *C. ferrea* treated (T) female rats ($p > 0.05$).



CONCLUSION

Considering the dose administered and the experimental model used, it is possible to suggest that *C. ferrea* does not alter the process of blastocyst implantation in rats.

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