Chinese Medicine, 2011, 2, 115-119
doi:10.4236/cm.2011.23019 Published Online September 2011 (http://www.SciRP.org/journal/cm)

The Effect of Aqueous Extract of *Cecropia glazioui* Snethlage (Embauba) in the Rat Fetal Development

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Received May 15, 2011; *revised May* 29, 2011; *accepted June* 8, 2011

Abstract

The effect of aqueous extract of *Cecropia glazioui* Snethlage (Embauba) in the rat fetal development. This study was to complement previous assays on the physical and neurobehavioral development of rats resulted from oral administration of 1 g/kg/day *Cecropia glazioui* Snethlage (*C. glazioui*) aqueous extract (LD50 > 5 $g \cdot kg^{-1}$) in pregnant rats (peri- and post-natal studies). In the present study, the effect of 2.5 g/kg/week *C. glazioui* aqueous extract, administered to pregnant rats during 15 days, was verified in the rat offspring development. No acute or chronic toxicity (no effects on mortality or weight average daily gain) were observed. In addition, no effects on reproductive parameters (offspring vitality, placenta and fetus weight, number of *corpora lutea* on each ovary, pre- and post-implantation loss) and on offspring external morphology were found. We concluded that *C. glazioui* aqueous extract administered during pregnancy did not cause abnormalities in rat offspring.

Keywords: Fetuses' Abnormalities, Medicinal Plants, Teratogenesis

1. Introduction

Several studies concerning the pharmacological effects of *Cecropia glazioui* Snethlage (Cecropiaceae), popularly known as "Embauba" in Brazil, were published in the last decade. The *C. glazioui* aqueous extract showed anxiolytic-like effect in mice [1], probable by blocking monoamines uptake in the central nervous system [2]. An anti-asthmatic property was confirmed in guinea pigs treated with *C. glazioui* purified fraction [3]. It also showed hypotensive activity [4], which is not related to angiotensin-converter enzyme [5], and it has antisecretory/antiulcer properties [6]. Plants are an important source of new molecules, which result mainly as consequence of their ontogenesis caused by internal and external forces [7]. They produce active primary and secondary metabolites to defend themselves against predators, microorganisms, and UV rays; and to attract seed-dispersive animals and insects [8]. Some of *C. glazioui* constituents, such as catechins, procyanidins and flavonoids, were observed by Tanae *et al.* [9]. Brazilian legal requirements of phytotherapics' marketing demand data on their pharmacological action and therapeutic efficacy. Safety is other legal aspect regarding phytotherapics [10-15]. However, few studies on phytotherapic safety are available in the literature and data regarding *C. glazioui* toxicity is still scarce. A previous study showed that the daily exposure to 1 g/kg *C. glazioui* aqueous extract showed low toxicity to pregnant rats and their litters [16]. The objective of the present study was to evaluate the influence of weekly oral administration of *C. glazioui* aqueous extract (2.5 g/kg) in pregnant rats, simulating the human sporadic use.

2. Material and Methods

2.1. Plants

The *Cecropia glazioui* Snethlage specimen were collected in Tapirai city (State of São Paulo, Brazil). A voucher specimen is deposited in University of Sorocaba (UNISO) herbarium, being identified by Dr Sérgio Romaniuc Neto (Botanic Institute of São Paulo, Brazil).

2.2. Preparation of the Aqueous Extract

Fresh leaves (450 g) of *C. glazioui* without petiole were dried, powdered and a 70% hydroalcoholic extract was obtained by percolation. The extract was concentrated under reduced pressure and lyophilized providing 102.3 g of powder (efficiency = 22.7%). It was stored at room temperature without light and humidity until the toxicological assays were performed. The *C. glazioui* aqueous extract was freshly prepared in distillated/deionizated water before oral administration.

2.3. Animals

Wistar rats (160 - 200 g) of both genders were obtained and kept at UNISO/Pharmacy School facilities according to "The Guide for the Care and Use of Laboratory Animal" (National Research Council 1996) and "European Community Guidelines" (EEC Directive of 1986; 86/609/EEC). All animals were maintained in groups (5 - 6 rats/cage) with food and water *ad libitum*. A twelve-hour light/dark cycle at constant temperature (23˚C ± 1˚C) was observed. All animals were previously adapted to laboratory conditions during one week before the experiments. The study design was approved by the UNISO Ethical Committee for Experiments.

2.4. Reproductive Ability Evaluation

Twelve sexually-naive rat females were mated with rat males (five females and one male per cage). Pregnancy was confirmed through the presence of spermatozoids in vaginal-washing rubbing observed by microscopy analysis [17]. The presence of spermatozoids was considered as the first day of pregnancy. Pregnant females were kept in separate cages. At the 1st, 5th, 10th and 15th days of pregnancy, six females received 2.5 mL/kg of deionized water by gavage (control group) and six females received 2.5 g/kg of *C. glazioui* solution by gavage (treated group). According to Gerenutti *et al*. [16], this *C. glazioui* dose is non-toxic for adult female rats. The posology of *C. glazioui* was based on rat-fetus development (in days from ovulation): blastocyst formation—3 to 5 days; implantation—5 to 6 days; organogenesis—7 to 17 days [18]. The weight gain of the pregnant rats exposed to any chemical agent during a specific period is one of the most used parameters to determine toxicological effects [19,20]. At the 18th day of pregnancy, each rat female was anesthetized by halothane (Halotano®, Cristalia, Brazil) inhalation and the uterus was rapidly excised. The following macroscopic parameters were evaluated in order to observe the reproductive performance [20,21]:

1) Offspring vitality;

2) Placenta and fetus weight (grams);

3) The number of corpora lutea on each ovary;

4) Pre-implantation loss (%) = corpora lutea number – implantation number/corpora lutea number

5) Post-implantation loss (%) = implantation number – alive fetus number/implantation number

Afterward, offspring animals were killed by halothane inhalation, fixed in Bouin's solution for 24 - 48 h, and kept in 70% hydroalcoholic solution in order to measure the following parameters (in cm): antero-posterior (A) and latero-lateral (B) of cranio; antero-posterior (C) and latero-lateral (D) of thorax; cranio-caudal (E) and tail (F), as showed in **Figure 1**.

2.5. Statistical Analysis

The data were analyzed by using Student's *t* test. The significance level was set at 5%. The results were expressed as mean ± standard error mean (SEM).

3. Results

Figure 2 shows the body weight (in grams) gain during pregnancy and killing period (18th day). At the 1st day of pregnancy, the females of *C. glazioui* group showed lower ($p < 0.05$) body weight gain when compared to control group. There was no influence ($p > 0.05$) of *C. glazioui* in body weight in the further periods. No other changes, such as morbidity or mortality, were registered during the experimental period.

Figure 3 shows the weight of placenta and fetus. No statistically significant difference ($p > 0.05$) was observed between control and treated groups for both parameters.

The **Table 1** shows the reproductive performance of pregnant rats exposed to *C. glazioui* ($n = 6$) compared to the control group ($n = 6$). *C. glazioui* did not influence ($p >$

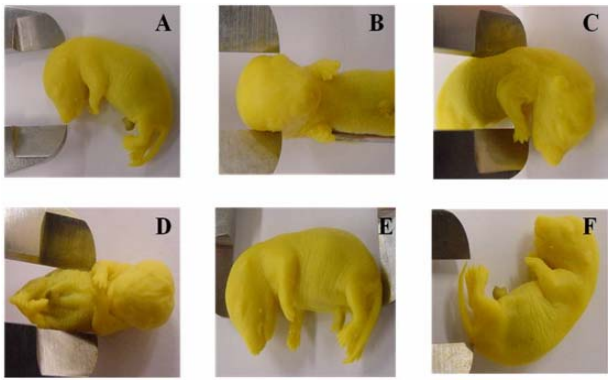

Figure 1. Measurement of external anatomy aspects: antero-posterior (A) and latero-lateral (B) of cranio; antero-posterior (C) and latero-lateral (D) of thorax; cranio-caudal (E) and tail (F).

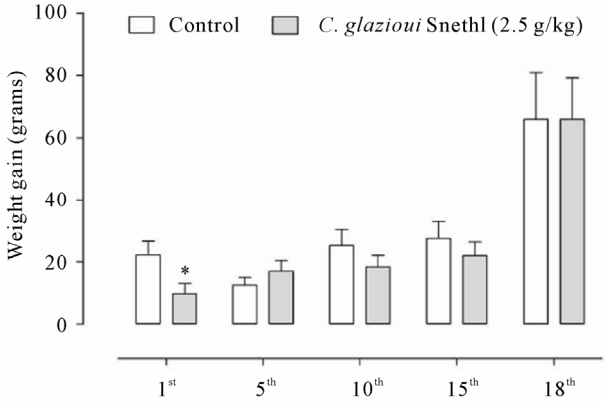

Figure 2. Mean (±SEM) body weight gain (in grams) of both control and *C. glazioui* treated groups. (*$p < 0.05$).

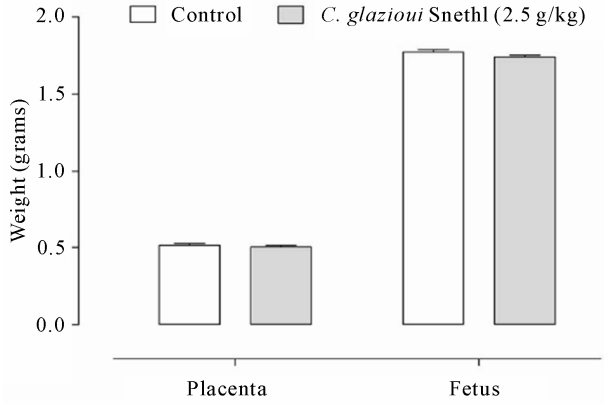

Figure 3. Mean (±SEM) of weight gain of placenta and fetus. No statistically significant changes were observed between groups ($p > 0.05$).

Table 1. Reproductive performance of pregnant rats exposed to *Cecropia glazioui* Snethl aqueous extract.

Parameters	Control group	*C. glazioui* 2.5 g/kg
Number of fetus	63	64
Number of corpora *lutea*	73	67
Pre-implantation loss (%)	0.38 ± 0.31	0.04 ± 0.04
Post-implantation loss (%)	0	0
Offspring vitality (%)	100	100

0.05) any of the five reproductive performance parameters.

Figure 4 shows the measurement (in cm) of offspring external anatomical parameters. No statistically significant difference between *C. glazioui* and control groups was observed regarding any of the measurements.

4. Discussion

Health governmental agencies and guidelines usually require developmental and reproductive toxicology (DART) tests for drugs destined for human use. DART studies require at least one of three segments of reproductive cycle [18]: 1) premating and mating through implantation (reproduction and fertility studies); 2) from implantation through major organogenesis (teratology and/or development toxicological studies); 3) late pregnancy and post-natal development (the perinatal/post-natal studies). In the present study, the segment 2 was observed, while segment 3 was previously studied by Gerenutti *et al*. [16]. Those authors observed the effect of *C. glazioui* extract (Cg) on physical and neurobehavioral development of rats. Female rats received 1 g/kg/day of Cg during pregnancy and they observed that LD_{50} was higher than 5 g/kg. In addition, latency of uprightness and negative geotaxis reflexes were enhanced by Cg in comparison to the control group, but the rearing frequency decreased [18]. They concluded that Cg showed low toxicity to the pregnant rats and their litters, and these effects were similar to the anxiolytic-like effect observed in mice by Rocha *et al*. [1]. In our study, the second segment of reproductive cycle, commonly referred as the major period of organogenesis (6 to 15 days in rats), was focused in a new experimental design. This included the period from implantation to closure of the hard palate, being the Cg extract administered at critical points (1st, 5th, 10th and 15th days of pregnancy). No signs of morbidity, mortality or abortion were registered during the pregnancy period, and no evidence of chronic toxicity, food intake changes, or body weight gain (except at the 1st day) was induced by Cg, which is similar to the results observed by Gerenutti *et al*. [16]. Abnormalities are usually classified as malformations or developmental variations. Malformations are structural anomalies altering general body conformity, causing dis-

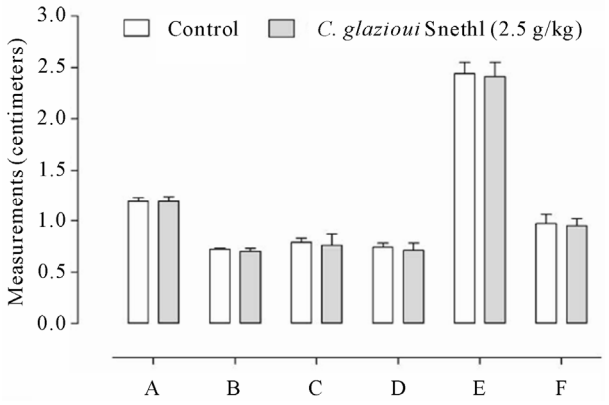

Figure 4. Offspring external morphological parameters (in cm): antero-posterior (A) and latero-lateral (B) of cranio; antero-posterior (C) and latero-lateral (D) of thorax; cranio-caudal (E) and tail (F). No statistically significant changes were observed between groups ($p > 0.05$).

ruption or interfering with body function, being generally incompatible with life. Developmental variations are defined as anatomical structure alterations having no significant biological effect on health or body conformity, usually representing slight deviations from normal [22]. Considering the offspring external morphological measurements in the present study, Cg extract did not induce significant effects in these measurements when compared with the control group. Studies carried out to establish the potential ability of drugs to induce adverse effects on the fetal development, which used acceptable and rationale experimental design, could provide accurate extrapolation of the potential risk in human beings of that particular drug. Considering the previous study performed by Gerenutti *et al*. [16], which used the same aqueous extract of Cg, it was possible to conclude that *Cecropia glazioui* Snethlage presents no toxicity on morphological development of rat offspring.

5. Acknowledgements

This work was supported by UNISO. R-M, P. was student of Scientific Initiation from UNISO.

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