Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
23(2): 374-378, Mar./Apr. 2013

Maternal exposure to *Cochlospermum regium*: a toxicological evaluation

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Short communication

Received 4 Oct 2012
Accepted 3 Dec 2012
Available online 1 Feb 2013

Keywords:
Cochlospermum regium
*medicinal plant*s
pregnant rat

ISSN 0102-695X
DOI 10.1590/S0102-695X2013005000005

Abstract: *Cochlospermum regium* (Schrank) Pilg., Bixaceae, is a Brazilian plant widely used as a folk medicine in the southwestern of the Brazil to treat inflammation and infection diseases. However, the effects of *C. regium* hydroethanolic extract on pregnant rats have not been assessed. To evaluate the effects of the *C. regium* on pregnant rats during the organogenic period, the hydroethanolic extract was administered via gavage at a dose of 11.5 mg/kg/day to rats from 6th to 15th day of pregnancy. No clinical signs of maternal toxicity were observed. The placenta's and fetuses' weight were similar in control and treated animals. The term fetuses dis not present malformations or anomalies although the number of live fetuses and birth rate were significantly decreased. In conclusion, the *C. regium* hydroethanolic extract is nontoxicant to the pregnant rat although it would be likely to interfere in the progress of the embryofetal development.

Introduction

Cochlospermum regium (Schrank) Pilg., Bixaceae, is a native plant of the Brazilian cerrado and it is widely used as a folk medicine in the southwestern of the Brazil (Nunes et al., 2003). *C. regium* has been employed to treat illnesses such as internal pain, inflammation, infection disease, rheumatoid arthritis and others (Correa, 1975; Oliveira et al., 1996). A recent study characterized the *C. regium* extract by the presence of flavonoids, triacylbenzenes and gallic acid derivatives, providing support to justify the popular use of this specie in treating infection (Solon et al., 2012). Additionally, in vitro studies have demonstrated the cytotoxicity of *C. regium* root extract against non-tumorigenic CHO-K1 cells (Ceschini & Campo 2006). Moreover, *C. regium* did not exhibit antimutagenic effects when evaluated in the mouse bone marrow (Andrade et al., 2008).

It is well established in the literature that exposure to xenobiotics during pregnancy may have different effects on embryo development depending on the conceptus phase and the maternal conditions (Wilson, 1977; Spritzer et al., 2001; Oliveira et al., 2009). Based on the finding that *C. regium* is one of the most often used plants by people in Campo Grande City (MS, Brazil) (Nunes et al., 2003), to treat inflammation, the aim of the present investigation was to evaluate the impact of the *C. regium* hydroethanolic extract on pregnant rats exposed during the organogenic period. In particular, our study sought to provide further insights into the effects of *C. regium* extract on maternal and fetuses toxicity and the *C. regium* single dose used in this experiment was based on the average consumption by the population.

Materials and methods

Preparation of extract

The underground system (xylopodium) of *Cochlospermum regium* (Schrank) Pilg., Bixaceae, was collected in Terenos (MS, Brazil), and identified by G. Hatschbach. A voucher specimen was deposited in the CGMS Herbarium (registration number 04375). The dried powder of the xylopodium (80 g) was extracted with 70:30 EtOH:H_2O (v/v) by exhaustive percolation, and the solvent was eliminated under reduced pressure. The resulting brown amorphous residue was maintained *in vacuum* to

dryness, and it yielded 26.4 g (33%, w/w yield) of crude extract. This extract was stored in the amber glass, at room temperature, in a desiccator.

Animals

Twenty four pregnant *Wistar* rats were used in the present investigation: twelve for the control and twelve for the *C. regium* exposed group. The females were mated with males and the gestational day 0 (GD0) was determined if there were sperm and estrus phase cells in vaginal smears. These animals were housed in a standard animal facility under controlled temperature (22 °C) and photoperiod (12 h light, 12 h dark) with access to water and rodent food *ad libitum*. All procedures and protocols followed approved guidelines for the ethical treatment of animals, according to the Ethics Committee in Animal Experimentation from the Federal University of Mato Grosso do Sul (Protocol # 48/2003).

Experimental procedure

The females of the *C. regium* treated group received 2.5 mg/kg/day of the extract suspended in 0.5 mL in distilled water, via gavage, during organogenic period, from 6th to 15th day of pregnancy (GD6 to GD15). This window of treatment was intended to evaluate possible embryotoxic effects of the plant. The chosen dose of *C. regium* used in this experiment corresponds to that used as a folk medicine for the treatment of diseases related to inflammation diseases related to inflammation and genitourinary infection (Correa, 1975; Oliveira et al., 1996). The control group received only the vehicle (5.5 mL/kg).

To observe maternal toxicity, during the treatment, the following clinical parameters were evaluated: body weight, food intake, piloerection, diarrhea, locomotor activity, and deaths (Mason & Kang, 1994).

The animals were weighed on the first day of treatment (GD6); on the last day of administration (GD15) and on the 20th day of pregnancy (GD20), when they were killed by ether inhalation and laparotomized. The ovaries and uterine horns were exteriorized, and the uteri were opened for counting of live and degenerated/dead fetuses. The corpora lutea were counted under a stereomicroscope. The ovaries, placenta and fetuses were weight. The fetuses (n=119 and 88, from control and *C. regium* exposed group, respectively) were examined for external malformations and fixed in Bouin's solution to perform liver morphometry (n=4-7 in each group). Maternal lung, spleen and liver were also weighted. For histological analysis of the liver, representative fragments were excited and fixed in Bouin's solution. Once fixed, the tissue fragments were dehydrated, cleared and embedded in paraffin wax. The samples were cut into 6 μm thick sections and stained with hematoxylin-eosin for histological analyses.

Hepatocytes parameters

The individual volume of maternal and fetuses hepatocytes were obtained from their nuclear volume and the proportion between nucleus and cytoplasm. To calculate the proportion between nucleus and cytoplasm an 850-point square lattice was placed over the sectioned material at 1000× magnification. At least four thousand points over hepatocytes were counted for each animal (n=4-7 in each group for mother and fetus). Because the hepatocyte nucleus in rats is spherical, its nucleus volume was obtained from the knowledge of the mean nuclear diameter. For this purpose, the diameters of forty nuclei were measured for each animal. Hepatocyte nuclear volume was expressed in μm^3 and obtained by the formula $4/3\pi r3$, where r=nuclear diameter/2.

Statistical analysis

Values are expressed as mean±SEM and data were analyzed using Student's t test in graphpad Prism (version 5; GraphPad Software Inc., San Diego, CA, USA). The significance level was set at $p<0.05$.

Results

The administration of *C. regium* to pregnant rats, during organogenic period, did not show clinical signs of toxicity since there was no alteration of locomotion activity, no death and no occurrences of piloerection or diarrhea. The maternal lung, spleen and liver weight of the treated animals were similar to the control group (data not shown). Also, there was no significant weight gain or loss during the experiment. However, the body weight of treated rats sacrifice on the 20th day of pregnancy was significantly decreased (Table 1).

Table 1. Body weight of control and *Cochlospermum regium* exposed rats from 6th to 15th day of pregnancy.

Group	6th	15th	20th
Control	244.5±9 (12)	286.5± 6(12)	312.6±5.5 (12)
C. regium	250.5±5 (12)	274.8±6 (12)	293.3 ±7.3 (12) *

Results expressed in mean±standard error (n); *$p<0.05$.

In comparison with the control group, the ovary weight was decreased ($p<0.05$) when the extract was administered during pregnancy. It is noteworthy that in this experimental lot, in the untreated group, an animal with a great number of corpora lutea (~17) was recorded. No significant difference was observed between control and *C. regium* treated group when the weight of placenta

and fetuses were analyzed (Table 2 and 3). The term fetuses presented a similar degree of development, no malformations were detected in both groups investigated.

The effects of *C. regium* extract on reproductive parameters are show in Table 2. The number of corpora lutea was similar ($p>0.05$) between the control and treated rats. However, the values obtained for live fetuses for the *C. regium* treated group presented a significant decrease ($p<0.05$), with a lower ($p<0.05$) birth rate (Table 2 and 3).

Table 2. Effect of *Cochlospermum regium* extract on maternal ovary and placenta weights, number of corpora lutea and birth rate (%).

Group	Ovary weight (mg)	Placenta weight (g)	Corpora lutea (number)	Birth rate[a] (%)
Control	112.1±5.3	5.5±0.4	11.7±0.7	84.4±3
C. regium	95.3± 5.2*	4.6±0.5	10.9±0.5	69±7.2*

Results expressed in mean±standard error; *$p<0.05$; aBirth rate: no. of live fetuses/no. of corpora lutea x 100.

Table 3. Effect of *Cochlospermum regium* extract on the fetuses parameters.

Group	Live fetuses (number)	Dead fetuses (number)	Fetuses size (cm)	Fetuses weight(g)
Control	9.9±0.7	1.8±0.3	2.8±0.02	1.9±0.02
C. regium	7.3±0.7*	3.5±0.9	2.8±0.03	1.9±0.03

Results expressed in mean±standard error; *$p<0.05$.

Table 4 summarizes the effects of *C. regium* extract in the hepatocytes parameters. The histophatological examination of maternal and fetuses livers showed similar ($p>0.05$) hepatocyte volumes between the control and treated group.

Table 4. Effect of *Cochlospermum regium* extract on maternal and fetus hepatocytes parameters.

Group	Nuclear volume µm^3	Cytoplasmic volume µm^3	Cellular volume µm^3
Control			
Mother	149±7	2249±174	2398±171
Fetus	88±7	940±44	1028±43
C. regium			
Mother	150±11	2259±361	2409±372
Fetus	95±4	913±42	1008±42

Results expressed in mean±standard error. $p>0.05$.

Discussion

C. regium extract is used as a folk medicine in the southwestern of the Brazil to treat inflammation and genitourinary infections. Many Brazilian women in this region have been using this plant. Some chemical and pharmacological evaluations of *C. regium* are available in the literature, but this is the first investigation to evaluate the impact of the *C. regium* hydroethanolic extract on pregnant rats within the organogenic period. At the dose given, maternal nontoxicity was evidenced by the body weight gain and by placenta's and fetuses' weight (Freitas et al., 2005). When testing possible fetal toxic effects of a specific substance, it is necessary to establish if these effects are due to direct action on the fetus or an indirect action trough the maternal organism that could secondarily interfere with the fetus (Chang et al., 2002). In this study, no clinical signs of maternal toxicity were observed. Additionally, maternal liver and spleen weights, in the treated rats, were similar to control and, reduction or increases in the weight of these organs suggest toxicity (Queiroz et al., 2012).

Although the body weight of the *C. regium* treated group sacrificed on the 20th day was decreased, this data might be explained by the reduction of the live fetuses in the treated animals. The birth rate was also lower in the *C. regium* treated group. Based on that, we can assume that the extract would interfere in the rate of postimplantation loss, which establishes the correlation between the number of implanted embryos and those which manage to develop normally (Almeida & Lemonica, 2000). There is a direct relation between the number of conceptuses and the corpora lutea in rats (Kato et al., 1979). In the present investigation, the number of corpora lutea in treated rats was similar to that in control and the term fetuses presented similar morphology. Taking all data together, it is possible to suppose that *C. regium* extract interferes in the progress of the embryo development, which justifies the decreased number of live fetus in the treated group. However, the dose tested in the present study, did not present a direct teratogenic effect because the live fetuses were completed normal and they did not show malformations. Moreover, there was a strong trend toward the increase in the number of dead fetus as well as reduced birth rate was an indication of the abortifacient activity (Yakubu & Bukoye, 2009) of the *C. regium* extract.

According to the literature the ovary weight is dependent of the number and volume of the corpora lutea (Guerra et al., 2000). The *C. regium* treated animals presented a significantly reduction at the ovary weight. The corpora lutea volume increases as pregnancy progress and it is correlated with the increase of the 20-hydroxyprogesterone concentration (Uchida et al., 1970; Waynforth, 1971). Additionally, there is a direct relationship between the number of conceptuses and the rate and/or degree of increase in the corpora lutea activity (Golos & Sherwood, 1982). As the corpora lutea number was similar in both groups analyzed in this study, it is possible to suggest that the corpora lutea volume was lower in the *C. regium* treated animals and consequently the progesterone production would be reduced. More

studies are necessary to evaluate the significance of this alteration.

This study confirms earlier findings that *C. regium* hydroethanolic extract does not induce histopathological alteration in the liver (Toledo et al., 2000). This organ was analyzed, in the present investigation, because its vulnerability to damage induced by different compounds (Tennant, 1997). Nevertheless, it should be emphasized that the liver vulnerability is increased in pregnancy due a decrease in hepatic metabolism (Hytten et al., 1984). Our results provide important morphological and stereological data related to maternal and fetus hepatocyte function. The absence of changes in the hepatocyte nuclear, cytoplasmatic and cellular volume, suggest normal liver function (Moreti et al., 2005).

In conclusion, although detailed understanding of the molecular and biochemical action of the *C. regium* hydroethanolic extract on pregnant rats during the organogenic period requires further characterization, the present study suggests that the indiscriminate use of this plant, by pregnant woman, would be likely to interfere in the progress of the embryofetal development, though maternal nontoxicity was observed.

Acknowledgments

We are grateful to Simone Bertozi de Souza Vasconcelos and Cláudio Gonçalves Oliveira for histological assistance. This work was financial supported by the Brazilian Foundation: FUNDECT-MS and CNPq. Authors JMS and MCV acknowledge CNPq for a research grant.

Authors' contributions

ALCL designed the study and contributed in running the laboratory work and analysis of the data. ALCB (graduate student) contributed to biological studies. JMS and MCV contributed in the obtaining of hydroethanolic extract and plant herbarium confection, respectively. RJO contributed to critical reading of the manuscript. SAA designed the stereological study and writing the manuscript. All the authors have read the final manuscript and approved the submission.

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