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Topic application of copaiba oil promotes wound healing without systemic toxicity in Wistar rats

Aplicação tópica de óleo de copaíba promove a cicatrização de feridas sem toxicidade sistêmica em ratos Wistar

La aplicación tópica de aceite de copaiba promueve la cicatrización de heridas sin toxicidad sistémica en ratas Wistar

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Abstract
Copaiba oil extracted from Copaifera spp., has been increasingly studied for its therapeutic benefits, including wound healing, anti-inflammatory, and anti-tumor properties. In a previous study, we demonstrated the positive impact of copaiba oil on skin wound repair in rats, notably enhancing lesion contraction and dermal annex approximation. However, despite its therapeutic potential, further toxicological evaluations are necessary to understand the safety of prolonged exposure to copaiba oil, particularly at low doses. To address this gap, the current study aimed to assess the effects of topical copaiba oil treatment on hematological, biochemical profile, and histopathological exams in male Wistar rats with surgical wounds. Forty-eight male Wistar rats with surgically induced skin wounds were randomly distributed into two groups (n=24): one group was treated with copaiba oil, while the other received mineral oil as a control. Topical treatments were administered once daily for a period of 21 days following the creation of the surgical wounds. Hematological and biochemical analyses revealed no significant alterations attributable to copaiba oil treatment. Similarly, histopathological evaluation of the heart, liver, and kidneys showed no evidence of tissue damage or morphological changes. These findings support the safety of topical copaiba oil, indicating it promotes wound healing without inducing systemic toxicity or hematological disturbances in rats.
Keywords: Copaiba oil; Blood profile; Rat; Toxicological evaluation.

Resumo
O óleo de copaíba, extraído de *Copaifera* spp., tem sido cada vez mais estudado por seus benefícios terapêuticos, incluindo cicatrização de feridas, propriedades anti-inflamatórias e antitumorais. Em um estudo anterior, demonstramos o impacto positivo do óleo de copaíba no reparo de feridas cutâneas em ratos, notavelmente

melhorando a contração da lesão e a aproximação do anexo dérmico. No entanto, apesar de seu potencial terapêutico, avaliações toxicológicas adicionais são necessárias para entender a segurança da exposição prolongada ao óleo de copaíba, particularmente em baixas doses. Para preencher essa lacuna, o presente estudo teve como objetivo avaliar os efeitos do tratamento tópico com óleo de copaíba nos perfis hematológicos e bioquímicos e, nos exames histopatológicos em ratos Wistar machos com feridas cirúrgicas. Quarenta e oito ratos Wistar machos com feridas cutâneas induzidas cirurgicamente foram distribuídos aleatoriamente em dois grupos (n=24): um grupo foi tratado com óleo de copaíba, enquanto o outro recebeu óleo mineral como controle. Os tratamentos tópicos foram administrados uma vez ao dia por um período de 21 dias após a criação das feridas cirúrgicas. As análises hematológicas e bioquímicas não revelaram alterações significativas atribuíveis ao tratamento com óleo de copaíba. Da mesma forma, a avaliação histopatológica do coração, fígado e rins não mostrou evidências de danos teciduais ou alterações morfológicas. Esses achados corroboram a segurança do óleo de copaíba tópico, indicando que ele promove a cicatrização de feridas sem induzir toxicidade sistêmica ou distúrbios hematológicos em ratos.
Palavras-chave: Óleo de copaíba; Perfil sanguíneo; Rato; Avaliação toxicológica.

Resumen
El aceite de copaiba, extraído de *Copaifera* spp., se ha estudiado cada vez más por sus beneficios terapéuticos, incluyendo propiedades cicatrizantes, antiinflamatorias y antitumorales. En un estudio previo, demostramos el impacto positivo del aceite de copaiba en la reparación de heridas cutáneas en ratas, mejorando notablemente la contracción de la lesión y la aproximación del anexo dérmico. Sin embargo, a pesar de su potencial terapéutico, se requieren más evaluaciones toxicológicas para comprender la seguridad de la exposición prolongada al aceite de copaiba, especialmente a dosis bajas. Para abordar esta deficiencia, el presente estudio tuvo como objetivo evaluar los efectos del tratamiento tópico con aceite de copaiba en los exámenes hematológicos, bioquímicos e histopatológicos de ratas Wistar macho con heridas quirúrgicas. Cuarenta y ocho ratas Wistar macho con heridas cutáneas inducidas quirúrgicamente se distribuyeron aleatoriamente en dos grupos (n=24): un grupo recibió aceite de copaiba, mientras que el otro recibió aceite mineral como control. Los tratamientos tópicos se administraron una vez al día durante 21 días tras la creación de las heridas quirúrgicas. Los análisis hematológicos y bioquímicos no revelaron alteraciones significativas atribuibles al tratamiento con aceite de copaiba. Asimismo, la evaluación histopatológica del corazón, el hígado y los riñones no mostró evidencia de daño tisular ni cambios morfológicos. Estos hallazgos respaldan la seguridad del aceite de copaiba tópico, lo que indica que promueve la cicatrización de heridas sin inducir toxicidad sistémica ni alteraciones hematológicas en ratas.
Palabras clave: Aceite de copaiba; Perfil sanguíneo; Rata; Evaluación toxicológica.

1. Introduction

Copaiba oil is a natural product composed of a solid, non-volatile resin, formed by acids obtained from the trunk exudation of various species of plants of the genus *Copaifera*, such as *C. multijuga* Hayne (Westphal et al., 2014), *C. reticulata* Ducke (Sachetti et al., 2009) and *C. langsdorfii* (Gushiken et al., 2023). *C. reticulata* is one of the most common species found in Central and Eastern Amazonia (Arruda et al., 2019). Theopaiba oil is commonly used in Brazil as anti-inflammatory, anti-tumor, and anti-septic agent and for treatment of skin disorders and wound healing. Several authors suggested the benefits of the oil resin in human health (Paiva et al., 2002; 2013; Arruda et al., 2019; Santana et al., 2024). The proportion of the chemical constituents present in the oil resin differs considerably according to the species (Veiga Junior et al., 2007, Arruda et al., 2019). Sesquiterpenes contribute to 78% of the composition of the oil resin from *C. reticulata*, being b-caryophyllene the main compound found (Veiga Junior et al., 2007). Diterpenes are present in a low concentration (21,8%) and the main compounds are methyl kaurenoate and methyl kolavenate. These major chemical constituents (diterpene and sesquiterpene acids) are responsible for its pharmacological activities (Gomes et al., 2007; da Trindade et al., 2018).

Several reports in the literature have already shown the therapeutic properties of copaiba oil including anti-inflammatory wound healing, and antibiotic effects (Veiga Junior et al., 2007; Corrêa et al., 2021). Also, there are reports of its antinociceptive and antimicrobial activities (Gomes et al., 2007) but none of those studies evaluated the effects of the treatments on hematological, biochemical, inflammatory, or anatomic pathological parameters. Some studies report side effects associated with the use of copaiba oil as anti-inflammatory and cicatricial agent.

Toxic effects such as thickening of visceral pleura, adherences between visceral and parietal pleura, intense exudation of fibrin and severe pleurisy were found in rats after the injection of copaiba oil extract into the pleural space (Westphal et al.,

2014). Kaurenoic acid, a diterpene isolated from the oleo-resin of *C. langsdorffii* was studied in developing sea urchin (*Lytechinus variegatus*) embryos, on tumor cell growth in microculture tetrazolium (MTT) test and on mouse and human erythrocytes in hemolysis assay. Continuous exposure of embryos to kaurenoic acid starting immediately after fertilization inhibited the first cleavage and progressively induced embryo destruction for blastulae and larvae stages, respectively (Costa-Lotufo et al., 2002). When administered to pregnant mice for 5 days, the copaiba oil extract of *C. langsdorffii* showed no maternal toxicity, but induced lower body weight and lower fetal length in the offspring of treated animals (Lourenço et al., 2009).

Sachetti et al. (2009) evaluated the acute oral administration of copaiba oleoresin extracted from *C. reticulata* in Wistar rats. There were no clinical signs of toxicity or neurotoxicity, change in food intake or body weight of the animals. Other studies also have found the use of copaiba oleoresin to be safe as a therapeutic agent (Sachetti et al., 2009; Teixeira et al., 2017). Despite that, the authors emphasize the need for additional toxicological studies to better clarify the safety of this medicine.

Traumatic injuries and surgical wounds are frequently seen in medical and veterinary medicine. Facilitating the healing process of these injuries, speeding up the repair of acute wounds, reducing scar formation and minimizing the aesthetic impact on the patient with a maximal restoration of tissue function remains a central concern in clinical care (Eming et al. 2014).

In our previous work, we demonstrated that copaiba oil resin healed the skin wounds induced in rats quickly and effectively, leaving minimal scars compared to control groups. Our data showed that copaiba oil resin induced faster contraction of the margins of the lesions at the 7th and 14th days and total healing of the wound within 21 days of treatment. Besides the minimal scar in the wound sites, there was a better approximation of dermal annexes (Corrêa et al., 2021).

The current study aimed to assess the effects of topical copaiba oil treatment on hematological, biochemical profile, and histopathological exams in male Wistar rats with surgical wounds. We seek to obtain relevant information to the safe use of copaiba oleoresin from *C. reticulata* as a wound healing agent.

2. Methodology

A laboratory research was conducted, of a quantitative nature (Pereira et al., 2018) and employing simple descriptive statistics using data classes and values of mean and standard deviation (Shitsuka et al., 2014) and statistical analysis.

Plant material and Copaiba oil resin extraction

The copaiba oil resin from *Copaifera reticulata* Ducke was obtained at km 67 of the Tapajós National Forest (municipality of Belterra, State of Pará, Brazil) and was kindly provided by Dr. Osmar Alves Lameira from Embrapa Amazônia Oriental, Belém, PA. The plant material was identified in the EMBRAPA Eastern Amazon Botany Laboratory, Brazil, where a voucher specimen is deposited and registered under number 183,939 (IAN Herbarium, Embrapa Amazônia Oriental). The harvesting procedure of the oil followed the International Guidelines recommended by the World Health Organization (WHO, 2003). The composition of the oleoresin was quantified through chromatography.

Animals

Forty-eight male Wistar rats (*Rattus norvegicus*), eight weeks old with a mean weight of 200g, were supplied by the animal facility, LaBIO, of the Universidade Estadual de Santa Cruz, Brazil. The animals were housed in cages (40x45x45cm) on a light/dark cycle of 12/12 hours and received commercial food and water *ad libitum*. Housing, anesthesia, and post-operative care adhered to the guidelines established by the Universidade Estadual de Santa Cruz for Animal Experimentation.

This experiment was in agreement with the Ethical Principles in Animal Experimentation adopted by the Ethics Commission for Animal Use of Universidade Estadual de Santa Cruz, Brazil (CEUA/UESC) (Protocol 016/13).

Experimentally Inflicted Wounds and Topical Treatment Protocol

All animals were premedicated with tramadol hydrochloride (5 mg/kg, intramuscularly). Fifteen minutes later, anesthesia was induced with halothane (Tanohalo®, Cristália Laboratory) using an open system with facial masks connected to a Baraka-type KTK Samurai apparatus (model 3304), at an initial concentration of 4% (vapor volume) for induction, followed by 1.5% for maintenance, adjusted according to Guedel's signs. The left dorsal-lumbar region was shaved and disinfected with povidone-iodine. A standardized 2 × 2 cm full-thickness excisional wound was created using a scalpel, exposing the subcutaneous muscle tissue.

Immediately after the procedure, wounds were covered with sterile gauze and bandage. Twenty-four hours after surgery, animals were randomly assigned to two groups (n=24 per group). Group 1 (control) received daily topical treatment with 0.2 mL of mineral oil, while Group 2 received 0.2 mL of copaiba oil (*Copaifera reticulata* Ducke), both applied directly to the wound bed using a calibrated dropper or syringe. In the presence of exudate, wounds were cleaned with sterile gauze and 0.9% saline solution prior to treatment. After application, lesions were re-covered with sterile gauze and elastic bandage. Treatments were repeated daily until euthanasia.

For postoperative analgesia, all animals received a second dose of tramadol hydrochloride (5 mg/kg, intramuscularly) 12 hours after surgery. Six animals from each group were euthanized under deep halothane anesthesia on the 3rd, 7th, 14th, and 21st days of treatment for subsequent clinical, hematology, biochemical, and histopathological analyses.

Hematology

Blood samples were collected immediately before euthanasia, stored in flasks with EDTA 10% or without anticoagulant for whole blood, plasma, and serum analysis. Hematological parameters including leukocytes (WBC), erythrocytes (RBC), platelet counts, hemoglobin (Hb), and packed cell volume (PCV), were assessed using a hemocytometer (ABC Vet Animal blood counter, Brazil). Red blood cell indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated. Blood smears were stained using the May-Grünwald-Giemsa technique to obtain a differential leukocyte count based on the analysis of 100 cells per smear (Thrall, 2004).

Biochemical Analysis

Renal and hepatic functions were assessed through the biochemical profile of the serum, measuring levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine using colorimetric analysis with commercial kits (Labtest, Brazil) and a chemistry analyzer (Bioplus® – Bio 2000). The plasmatic levels of alkaline phosphatase (ALP), creatine kinase (CK), and gamma-glutamyl transferase (GGT) were analyzed using commercial kits from Bioclin (Brazil) and a chemistry analyzer (TP Analyzer Basic, Thermoplate).

Plasma protein concentration was measured using a refractometer (Ningbo Utech International CO Ltda, model 301). Plasma protein concentration was measured using a refractometer (Ningbo Utech International CO Ltda, model 301). Plasmatic protein fractions were obtained by electrophoresis using an agarose gel electrophoresis system (CELM SDS-60, Brazil). Briefly, a 1.0 µL sample of plasma was applied to the gel, which was exposed to 100V for 32 min. The gel was stained (Ponceau S, Vetec Química Fina Ltda, Duque de Caxias-RJ, Brazil), fixed, and dried. Bands were scanned and measured using the software Celm SE-250. Percentage and absolute values (g/dL) for the protein fractions were determined based on total

protein concentration. The albumin/globulins ratio was calculated as albumin/globulins (α1+α2+β+ɣ globulins).

Anatomic pathology parameters: pathological analysis

After euthanasia, gross examination was performed, and the spleen, kidney, and liver were collected, fixed in 10% buffered formalin, paraffin-embedded, then sectioned at 5µm, and stained with hematoxylin and eosin (HE) for histopathological assessment.

Statistical Analysis

Data were presented as mean±SD and analyzed using one-way ANOVA. The Tukey test was applied to compare the differences between treatment groups at each time point**.** The Bonferroni test was used to compare the differences across time points within the same treatment group**.** P values < 0.05 were considered significant. Statistical analysis was performed using GraphPad Prism (version 8), (GraphPad Software Inc., San Diego, CA, USA).

3. Results and Discussion

Oil-Resin Composition

Chromatographic analysis of the *Copaifera reticulata* oil-resin revealed a range of volatile components, with the most prominent being β-caryophyllene (37.6%), followed by β-bisabolene (13.9%), trans-α-bergamotene (9.3%), α-humulene + (E)-β-farnesene (5.3%), β-selinene (4.9%), and other minor compounds, as shown in Table 1.

Table 1 - Percentage of volatile components present in *Copaifera reticulata* Ducke oleoresin.

Components %		Components %	
β-caryophyllene	37,6	Ciclosativeno	0,9
β-bisabolene	13,9	β-chamigrene	0,9
Trans-α-bergamotene	9,3	γ-gurjunene	0,6
α-humulene + (E)-β-farnesene	5,3	γ-curcumene	0,6
β-selinene	4,9	α-copaene	0,5
β-elemene	3,3	α-gurjunene	0,4
α-selinene	3,1	β-curcumene	0,4
α-bulnesene	2,1	Caryophyllene oxide	0,2
(Z)-α-bisabolene	1,8	δ-elemeno	0,2
(E)-γ-bisabolene	1,3	β-bisabolol	0,2
β-sesquiphellandrene	1,1	*Eepi*-β-santalene	0,1
Aromadendrene	0,9	*Epi*-β-bisabolol	0,1

Source: Research data (2025).

Copaiba oil resin is composed of diterpenic acids and sesquiterpenes that are responsible for healing, anti-inflammatory, antinociceptive and antimicrobial activities (Paiva et al., 2002). The pharmacological activities of this oleoresin have been attributed to sesquiterpenes because they are present in greater quantities, but all components act synergistically. Therefore, it is very important to test the toxicity of this oil resin.

Effects of topic copaiba oil treatment on hematology of Wistar rats

The study of hematology is a fundamental tool for observing the response of animals to treatments. We evaluated

several hematological parameters to assess the effects of topical copaiba oil treatment on blood health over a 21-day period in Wistar rats with surgical wounds. Erythrogram (RBC, Hb, PVC, MCV, MCH, and MCHC) and platelets were assessed on days 3, 7, 14, and 21 of treatment with copaiba oil and mineral oil (Table 2).

Table 2 - Effect of copaiba oil treatment for 21 days on erythrogram parameters (red blood cells – RBC; hemoglobin – Hb; packed cell volume – PCV; mean corpuscular volume – MCV; mean corpuscular hemoglobin – MCH; mean corpuscular hemoglobin concentration -MCHC) and platelets of Wistar rats with surgical wounds.

	Day 3		**Day 7**		**Day 14**		**Day 21**	
Blood parameter	**Copaiba oil**	**Mineral oil**	**Copaiba oil**	**Mineral oil**	**Copaiba oil**	**Mineral oil**	**Copaiba oil**	**Mineral oil**
RBC	5.85±0.31	6.83±0.41	5.91±0.24	6.59±0.52	5.57±0.44	6.14±0.24	6.39±0.12	6.29±0.39
x10^6 µL^{-1}	aAB	bA	aAB	bAB	aA	aB	aB	aAB
Hb (g dL^{-1}	11.84 ±0.69	13.74 ±0.51	11.98± 0.52	12.68± 0.68	11.14 ±0.49	11.72±1.33	11.78 ±0.56	11.78± 0.56
	aA	bA	aA	aAB	aA	aB	aA	aB
PCV (%)	33.78±2.96	37.92±1.99	33.66±1.62	37.6±2.30	31.82±2.56	33.58±2.68	33.2±0.81	34.08±1.5
	aA	bA	aA	bAC	aA	aB	aA	aBC
MCV fL	57.81±4.60	55.57±1.55	57.02±4.94	57.22±4.40	57.28±4.94	54.74±4.43	52.03±1.33	54.27±3.54
MCH pg	20.25±0.43	20.17±1.28	20.28±0.85	19.31±1.59	20.25±0.92	19.08±2.04	18.46±0.95	18.80±1.80
MCHC %	35.17±2.27	36.32±2.42	35.67±2.51	33.74±0.53	35.11±1.85	34.84±1.71	35.48±1.44	34.64±2.60
Platelets	830,8± 100,9	805,8± 55,1	756± 84,7	874± 44,4	744,6± 44,1	706,3± 84,3	792,8± 88,8	759,8±101,6
x10^3 µL^{-1}	A	AB	A	A	A	B	A	AB

Data presented as mean±SD ($p \leq 0.05$). Values with different letters in the same line are significantly different. Small letters are used to compare treatments at the same time point, and capital letters compare the same group at different time points. Source: Research data (2025).

At the initial evaluation time points (days 3 and 7), animals treated with copaiba oil showed a lower RBC count, hemoglobin concentration, and packed cell volume ($p \leq 0.05$) compared to the mineral oil-treated group (control). However, at days 14 and 21, the erythrogram results were statistically similar. Although the values were slightly below the minimum reference limit for the species, they did not alter the hematimetric indices, which remained within the reference ranges (MCV: 50–70 fL; MCHC: 32–36 g/dL) as reported by Kaneko et al. (2008) and Thrall et al. (2015). Changes in hematological constituents reflect the animal's physiological response to changes in the internal and external environment, including the effects of drug treatments (Kaneko et al., 2008). Exposure to plant extracts has already been associated with erythrocyte destruction and hemolytic anemia as part of a clinical syndrome associated with poisoning. Interaction with sulfhydryl groups, inhibition of enzymes and immune mechanisms, and fragmentation of erythrocytes as they pass through the platelet-fibrin mess are some mechanisms by which natural compounds can cause hemolysis (Costa-Lotufo et al., 2002). Changes in RBC indices associated with a decrease in RBC production in rodents include a decrease in MCV, MCHC, and RDW (red blood cell distribution width). Our data showed no reduction in MCV or MCHC in those groups. Therefore, the decrease of RBC in copaiba oil groups, in the first week of treatment, was associated with hemorrhage on the site of lesion in the skin.

Previously, our group demonstrated that copaiba oil stimulates wound healing with a better contraction of the wounds

and approximation of the skin margins and annexes when compared to the control group (Corrêa et al., 2021). In the macroscopic and microscopic evaluation, there was hyperemia and multifocal hemorrhage in the wounds especially on the third and seventh day. Those hemorrhages were attributed to injuries caused by the daily removal of the gauze that adhered to the granulation tissue, causing vessel rupture during their removal. It is also important to note that, at 14 days, both groups showed the same erythrogram profile, i.e., lower red blood cell counts, hemoglobin, and packed cell volume. Also, the number of platelets was lower on day 14 in both groups. This means the treatment did not interfere, but the proposed challenge (surgical wound). Our result suggests the absence of toxicity of copaiba oil on red blood cells following its topic administration for 21 days.

No significant differences were observed in the WBC count between the copaiba oil and mineral oil groups at any time point (Table 3).

Table 3 - Effect of copaiba oil treatment for 21 days on leukogram parameters (white cell count – WBC; and relative counts (%): lymphocytes, monocytes, neutrocytes and eosinophils) of Wistar rats with surgical wounds.

	Day 3		Day 7		Day 14		Day 21	
WBC x10^3 µL^{-1}	9.3±4.1	7.7±3.5	8.5±4.3	11.6±5.3	9.3±2.7	9.4±3,.2	7.2±1.1	5.8±1.3
Lymphocyte %	69.2±5.6	65.4±1.4	59.6±17.5	58.6±9.6	65.0±13.4	51.1±9.3	67,6±9.4	55.4±9.3
Monocytes %	4.7±1.8	4.6±2.1	3.6±2.9	2.5±1.9	3.0±1.6	3.3±1.5	3.3±0.8	4.5±2.9
Neutrophils %	23.3±5.8	27.8±15.3	35.1±16.4	37.1±11.4	31.6±12.4	41.7±7.6	26.5±8.9	35.0±7.4
Eosinophils %	2.8±1.8	2.2±0.5	1.7±0.5	1.8±1.5	0.4±3.6	3.9±0,1	2.6±1.5	4.8±2.2

Data presented as mean±SD ($p>0.05$). Source: Research data (2025).

Values remained within the reference range for Wistar rats (6000–17000/µL) as reported by Kaneko et al. (2008) and Thrall et al. (2015). Similarly, the differential leukocyte count (lymphocytes, monocytes, neutrophils, and eosinophils) showed no significant changes between the groups across the 21-day period, with values consistent with the reference range of Wistar rats (Kaneko et al., 2008; Thrall et al., 2015). This indicates that copaiba oil did not significantly affect leukocyte populations during the treatment.

Effects of topic copaiba oil treatment on biochemical profile of Wistar rats

The enzymes AST and ALT are widely used as biomarkers for assessing liver integrity and damage, with their concentrations providing valuable information for diagnosing liver toxicity during treatment. Topical treatment with copaiba oil for 21 days did not significantly affect AST and ALT at any time point evaluated (Table 4). The plasma concentrations of ALT and AST in the copaiba oil-treated Wistar rats ranged from 29.0 to 55.6 IU/L and 60.0 to 100.6 IU/L, respectively, which are within the physiological limits for this specie. These findings align with the reference intervals established by Kaneko et al. (2008) and Thrall et al. (2015), indicating that the topical application of copaiba oil did not induce significant changes in these liver enzymes.

Table 4 - Effect of copaiba oil treatment for 21 days on serum/plasma biochemical enzymes (IU/L) aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), and urea and creatinine (mg/dL) of Wistar rats with surgical wounds treated with copaiba oil for 21 days.

	Day 3		**Day 7**		**Day 14**		**Day 21**	
Plasma profile	**Copaiba oil**	**Mineral oil**	**Copaiba oil**	**Mineral oil**	**Copaiba oil**	**Mineral oil**	**Copaiba oil**	**Mineral oil**
ALT IU/L	29.0±9.9	34.6±6.9	42.20±6.3	55.0±28.7	39.0±5.8	55.6±17.9	32.2±7.7	47.6±8.4
AST IU/L	69.4±20.5	68.6±6.7	92.0±9.4	100.6±36.8	88.4±21.7	97.6±26.6	60.0±22.2	75.40±17.1
GGT IU/L	3.32±3.41	3.18±0.56	3.18±1.69	3.08±0.45	4.56±0.45	3.83±1.79	3.25±1.93	2.62±1.41
ALP IU/L	208.48±36.18	210.36±54.11	285.78±46.18	314.68±121.87	323.77±111.70	369.22±72.28	284.47±49.20	297.92±81.4
CK U/L	84.50±75.15	28.00±15.51	83.03±45.97	47.44±26.56	84.84±50.70	47.12±22.48	118.11±79.80	26.36±13.09
Urea mg/dL	34.80±8.01	34.20±3.56	37.40±5.03	37.40±5.81	33.60±6.23	34.20±7.82	37.80±9.86	44.80±4.97
Creatinine mg/dL	0.30±0.07	0.30±0.00	0.30±0.00	0.36±0.05	0.34±0.05	0.30±0.00	0.30±0.07	0.36±0.05

Data presented as mean±SD ($p>0.05$). Source: Research data (2025).

Gamma-glutamyltransferase (GGT) is an induction enzyme synthesized by almost all body tissues, with highest concentrations in the liver (bile ducts) and kidneys. Acute liver injury can cause an immediate increase in serum activity, possibly due to the release of membrane fragments containing GGT. In cases of cholestasis, increased production and release, and consequently increased activity, are observed (Thrall et al. 2015). Topical treatment with copaiba oil for 21 days did not significantly affect GGT at any time point evaluated (Table 4). The concentrations of GGT in the copaiba oil-treated Wistar rats ranged from 3.18 to 4.56 IU/L, which are within the physiological limits for this specie.

Serum alkaline phosphatase (ALP) activity is frequently measured in toxicity studies. Principal organs which contribute to the source of serum alkaline phosphatase are bone, intestines, and liver. Elevations in the serum can be due to pathological injury; failure of removal due to excretory obstruction; or due to an increased synthesis and release into the circulation (Kaneko et al. 2008). It can be observed that at all times (Table 4), animals treated with copaiba oil showed lower serum alkaline phosphatase values when compared to animals treated with mineral oil, although without a significant difference ($p>0.05$).

Creatine kinase (CK) is a key enzyme for energy metabolism during contraction and relaxation in skeletal muscle. This enzyme is also correlated with mitochondrial oxidative phosphorylation. Its distribution is quite broad, including skeletal muscle, myocardium, central nervous system, and smooth muscle (Kaneko et al., 2008). CK plays an important role in soft tissues, and the CK-BB isoform is overexpressed in keratinocytes during wound healing (Schlattner et al., 2002). When observing CK concentrations in the study groups, animals treated with copaiba oil had higher concentrations compared to animals treated with mineral oil (control) at all time points, although no significant differences were found ($p>0.05$). Since keratinocytes overexpress CK-BB during wound healing (Schlattner et al., 2002), this finding may reflect increased cellular metabolic activity related to tissue repair. Further studies are needed to confirm the involvement of CK-BB in the acceleration of copaiba-induced wound healing.

The mean serum urea concentrations in all groups ranged from 34.20 to 44.80mg/dL considering all time points tested. Values found for urea concentration were above the physiological limit for the strain described in the literature (19 mg/dL). However, it probably reflects the concentration of dietary protein. It is well known that high-protein diets induce its oxidation for energy, thus increasing urea synthesis in liver and it was already shown an increase in blood urea content with increasing the protein content of diet in growing rats (Wang et al., 2014). The absence of changes in liver and kidney tissue and measured biomarkers enzymes lead to the hypothesis that increased urea concentrations found in this study are associated with diet rather than a possible toxic effect of copaiba oil in those organs. It is important to note that there were no differences between groups at the same time point. Furthermore, no significant difference in urea concentration was observed within the same group when comparing different times. Regarding creatinine, it is important to emphasize that the values obtained (ranging from 0.30 to 0.36 mg/dL) are within the reference range for Wistar rats, which, according to Kaneko et al. (2008) and Thrall et al. (2015), is approximately 0.15 to 0.50 mg/dL. Therefore, topical application of copaiba oil for 21 days did not induce significant changes in the biochemical parameters evaluated, suggesting that it did not negatively impact renal or soft tissue function in Wistar rats under the conditions of this study.

Effects of topic copaiba oil treatment on serum protein levels of Wistar rats

Agarose gel electrophoresis was conducted to analyze plasma protein levels in Wistar rats with surgical wounds treated with copaiba oil for 3, 7, 14, and 21 days. The electrophoretograms revealed five distinct protein bands: albumin, α1-globulin, α2-globulin, β-globulin, and γ-globulin. The relative and absolute plasma protein concentrations are summarized in Table 5.

Monitoring acute phase proteins and acute phase response is a quick and easy way to assess the response of the innate immune system to organic disorders and has been considered an emerging biomarker for use in animals for health monitoring (Kaneko et al., 2008). Therapies applied can cause changes in plasma protein concentrations. In this study, we aim to investigate the safe use of copaiba oil resin as a healing medicine and better understand its interventions on acute and chronic inflammatory responses.

Table 5 - Mean, standard deviation and statistical analysis results of protein levels in adult Wistar rats treated with copaiba oil.

	Day 3		**Day 7**		**Day 14**		**Day 21**	
	Copaiba oil	**Mineral oil**	**Copaiba oil**	**Mineral oil**	**Copaiba oil**	**Mineral oil**	**Copaiba oil**	**Mineral oil**
Total protein (g/dL)	5.90±0.24 aA	5.58±0.29 aA	6.06±0.31 aAB	5.98±0.38 aAB	6.00±0.14 aAB	6.24±0.33 aB	6.36±0.17 aB	6.04±0.09 aB
Albumin (%)	43.92±5.27 aA	50.26±2.82 aA	47.38±4.30 aAB	49.82±4.87 aA	46.92±2.50 aAB	54.86±4.90 bAB	53.94±3.66 aB	58.60±5.57 aB
Albumin (g/dL)	2.80±0.32 aA	2.91±0.31 aA	2.79±0.26 aA	3.23±0.49 aAB	2.97±0.26 aA	3.84±0.32 bB	3.39±0.17 aA	3.29±0.85 aAB
α1-globulin (%)	23.58±3.80 aA	23.20±1.68 aA	21.14±2.25 aA	20.24±3.35 aAB	20.42±4.10 aA	17.72±2.17 aB	19.24±1.71 aA	15.94±3.18 aB
α1-globulin (g/dL)	1.51±0,28 aA	1.34±0.08 aA	1.32±0.17 aA	1.31±0.22 aA	1.29±0.25 aA	1.27±0.23 aAB	1.21±0.13 aA	0.91±0.36 aB
α2-globulin (%)	5.64±1.03 aA	4.76±1.11 aA	5.52± 0.85 aA	3.72± 1.17 aA	3.12± 1.52 aB	3.54± 2.35 aA	3.30±1.06 aAB	3.08± 1.41 aA
α2-globulin (g/dL)	0.36±0,05 aA	0.27±0.06 aA	0.34±0.07 aAB	0.24±0.09 aA	0.19±0.09 aB	0.25±0,17 aA	0.21±0,07 aAB	0.17±0,06 aA

β-globulin (%)	18.60±1.71 aAB	15.90±0.82 aA	18.14±2.22 aAB	19.68±3.04 aB	21.24±2.55 aA	16.86±1.46 bAB	17.46±2.02 aB	15.58±1.82 aA
β-globulin (g/dL)	1.20±0.16 aA	0.92±0.10 aA	1.13±0.16 aA	1.26±0.14 aB	1.34±0.21 aA	1.20±0.21 aAB	1.11±0.18 aA	0.89±0.30 aA
γ-globulin (%)	8.18±1.17 aA	5.88±1.61 aA	7.82± 1.40 aA	6.54± 1.71 aA	8.30± 2.02 aA	7.02± 1.26 aA	6.06± 0.82 aA	6.80± 0.79 aA
γ-globulin (g/dL)	0.52±0.10 aA	0.34±0.08 bA	0.48±0.08 aA	0.42±0.11 aAB	0.53±0.13 aA	0.50±0,09 aB	0.38±0,05 aA	0.38±0,08 aAB
A/G ratio	0.80±0.18 aA	1.01±0.11 aA	0.87±0.23 aAB	1.01±0.20 aA	0.89±0.08 aAB	1.22±0.23 aAB	1.18±0.18 aB	1.45±0.30 aB

Data presented as mean±SD. ($p \leq 0.05$). Values with different letters in the same row are significantly different. Small letters compare treatments at the same time point, and capital letters compare the same group across different time points. Source: Research data, 2025.

Cutaneous wound healing is a complex process involving different cell types and sequential steps and overlapping phases of inflammation, proliferation, and tissue remodeling (Eming et al., 2014). The concentration of acute phase proteins can increase or decrease during this process (Kaneko et al., 2008). The results of this study indicated small changes in the protein profile of animals treated with copaiba oil resin or mineral oil.

Acute phase proteins modulate the inflammatory and immunological reactions and generally, it is considered that acute phase proteins have anti-inflammatory activities and negative proteins have pro inflammatory activities. Early tissue damage causes the release of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α, which together with nitric oxide and glucocorticoids modulate the acute phase reaction and acute phase protein production in the liver (Gruys et al., 2005).

The serum protein profile revealed no significant alterations in total protein levels, but demonstrated changes in specific protein fractions consistent with inflammatory resolution. Notably, albumin levels increased significantly over time in both treatment groups, aligning with its role as a negative acute phase protein and marker of inflammation resolution (Gruys et al., 2005). The total protein concentration ranged from 5.58 to 6.36 g/dL in the copaiba oil group and from 5.58 to 6.24 g/dL in the mineral oil group, which falls within the reference range for Wistar rats (5.5–7.5 g/dL; Kaneko et al., 2008; Thrall et al., 2015).

The mineral oil group showed a significant increase in albumin percentage on day 14 compared to the copaiba oil-treated group. On the 21st day, albumin percentage in the copaiba oil group increased from 43.92% (day 3) to 53.94%, whereas the mineral oil group exhibited a rise from 50.26% to 58.60%. Absolute albumin concentrations ranged from 2.80 to 3.39 g/dL in the copaiba oil group and from 2.91 to 3.84 g/dL in the mineral oil group. Significant increases in albumin values were observed between day 3 and day 21 for both treatment groups ($p \leq 0.05$).

Albumin is a negative acute phase protein and will decrease with inflammation. As it could be expected the mean values of albumin increased at the end of the experiment, when the wounds were healed and the inflammatory process ceased.

On the third day of treatment, there were no significant differences between the groups for α1-globulin (ranging from 1.34 to 1.51 g/dL in the mineral oil group and 1.51 to 1.34 g/dL in the copaiba oil group), α2-globulin (ranging from 0.27 to 0.36 g/dL in the mineral oil group and 0.36 to 0.27 g/dL in the copaiba oil group), and β-globulin (ranging from 0.92 to 1.20 g/dL in both groups). Although there were no statistical differences, the copaiba oil group had slightly higher mean values compared to the mineral oil group for all three globulin fractions. For α1-globulin and β-globulin, the values in their study were within the reference ranges cited by Kaneko et al. (2008) and Thrall et al. (2015). For α2-globulin, the values were slightly below the reference range but still relatively close to the lower limit. There was a decreasing trend in the α_1- and α_2-globulin fractions over the 21-day period, corroborating a shift from acute inflammation to the proliferative phase of healing. The only significant difference was observed in γ-globulin (ranging from 0.34 to 0.48 g/dL in the mineral oil group and from

0.52 to 0.34 g/dL in the copaiba oil group), where the copaiba oil group had a higher mean value compared to the mineral oil group. It is known that inflammatory diseases increase levels of the positive acute phase proteins that either migrate in the α-globulin region for example α2-macroglobulin and haptoglobin, or in the β-globulin region for example C reactive protein, serum amyloid A and fibrinogen. We hypothesize that the pattern of decreasing mean values presented here is linked to the resolution of the inflammatory process and the healing of the wounds. The α1-antitrypsin is a moderate acute phase protein and increases only two to three times its normal value during the acute response phase. It represents 90% of the α1-globulin band proteins, with the remaining 10% represented by α-1-acid glycoprotein (AGP) and alpha-fetoprotein, among others (Eckersall, 2000). The α1-antitrypsin is the most important component of the group of protease inhibitors with the function of neutralizing bacterial or leukocyte proteolytic enzymes during the acute inflammatory process. Its antiprotease activity minimizes damage to normal tissues (Kaneko et al., 2008). Haptoglobin, found in an α2-globulin band, has cleaning activities and binds to metabolites arising from cellular protein degradation, allowing them to be reinserted into the host's metabolic processes. It binds to free hemoglobin in the blood, preventing oxidative damage to the tissue, and indirectly exerts antibacterial activity by reducing the availability of heme and iron (Kaneko et al., 2008). Based on this information our data could suggest a decrease in the plasmatic concentration of these proteins and consequently in the protein bands with the resolution of the inflammatory process on the skin.

While γ-globulin levels remained stable throughout the study, their early increase in the copaiba group suggests a modest immune activation during the initial inflammatory phase. Overall, the protein dynamics observed are consistent with the expected physiological trajectory of wound healing and support the hypothesis that copaiba oil modulates the acute phase response favorably.

Despite slight differences in albumin and globulin values, no significant difference was found in the albumin/globulin ratio between the treatment groups. The ratio showed a trend toward an increase over time, with the highest value observed at day 21 for both groups (copaiba oil: 1.18, mineral oil: 1.45), suggesting a potentially more efficient resolution of inflammation.

Effects of topic copaiba oil treatment on anatomic pathology parameters of Wistar rats

Regarding the macroscopic findings of the kidneys, liver, and spleen, no significant changes in their appearance, size, or volume were observed in the study groups. There were also no microscopically visible changes in the kidneys, spleen, or liver of the animals in either group, reinforcing the systemic safety of topical copaiba oil. All tissues presented normal morphology. Histopathological analysis of the spleen and liver revealed no increased macrophage activity, hemosiderin accumulation, or morphological abnormalities, further corroborating the absence of systemic hemolysis. Therefore, the transient decrease in erythrocytes is most plausibly linked to local hemorrhage during the early stages of healing, potentially exacerbated by the daily removal of gauze from the wound site. Serum liver enzymes (ALT, AST, GGT, and ALP) remained within physiological limits. Although urea levels slightly exceed typical reference values (Kaneko et al., 2008), this increase likely reflects dietary protein intake rather than nephrotoxicity, as evidenced by normal creatinine levels and renal histology.

4. Conclusion

Hematological, biochemical, protein profile, and histopathological findings provide strong evidence that topical application of *Copaifera reticulata* oleoresin does not induce systemic toxicity in Wistar rats. The oil appears to modulate the inflammatory response during wound healing, as evidenced by favorable shifts in acute phase proteins and stable organ function markers. These results support the safe therapeutic use of copaiba oil in wound management and warrant further investigation into its mechanisms of action and clinical applications in chronic wound models.

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