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Non-clinical toxicity study of the oil-resin and vaginal cream of Copaiba (*Copaifera duckei*, Dwyer)

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Abstract: The toxic effects of copaiba oil-resin (ORC) and the copaiba oil-resin vaginal cream (CVC) were evaluated in subacute treatment phase, in wistar rats, which were treated orally (p.o) and intravaginal (ivag). Treated groups received the dose of, 0.04 mg/kg, 28 mg/kg, and 32 mg/kg, respectively, with ORC (p.o), *Copaifera duckei* oil-resin (ORCV-ivag) and vaginal cream with oil-resin *C. duckei* (CVC – ivag). The treatment for 22 days with ORC and CVC did not cause clinical signs of toxicity, no deaths have been reported, and did not change the development weight of the animals. The females treated with ORC (p.o), exhibited greater intake of water, but the feed intake was not different in males and females. The use of CVC did not change the water intake of females, but altered feed intake. The ORCV was capable of causing hypoglycemic effect and elevated serum creatinine levels. The hematology parameters of the animals were not changed by ORC (p.o, 32 mg/kg). The use of CVC changed hematocrit, lymphocytes, and the concentration of hemoglobin. The use of CVC and ORCV (ivag), did not altered the biochemicals parameters. Females treated with ORC (p.o, 32 mg/kg) showed some kind of susceptibility to specific use (elevation of total cholesterol, HDL and alkaline phosphatase). The elevation of serum ALT and AST enzymes was not attributed to the use of ORC orally and intravaginal and

ABOUT THE AUTHOR
Clarissa Silva Lima, is a specialist in reproductive toxicology, has dedicated herself to the topics related to the use of drugs in the reproductive age, evaluating the toxicity on the reproductive parameters. She is currently a professor at the Pharmacy Faculty of the Department of Biological Sciences and Health (DCBS) of the Federal University of Amapá. She is the coordinator of the Clinical Cytology Laboratory at DCBS. Has been dedicated to the research of new cytological techniques, using natural products. Reproductive toxicology is the main theme, which has been dedicated to basic and applied research.

PUBLIC INTEREST STATEMENT
The Research Group on Pharmaceuticals of the Federal University of Amapá, in collaboration with the Group of Pharmacology of Natural Products of the National Autonomous University of Mexico, has been dedicated to the study of natural products of plant species of the Brazilian Amazon flora and Mexican flora, mainly those species that have reported use in folk medicine. These groups have developed and studied the pharmacological effects of nanotechnological products of natural origin on inflammatory processes, smooth muscle, gastritis, gastric ulcer and repellent and larvicidal actions. Amazon has a richness in terms of biodiversity, and together with nanobiotechnology development, it is possible to obtain products with a degree of efficacy better than those existing in the pharmaceutical market today. Reproductive toxicology is a strong theme developed by groups.
the use of CVC, as well as the product of its formulation (BVC). The subacute treat-
ment with C. duckei oil-resin and cream (CVC) did not cause clinical signs of toxic-
ity, no deaths have been reported and did not change significantly the parameters 
evaluated in this study.

**Subjects:** Laboratory Animal Science; Drug Discovery; Natural Products

**Keywords:** *Copaifera*; copaiba vaginal cream; non-clinical toxicity; subacute; oil-resin

1. Introduction

The copaibeiras are common trees of Latin America and Western Africa (Cascon & Gilbert, 2000; Francisco, 2005; Maciel et al. 2002). In Brazil, they are found in the regions Southeast, Midwest, and Amazonic.

The copaiba oil is a natural product composed of a solid, non-volatile resin, formed by acids. These acids are responsible for 55 to 60% of the oil, diluted in the other part, an essential oil, compound of sesquiterpenes, which can be divided into oxygenated sesquiterpenes (alcohols) and hydrocarbons sesquiterpenes (Cascon & Gilbert, 2000; Maciel, Pinto, Veiga-Júnior, Grynberg, & Echevarria, 2002).

The lower genital tract infections have been the most frequent cause of gynecological consulta-
tion (50–70% of complaints). It is believed that all sexually active women have encountered at least one episode of bacterial vaginosis and/or vulvovaginitis. Several microbial infections (bacterial, viral, or fungal) are reported in the literature. In many cases, organisms responsible for the infections, bacterial or fungal, acquire resistance to the administered drugs, and for this reason, a large number of antibiotics and/or chemotherapeutic agents have been developed (Koshi & Cherian, 1995).

The increased resistance of bacteria and fungi to antibiotics/conventional chemotherapeutic 
agents has stimulated intensive efforts to develop new effective antimicrobials. Thus, the natural 
products can be used as an alternative to the current existing antibiotics. This scenario has aroused 
the interest of the scientific class and the pharmaceutical industry, especially in the plant molecules, 
since plants have great potential in synthesizing chemicals substances with diverse structures, such 
as a defense system against pathogenic agents (Nakamura et al., 2008; Pacheco, Barata, & Duarte, 
2006).

The copaíba resin oil is one of the most known Amazonic herbal product, and it is recognized for 
its wide use in folk medicine due to its property to restore mucous, especially in the case of gyneco-
logical inflammation and disinfectant action in the human pulmonary tract secretions (Francisco, 
2005). It has diuretic properties, which explains its beneficial effects on cystitis (Brito, Tavares, 
Moura, & Lima, 2001). Thus, it is also used in the treatment of leukorrhea and gonorrhea topically 
(vaginal capsules) and orally, as the oil is eliminated through the kidney and has urethral transit 
(Brito et al., 2000; Brito et al., 2001). Currently, the traditional knowledge, mainly on the Amazon bio-
diversity products, has become an important tool in developing new pharmaceuticals products. 
Despite the existence of various vaginal creams on the market, copaíba ovule never ceased to be 
used by the population (Lima et al., 2011).

In the literature, it was already described to the oil resin copaíba: anti-inflammatory effect 
(Carvalho et al., 2005; Kobayashi et al., 2011), analgesic (Carvalho et al., 2005; Pacheco et al., 2006; 
Veiga-Júnior & Pinto, 2002), antimicrobial (Nakamura et al., 2008; Pieri, Mussi, Fiorini, Moreira, & 
Schneedorf, 2012; Santos et al., 2009); antibacterial and bacteriostatic (Pieri et al., 2012; Souza et al.,
2011); anti-helmintic and trypanosomicide (Izumi, Ueda-Nakamura, Veiga-Junior, Pinto, & Nakamura, 2012), antifungal, anticancer (Santos et al., 2009; Veiga-Junior & Pinto, 2002), healing (Eurides et al., 1998), antitumor (Lima, Veiga-Junior, Christo, Pinto, & Fernandes, 2003), germicide (Blaisse, 2003), expectorante, and diuretic (Freire, Brito-Filha, & Carvalho-Zilse, 2006; Maciel et al., 2002).

Other indications are also known and cited, as the antiviral action (Veiga-Júnior & Pinto, 2002), repellent, larvicide and insecticide (Geris et al., 2008; Kanis et al., 2012), syphilis (Pacheco et al., 2006), anti-gonorrhea, anti-leukorrhoea, and cercaricide (Maciel et al., 2002). Other actions related to copaiba oils have also been discovered as vasorelaxant, cytotoxic and embryotoxic (Costa-Lotufo et al., 2002), and the isolated diterpene, kaurenoic acid, showed anti-inflammatory activity and protective actions on colitis induced by acetic acid (Paiva et al., 2004).

The pharmacological action of the oils Copaifera sp. is attributed to the chemical structure of their constituents, whose main components responsible for the sesquiterpene hydrocarbons, especially the β-bisabolene and β-caryophyllene (Carvalho et al., 2015; Veiga-Júnior & Pinto, 2002). However, the exact mechanism of action of these compounds is not well elucidated (Souza et al., 2011).

Thus, it is important to note that the population has used this oil-resin for many therapeutic purposes. However, there is a need for more research to better clarify the safety of this herbal medicine. Lima et al. (2011) conducted a study on the reproductive performance of rats submitted to the treatment with a vaginal cream containing copaiba oil. The results of this study demonstrated no maternal toxicity and no embryo or fetus toxicity based on the administered dose, equivalent to 10 times the preconized for humans. Reproductive toxicology studies of this magnitude are necessary to give subsidies to pregnant women prescription. Based on the application of vaginal cream of oil-resin copaiba, this study aimed to evaluate the toxic effects of the subchronic treatment phase.

2. Materials and methods

2.1. Obtainment of copaíba oil-resin
The copaiba oil-resin for pharmaceutical development and testing was obtained from the company Beraca/Brasmazon – oil seeds and Amazon products Industry, located in the city of Ananindeua, in the state of Pará, Brazil. According to the company report, the oil was obtained from the species, Copaifera duckei Dwyer.

The oil-resin was previously analyzed by Gas Chromatography coupled to mass, and presented β-caryophyllene as majority compound (Lima et al., 2011). The CVC was standardized, considering as phytochemical marker, β-caryophyllene.

2.2. Vaginal cream of copaíba oil-resin
The samples, vaginal cream base and copaiba vaginal oil-resin cream were produced and provided by enterprise Laboratorio Almeida Prado Co., located in São Paulo city, State of São Paulo, Brazil, appearing in the form of a vaginal cream, lot number 1062/08 12/2010 as manufacturing date.

3. Study of toxicity of copaiba oil-resin and copaiba vaginal cream

3.1. Animals
The initial project was approved by the Research Ethics Committee of the Federal University of Amapá, Amapá, Brazil, receiving the certificate number 008A/2011.
Rats (Rattus norvegicus,) Wistar strain (male and female) were used, weighing 145 to 220 g, from the Bioterium of the Federal University of Juiz de Fora, Minas Gerais. After the arrival of the animals in the Laboratory, they went through an adjustment period and were kept in a room with controlled humidity and had temperature controlled at around 23 °C ± 2 °C, with cycle light/dark 12 h with food and water ad libitum.

3.2. Administration of the oil-resin and vaginal cream
The doses were based on the possible clinical application of the oil-resin by intravaginal route, described by Lima et al. (2011), and the treatment time was based on the fact of the clinical indication of use, being for seven days of treatment. To study the toxicity of subacute treatment phase (22 days of treatment) the routes which were used were: orally (p.o), by gavage and intravaginal (ivag) with application micropipette. The treatment groups received the doses 0. 0.04 mg/kg, 28 mg/kg, and 32 mg/kg, respectively, with ORC (p.o), ORCV (ivag) and CVC (ivag) (Lima et al., 2011). For the control groups was used 0.5 ml of distilled water (p.o) and 220 mg of vaginal cream base (ivag) (Lima et al., 2011).

3.3. Experimental groups
Thirty-five animals were randomly divided according to with the type of treatment. Thus, 20 animals were divided into two groups (n = 10/group, five males and five females) and treated orally with the C. duckei oil-resin and distilled water (ORC and Control). Other 15 animals (female) were divided into three groups (n = 5/group), which received intravaginally treatment with C. duckei vaginal cream oil-resin (CVC), only oil-resin C. duckei (ORCV) and the control group, with the base vaginal cream (control BVC).

4. Toxicity assessment of subacute treatment stage of copaiba oil-resin (oral and intravaginal)

4.1. Ponderal development rate and water-feed consumption
The animals were weighed every five days (d1, d5, d10, d15, d20) using balance Gehaka BG4000, with a capacity of 4,200 g and accuracy of 0.1 g. The water and feed consumption were measured daily from initiation of treatment until the d20, using the beaker and the balance, respectively. Daily, it was offered 100 g of food and 250 ml of water to the group.

4.2. Sample collection
After the end of the treatment, the animals were subjected to fasting for 12 h and after, were anesthetized with sodium pentobarbital (45 mg/kg, ip, Cristalia Co., Brazil). It was collected 5 ml of blood through the hepatic portal vein, being packed in two types of tubes: one with EDTA anticoagulant for determination of hematological parameters and another without anticoagulant to obtain serum and evaluation of biochemical parameters.

4.3. Biochemical parameters evaluation
For biochemical analysis, the blood samples were centrifuged at 3,500 rpm for 10 min to separate the serum from the blood cells. Subsequently, it proceeded the dosage of plasma enzymes such aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total cholesterol and the High-Density Lipoprotein (HDL), triglycerides, alkaline phosphatase, albumin, glucose and creatinine, using Bioclin® kits (colorimetric enzymatic assays and kinetic colorimetric) and multiparametric equipment (Alizé, Biomerieux).

4.4. Hematological parameters evaluation
For hematological analysis, complete blood count was performed by determining the values of erythrocytes, hemoglobin, hematocrit, white blood cells, platelets and RBC indices, mean
corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and concentration of mean corpuscular hemoglobin (MCH) using automatic blood analyzer logic cells HumaCount-Plus. The leucocyte count was performed on extensions stained with May-Grunwald-Giemsa, in each assay were analyzed and counted 100 cells.

4.5. Statistical analysis
From the results obtained in the several tests, were expressed as standard error mean (Mean ± SEM) of each experimental group. To compare the data of water consumption, diet and weight development, we used the “t” test (unpaired). For biochemical and hematological analysis, we applied the Mann-Whitney test. For analysis of the intravaginal treated groups, ANOVA was used, followed by Tukey test. The significance level was 5% (p < 0.05). The software used was GraphPad Prism® (version 5.03).

5. Results

5.1. Toxicity assessment of subacute treatment stage of copaiba oil-resin (oral and intravaginal)

5.1.1. Ponderal development rate and water feed consumption
During the oral treatment, it was not observed clinical signs of toxicity and none death was recorded. Treatment for 22 days with the ORC (p.o) did not affect the weight development of the animals (male and female), and there was no significative statistical difference between the control group and treated with ORC (p.o, 32 mg/kg) (Figure 1).

The daily intake of water was higher in the groups treated with oil-resin (ORC, 32 mg/kg), both in males and females. However, this was only significant in females (Figure 2). The daily feed intake was higher in the control groups (males and females), but there were no significance statistics between the groups (Figure 3).

Regarding the subacute treatment with CVC (ivag), there was no change in the weight development of females. However, the group treated with ORC (ivag) (Figure 4) presented alterations. The daily water consumption was not different between the treated groups (CVC 28 mg/kg and ORCV 0.04 mg/kg) and Control group BVC (Figure 5). However, the feed intake was higher in the treated group (CVC 28 mg/kg). It was also observed differences between groups CVC (28 mg/kg) and treated with ORCV (0.04 mg/kg) (Figure 6).
Figure 2. Effect of administration (vo) \textit{C. duckei} oil-resin (ORC, 32 mg/kg) and distilled water (control) on the daily consumption of water Wistar rats (males and females).

![Figure 2](image2.png)

Figure 3. Effect of administration (vo) \textit{C. duckei} oil resin (ORC, 32 mg/kg) and distilled water (control) on the dietary intake of Wistar rats feed (males and females). Values represent the mean ± SEM (\(n = 5\)/group).

![Figure 3](image3.png)

Figure 4. Effect of administration (ivag) of vaginal cream \textit{C. duckei} oil-resin (CVC, 28 mg/kg), oil-resin \textit{C. duckei} (ORC, 0.04 mg/kg) and based vaginal cream (BVC) on the Wista rats weight development. The points represent the mean ± SEM (\(n = 5\)/group).

![Figure 4](image4.png)

Figure 5. Effect of administration (ivag) of vaginal cream \textit{C. duckei} oil-resin (CVC, 28 mg/kg), \textit{C. ducki} oil-resin (ORCV, 0.04 mg/kg) and based vaginal cream (control BVC) on daily Wistar rats’ water consumption. The bars represent the mean ± SEM (\(n = 5\)/group).

![Figure 5](image5.png)
5.1.2. Biochemical parameters evaluation

The subacute treatment with the ORC (p.o), has shown some biochemical changes in females (total cholesterol = 76.8 ± 2.2 mg/dl; HDL = 37.1 ± 1.5 mg/dl and 112.6 ± 16.6, Alkaline Phosphatase = U/l). These parameters were higher when compared to the control group. It was observed in the treated groups a decrease blood glucose (83.3 ± 4.2 and 52.7 ± 10.0 mg/dl) and a significant increase in serum creatinine levels (0.5 ± 0.0 and 1.2 ± 0.3 mg/dl), respectively, in males and females (Table 1). The intravaginal treatment with ORC and CVC produced no interference on the biochemical parameters of Wistar rats, as there was no significant difference when compared to the group control BVC (Table 2).

5.1.3. Hematological parameters evaluation

Hematological parameters were assessed at 23º day after the end of the treatment with ORC (p.o and ivag) and CVC (ivag). The oral treatment did not produce interference (male and female), because the blood parameters were within the normal range (Clifford; Giknis, 2008), except platelets of the female group treated (1,400.3 ± 130.7 × 10³/mm³) (Table 3).

Table 1. Effect of subchronic administration (p.o) of C. duckei oil-resin (ORC, 32 mg/kg) and distilled water (control) on Wistar rats’ biochemical parameters (males and females)

|                | Female | Male                  |
|----------------|--------|-----------------------|
|                | Control | ORC 32 mg/kg | Control | ORC 32 mg/kg |
| AST (U/L)      | 241.6 ± 50.0 | 304.0 ± 9.2 | 193.2 ± 27.6 | 198.4 ± 29.3 |
| ALT (U/L)      | 83.6 ± 22.1 | 95.0 ± 21.7 | 53.0 ± 4.8 | 50.6 ± 6.0 |
| Total cholesterol (mg/dL) | 66.5 ± 1.8 | 76.8 ± 2.2* | 61.6 ± 3.3 | 67.8 ± 8.0 |
| HDL-Cholesterol (mg/dL) | 27.9 ± 1.0 | 37.1 ± 1.5* | 31.6 ± 1.2 | 30.0 ± 2.0 |
| Triglycerides (mg/dL) | 82.0 ± 4.5 | 75.0 ± 7.2 | 55.5 ± 2.3 | 56.9 ± 12.4 |
| Alkaline Phosphatase (U/L) | 23.6 ± 2.4 | 112.6 ± 16.6* | 90.8 ± 8.9 | 114.4 ± 9.4 |
| Albumin (g/dL) | 3.5 ± 0.0 | 3.6 ± 0.0 | 3.7 ± 0.0 | 3.7 ± 0.0 |
| Glucose (mg/dL) | 111.7 ± 20.9 | 52.7 ± 10.0* | 154.5 ± 6.7 | 83.3 ± 4.2* |
| Creatinine (mg/dL) | 0.5 ± 0.0 | 1.2 ± 0.3* | 0.4 ± 0.0 | 0.5 ± 0.0* |

Notes: The average values ± SEM (n = 5/group). AST: aspartate aminotransferase; ALT: alanine aminotransferase.

*p < 0.05 (Mann-Whitney test).
However, in the treatment with CVC, it was observed different values compared to control group BVC (hemoglobin, hematocrit and lymphocytes). The monocytes values (Control BVC = 7.0 ± 3.6% and Treaty CVC = 12.4 ± 4.5%), eosinophils (Control BVC = 6.0 ± 1.7%; Treaty CVC = 9.6 ± 4.5% and treaty ORCV = 7.4 ± 2.2%) and platelets (Treaty CVC = 1,256.4 ± 64.8 × 10³/mm³) are above the reference value (Clifford & Giknis, 2008). In ORCV group 0.04 mg/kg, there was a decrease in HCM hematiometric index (18.9 ± 0.3 pg) and red blood cells (8.1 ± 0.0 × 10⁶/mm³) compared to the group treated with CVC (28 mg/kg), however, they are in the normal range (Clifford, & Giknis, 2008) (Table 4).

### Table 2. Effect of subchronic administration (ivag) of vaginal cream *C. duckei* oil-resin (CVC, 28 mg/kg), *C. dukei* oil-resin (ORC, 0.04 mg/kg) and based vaginal cream (BVC, control) on biochemical parameters in Wistar rats

| Female | Control BVC | CVC 28 mg/kg | ORC 0.04 mg/kg |
|--------|-------------|--------------|----------------|
| AST (U/L) | 435.6 ± 83.8 | 325.0 ± 13.9** | 505.4 ± 56.1 |
| ALT (U/L) | 106.2 ± 25.5 | 63.6 ± 11.3** | 134.6 ± 26.8* |
| Total cholesterol (mg/dL) | 77.4 ± 3.9 | 82.0 ± 5.5 | 79.9 ± 3.8 |
| HDL cholesterol (mg/dL) | 35.6 ± 2.2 | 34.0 ± 1.4 | 33.5 ± 2.0 |
| Triglycerides (mg/dL) | 79.6 ± 3.1 | 67.6 ± 7.8 | 78.3 ± 1.8 |
| Alkaline Phosphatase (U/L) | 36.2 ± 6.6 | 28.8 ± 4.8 | 28.2 ± 5.0 |
| Albumin (g/dL) | 3.8 ± 0.1 | 3.7 ± 0.0 | 3.7 ± 0.0 |
| Glucose (mg/dL) | 95.6 ± 9.5 | 133.9 ± 17.1** | 110.3 ± 6.8* |
| Creatinine (mg/dL) | 0.6 ± 0.0 | 0.6 ± 0.0 | 0.6 ± 0.0 |

Notes: The values represent mean ± SEM (*n* = 5/group). AST: aspartate aminotransferase; ALT: alanine aminotransferase.

* *p < 0.05 compared to control group BVC.

** p < 0.05 ORC group compared to the CVC group (ANOVA followed by Tukey test).

### Table 3. Effect of subchronic administration (p.o) of *C. duckei* oil-resin (ORC, 32 mg/kg) and distillated water on hematological parameters in Wistar rats

| Female | Control | ORC 32 mg/kg | Male | Control | ORC 32 mg/kg |
|--------|---------|--------------|------|---------|--------------|
| Hm (×10⁶/mm³) | 7.9 ± 0.1 | 7.9 ± 0.1 | 9.2 ± 0.0 | 8.9 ± 0.2 |
| Hb (g/dL) | 14.8 ± 0.1 | 14.7 ± 0.2 | 15.7 ± 0.1 | 15.7 ± 0.1 |
| Ht (%) | 41.3 ± 0.4 | 40.4 ± 0.6 | 48.0 ± 0.3 | 46.7 ± 1.1 |
| VCM (fl) | 52.2 ± 0.6 | 51.2 ± 0.4 | 52.0 ± 0.3 | 52.6 ± 0.5 |
| HCM (pg) | 18.6 ± 0.1 | 18.5 ± 0.2 | 17.0 ± 0.1 | 17.6 ± 0.4 |
| CHCM (g/dL) | 35.7 ± 0.3 | 36.3 ± 0.3 | 32.7 ± 0.1 | 32.9 ± 0.4 |
| Leukocytes (×10³/mm³) | 3.7 ± 0.6 | 6.1 ± 1.9 | 3.6 ± 0.6 | 4.0 ± 0.6 |
| Lymphocyte (%) | 56.2 ± 3.8 | 66.6 ± 3.5 | 63.8 ± 2.1 | 68.4 ± 2.8 |
| Monocyte (%) | 24.0 ± 6.6 | 2.2 ± 0.5 | 1.6 ± 0.4 | 2.4 ± 0.6 |
| Neutrophil (%) | 40.0 ± 3.5 | 28.4 ± 2.7 | 32.6 ± 2.2 | 27.8 ± 2.7 |
| Eosinophil (%) | 1.4 ± 1.4 | 2.8 ± 0.9 | 2.0 ± 0.8 | 1.2 ± 0.8 |
| Platelets (×10³/mm³) | 1,069.0 ± 35.5 | 1,400.3 ± 130.7 | 1,026.3 ± 159.0 | 1,059.6 ± 62.4 |

Notes: Values represent the mean ± SEM (*n* = 5/group). Hm: Red blood cells; Hb: Hemoglobin; Ht: hematocrit, MCV: Volume Corpuscular East, HCM: Mean Corpuscular Hemoglobin, MCHC: Concentration Mean Corpuscular Hemoglobin.

However, in the treatment with CVC, it was observed different values compared to control group BVC (hemoglobin, hematocrit and lymphocytes). The monocytes values (Control BVC = 7.0 ± 3.6% and Treaty CVC = 12.4 ± 4.5%), eosinophils (Control BVC = 6.0 ± 1.7%; Treaty CVC = 9.6 ± 4.5% and treaty ORCV = 7.4 ± 2.2%) and platelets (Treaty CVC = 1,256.4 ± 64.8 × 10³/mm³) are above the reference value (Clifford & Giknis, 2008). In ORCV group 0.04 mg/kg, there was a decrease in HCM hematiometric index (18.9 ± 0.3 pg) and red blood cells (8.1 ± 0.0 × 10⁶/mm³) compared to the group treated with CVC (28 mg/kg), however, they are in the normal range (Clifford, & Giknis, 2008) (Table 4).
6. Discussion

Non-clinical studies of herbal medicines are required to be registered. This has led many countries to issue legal norms, supporting the development of scientific studies to prove the efficacy and safety of these drugs (WHO, 1993).

Lima et al. (2011) conducted a study on the reproductive performance of rats subjected to treatment with vaginal cream containing *C. duckei* oil-resin and standardized the ORC in terms of β-caryophyllene phytochemical marker, and according to the survey by Carvalho et al. (2015), β-caryophyllene is the terpenoid most frequently found in the studied copaiba oils.

In the present study, during subacute treatment with *C. duckei* oil-resin, both orally or intravaginally, didn’t produce clinical signs of toxicity and death. Similarly, with the treatment with a vaginal cream containing oil-resin and vaginal cream base used in the formulation. In assessing the weight development of the females treated with the oil-resin (intravaginal), it revealed that the 10th day after treatment, there was no weight gain, but this fact was not related to the use of oil-resin. In general, the *C. duckei* oil-resin, administered orally, was not able to change the weight development of the animals (males and females) and, consequently, the treated females (ivag) with the oil-resin and CVC (ivag) (Figure 1).

The daily feed intake was higher in the control groups (males and females), but not statistically significant. Despite the daily water intake being greater in the groups treated with *C. duckei* oil-resin (male and female), only in the group of females this may be related to the treatment, indicating, perhaps, the greater specific susceptibility of females. Sachetti, Fascineli, Sampaio, Lameira, and Caldas (2009) studying the toxicity, acute oral, of *C. reticulata* oil-resin (300 and 2,000 mg/kg) in female rats, didn’t observed a decrease in body weight and clinical signs of toxicity, as well as changes in feed intake (Figures 2–6).

The elevation of total cholesterol and HDL-cholesterol in p.o – ORC (32 mg/kg) treated female was not very significant, because the values are in the normal range (Dantas, Ambiel, Cuman, Baroni, & Bersani-Amado, 2006). Therefore, it should not be assigned as an important clinical change.

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### Table 4. Effect of subchronic administration (ivag) of vaginal cream *C. duckei* oil-resin (CVC, 28 mg/kg), *C. duckei* oil-resin (ORC, 0.04 mg/kg) and base vaginal cream (BVC, control) on hematologic parameters of Wistar rats

|                | Control BVC | CVC 28 mg/kg | ORCV 0.04 mg/kg |
|----------------|-------------|--------------|-----------------|
| Hm (×10⁶/mm³)  | 7.9 ± 0.2   | 7.4 ± 0.1    | 8.1 ± 0.0**     |
| Hb (g/dL)      | 15.4 ± 0.2  | 14.6 ± 0.1*  | 14.8 ± 0.0      |
| Ht (%)         | 41.5 ± 1.3  | 38.8 ± 0.7*  | 42.2 ± 0.4      |
| VCM (fl)       | 52.4 ± 0.5  | 52.2 ± 0.3   | 51.2 ± 0.2      |
| HCM (pg)       | 19.4 ± 0.5  | 19.7 ± 0.3   | 18.9 ± 0.3**    |
| CHCM (g/dL)    | 37.1 ± 0.7  | 37.7 ± 0.5   | 35.2 ± 0.5      |
| Leukocytes (×10⁹/mm³) | 6.1 ± 2.8 | 3.3 ± 0.5    | 2.3 ± 0.1       |
| Lymphocyte (%) | 64.8 ± 5.1  | 45.4 ± 5.9*  | 58.8 ± 3.0      |
| Monocyte (%)   | 7.0 ± 3.6   | 12.4 ± 4.5   | 2.2 ± 0.8       |
| Neutrophil (%) | 22.2 ± 2.5  | 32.6 ± 4.2   | 31.6 ± 3.4      |
| Eosinophil (%) | 6.0 ± 1.7   | 9.6 ± 4.5    | 7.4 ± 2.2       |
| Platelets (×10³/mm³) | 1,078.6 ± 140.4 | 1,256.4 ± 64.8 | 1,005.2 ± 71.2 |

Notes: Values represent the mean ± SEM (n = 5/group). Hm: Red blood cells; Hb: Hemoglobin; Ht: Hematócri-to, MCV: mean cell volume, MCH: Mean corpuscular hemoglobin, MCHC: Concentration of corpuscular hemoglobin Ages.

*p < 0.05 compared to control group BVC.

**p < 0.05 ORCV group compared to the CVC group (ANOVA followed by Tukey test).
sub-chronic use of oil-resin, orally, may be associated with some specific susceptibility to females, since there were no equivalent manifestations in males (Tables 1 and 2).

The aminotransferase levels indicate structural or functional alterations of the liver cell and may be used to evaluate liver function in a reliable way. Most of AST is in the hepatocytes’ mitochondria, while the ALT is located mainly in the cytoplasm (Hessel, De Santi-Neto, & Collares, 1996). The designated hepatocellular liver injury is featured by ALT twice greater than the threshold value or if the ratio ALT/Alkaline phosphatase ≥ 5; cholestatic if alkaline phosphatase is twice greater than the threshold value, or ratio ALT/Alkaline phosphatase ≤ 2; and mixed (hepatocellular and cholestatic) the proportion ALT/Alkaline phosphatase vary from 2 to 5 (Kaplowitz, 2001).

The alkaline phosphatase was also higher in females treated with *C. duckei* oil-resin. However, it should also not be considered as an indicative of an important clinical change, because serum levels are in the reference range.

Moreover, Botelho, Carvalho, Matos, Lobato, and Corrêa (2010) also noted an increase in serum alkaline phosphatase after treatment subacute with *C. officinalis* oil-resin. In this study, serum levels of enzymes (AST and ALT) did not differ statistically between the control and treated groups, both orally (male and female) and intravaginally (CVC, BVC and ORCV). Importantly, in all groups, the values were above the reference range (Dantas et al., 2006), except for the ALT of the CVC group (Tables 1 and 2).

The elevation of ALT and AST as well as the ratio of ALT/Alkaline phosphatase ≤ 2 (cholestatic hepatic injury) (Kaplowitz, 2001), seems not to be the related to the use of *C. duckei* oil-resin and of CVC. However, it is necessary additional studies, as the histopathology of the liver, to better clarify the existence of liver damage. Araújo-Júnior, Braz, and Rocha Neto (2005) corroborate with the results obtained in this study, because the *C. officinalis* oil-resin, too, was not able to alter the serum levels of AST and ALT after acute treatment (7 days) without significant damage to the males Wistar rats’ hepatocytes. However, Noguchi et al. (2002) observed a reduction in serum levels of liver enzymes (AST and ALT) after the treatment with *C. reticulata* oil-resin. A study conducted by Castro-E-Silva et al. (2004) identified antiproliferative activity during liver regeneration in rats after using oil-resin *C. duckei*. According to the author, this oil-resin can alter mitochondrial function, decoupling and causing a relative decrease of regeneration and liver function.

Serum creatinine and blood urea nitrogen are two important factors to assess the glomerular filtration rate, which can be elevated or decreased in certain situations. Creatinine is an endogenous compound obtained by the creatine muscle metabolism, and their production is continuous by the organism. The value of this serum metabolite, besides renal function, depends on muscle mass, nutrition and the occurrence of edema (Pasupathy, Dhanalakshmi, Ponnusha, Ambika, & Cystatin, 2011). In this study, serum creatinine levels (male and female) were higher in the control group. However, in males the values were within the reference range (Dantas et al., 2006), and it is not associated with any significant clinical change, but there is some susceptibility in females. This finding may be associated with the use of subacute *C. duckei* oil-resin, however, further studies, such as dosage of urea and morphological and histological analysis of the kidneys, must be carried out to confirm this change in renal function and/or kidney damage (Tables 1 and 2).

Brito et al. (2001) evaluated the renal function of rats, dosing urea and creatinine, after oral treatment, subacute (14 days), with *C. reticulata* oil-resin (0.06 ml/kg and 0.63 ml/kg), and concluded that oil-resin cannot alter the renal function of Wistar rats. In another study (Brito et al., 2005), it was noted that the improvement of renal function in rats with the syndrome of ischemia and reperfusion after administration, during seven days, of *C. multijuga* oil-resin (0.63 ml/kg).
Moreover, the use of *C. officinalis* oil-resin caused diffuse glomerular vasocongestion, which can pathologically alter renal function; however, urea and creatinine levels were not measured in the study (Botelho et al., 2010). According to Sá et al. (2015), changed urea plasma levels in rats are not good indicators of renal damage, but changes in creatinine levels can be an entrust level indicator to assess the presence of injury, because serum levels are not influenced by diet, age, and sex.

The oral treatment with *C. duckei* oil-resin caused a hypoglycemic effect in both males and females. This pharmacologic action may be associated with the presence, in this oil-resin, of Kaurenoic acid. This constituent is a diterpene acid present in the resinous fraction of the *Copaifera* sp oils (Cascon & Gilbert, 2000; Leandro et al., 2012; Veiga-Júnior & Pinto, 2002). According to Bresciani et al. (2004), the hypoglycemic potential effect of *Wedelia paludosa* may be associated, paired up, to the kaurenoic acid present in several parts of the plant.

The subacute use the CVC has not altered the biochemical parameters analyzed. Nevertheless, the results confirm the absence of systemic toxicity, because most of the parameters were within normal limits, probably due to the no occurrence of drug absorption. The use of topical medications aimed a local action, e.g. to the skin and mucous membranes, and have low toxicity. According to Robert and Scialli (1994), the skin may function as a depot of active substances. However, if the skin is full, rarely the drug will be absorbed in a significant amount.

In the assessment of hematological parameters, *C. duckei* oil-resin, administered orally, did not interfere in the complete blood count values (erythrocyte and leukocyte count) in mice (males and females). The subacute CVC use modified hemoglobin, hematocrit and lymphocyte. The MCV and MCHC values, commonly used in the morphological classification of anemia, were within the normal range in this study (Dantas et al., 2006). Chronic renal and hepatic insufficiencies can cause anemia, normochromic normocytic type, characterized by MCV and MCHC within the reference range. However, despite the decrease of the hemoglobin concentration in the blood of rats treated with CVC, the hypothesis of anemia can be ruled out; because although the difference between groups was significant, hemoglobin and hematocrit values are within the normal range (Dantas et al., 2006) (Tables 3 and 4).

Conversely, the values of monocytes, eosinophils, and platelets of control and treated groups (CVC and ORCV) are above the reference values (Dantas et al., 2006), but this result is acceptable, since variations on several parameters, both hematological and biochemical may exist in rats and mice (Tables 3 and 4). According to Sá et al. (2015), the differences may be related to gender, lineage, genotype and also can be influenced by the environment, manipulation, diet, age, and other factors.

**7. Conclusion**

The subacute treatment with *C. duckei* oil-resin and cream (CVC) did not cause clinical signs of toxicity, no deaths have been reported and did not change significantly the parameters evaluated in this study. These results corroborate and support the results obtained by Lima et al. (2011). However, it is necessary to further studies, as the histopathology of organs after subacute use of *C. duckei* oil-resin.
List of abbreviations

- ALT: alanine aminotransferase
- ANOVA: Analysis of Variance
- AST: aspartate-aminotransferase
- BVC: base vaginal cream
- BVC (ivag): base vaginal cream treated intravaginally
- CVC: Copaiba oil-resin vaginal cream
- CVC (ivag): Copaiba oil-resin vaginal cream treated intravaginally
- HDL: High-Density Lipoprotein
- ivag: treated intravaginal
- MCH: Mean corpuscular hemoglobin
- MCV: mean corpuscular volume
- ORC: Copaíba oil-resin (C. duckei)
- ORC (p.o): Copaíba oil-resin (C. duckei) treated orally
- ORCV: Copaíba oil-resin (C. duckei) treated intravaginally
- ORCV (ivag): Copaíba oil-resin (C. duckei) treated intravaginally
- p.o: treated orally
- RBC: red blood cells
- WBC: white blood cells

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Competing Interests
The authors declare no competing interest.

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