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Phytochemical profile, toxicological evaluation of *Rhipsalis baccifera* (Sol.) Stearn (Cactaceae) extract and their antitumor activity in Ehrlich carcinoma-bearing mice

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ABSTRACT: *Rhipsalis baccifera* (Sol.) Stearn is a typical cactus from tropical regions with wide geographic distribution, and its therapeutic potential is not yet fully understood, such as antitumor property. Thus, this study evaluated the cytotoxic ethanolic extract of *R. baccifera* (EERB) and its antitumor activity against Erlich's tumor in mice. The EERB was obtained, and its phytochemical profile was filed by thin-layer chromatography. The toxicity was evaluated *in vitro* and *in vivo* using the microcrustacean *Artemia salina* Leach and mice. The lethal dose was determined after implantation of a tumor cell suspension, with subsequent treatment with EERB (200 mg/kg and 100 mg/kg) 48 h after implantation. These values represent the tenth part of the DL₅₀ and CL₅₀, respectively. The presence of phenols, tannins and triterpenes were demonstrated in the phytochemical results. Toxicity was dose-dependent, and the tumor inhibition was 84.1% and 75.8% at doses of 200 mg/kg and 100 mg/kg, respectively. We can highlight that the growth of Erlich's carcinoma suffered inhibitory effects against the EERB. EERB was found to have low acute toxicity and a high potential for use in antitumor therapy. Thus, new studies involving pre-clinical and clinical analyses of the extract are essential to determine the safe dose.

1. INTRODUCTION

Natural products, made up of chemical compounds and pharmacological properties, are highlighted nowadays, but their use dates back to ancient times. Therefore, they are recognized as an essential alternative in the treatment of various pathologies. As a highlight, we use active principles derived from plants, for

example, which date back to the first discoveries of modern medicine, and, today, plants continue to be the predominant source of natural medicine due to their secondary metabolites. About seventy-five percent of herbal medications recently in clinical use have their origins in traditional medicine (David et al., 2015; Hosseinzadeh et al., 2015; Maia-Neto et al., 2020).

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”Complementary”, ”alternative”, or ”unconventional” therapies for primary health care are more used than traditional medicines. It is estimated that 75% to 90% of citizens in most developing countries perform this approach, according to the World Health Organization (WHO) (Who, 2018). In the Brazilian territory, several public health projects are intended to encourage medicinal plants and advance alternatives for the insightful and low-cost treatment of various pathologies. The main objective is to provide treatments that would not be dependent on imported products and could be acquired locally through the development of family farming (L.F.G. Oliveira et al., 2017). Covering one of the most abundant biodiversity on the planet, Brazil encompasses more than 39.000 plant species (20-22% of the planet’s total value). Brazilian inhabitants have an extensive tradition of using medicinal plants to treat different acute and chronic pathologies, and many varieties of active principles have been isolated from medicinal plants in the Brazilian territory (Dutra et al., 2016).

In this way, the choice of plant species for analysis based on a therapeutic principle can be optimized to reveal new, more efficient drugs, less toxic, and/or with new mechanisms of action (L.P. Oliveira et al., 2010). Due to the severe environmental conditions (scarcity of water, intense radiation, temperature differences and poor soils), which only the arid and semi-arid areas of Brazil have, the plants of the Cactaceae family are developed and adapted to resist these unfavourable conditions Harlev, Nevo, Solowey, and Bishayee (2013). Phytochemicals that are produced to combat these stressors include alkaloids, flavonoids, terpenes and tannins. These metabolites concentrate the active principles of the plant that can present bioactivity against human diseases, such as cancer (Harlev et al., 2012) and diabetes Harlev, Nevo, Mirsky, and Ofir (2013).

Rhipsalis baccifera (Sol.) Stearn of the Cactaceae family is the only species to develop beyond the American territory due to its vast growth in humid tropical regions of the African continent and part of the Asian continent (Barthlott, 1983; Cassels, 2019; Cota-Sánchez & Bomfim-Patricio, 2010). Comprises 124 genera and 1,438 species, among which we have *R. baccifera*, also known as macaroni cactus (Anderson, 2001; Hunt et al., 2006; Taylor, 1997). This family has considerable economic importance, where many species are used as fodder in food (stems, leaves and fruits). In addition to its use in popular medicine, several studies with plants of the Cactaceae family are showing positive results in research in the area of healing, treatment of inflammatory processes and toxic against several tumor cell lines (Anderson, 2001; C.T.S. Andrade et al., 2006; T.C. Andrade et al., 2021; Barthlott & Hunt, 1993; Taylor, 1997).

According to the WHO, we can still highlight neoplastic pathologies as one of the leading causes of worldwide death. It is estimated that for Brazil, 625,000 new cancer cases (450,000, excluding cases of non-melanoma skin cancer) occurred for each year of the 2020-2022 triennium. Non-melanoma skin neoplasms will be the most frequent ones (177,000),

followed by breast and prostate (66,000 each), colon and rectum (41,000), lung (30,000) and stomach (21,000) cancers (INCA, 2020). Progress in cancer research and understanding the numerous mechanisms related to its etiology, progression, diagnosis, and treatment have been achieved, primarily due to experimental oncology. Transplantable or transmissible neoplasms started this fascinating branch of experimental oncology. Thus, they can be manipulated and preserved in the laboratory by ordered inoculation in susceptible animals or cell cultures (Guerra, 2002). Ehrlich’s tumor is a natural adenocarcinoma originating from the mammary gland of female mice, discovered by Paul Ehrlich in 1896. We can classify it in the ascitic form when inserted intraperitoneally or in its solid form when cells of this tumor are introduced subcutaneously or intramuscularly in mice (Dagli et al., 1992). The benefits of transplantable neoplasms, compared to other techniques, are concentrated in the advanced knowledge of the quantity and particularity of tumor cells to be implanted and in the accelerated development that improves the study time (Palermo-Neto et al., 2003).

Depending on the extension, type and location, one can select a single or a combination of treatments, such as chemotherapy, surgery and radiotherapy (Araújo et al., 2007). The current alternative strategies for cancer therapy remain on the rise, and analysis in divergent cell lines in epidemiological studies in humans and animals indicate a defending role of many plant molecules against different cancer lineages (Liu et al., 2013; Ullah et al., 2014). Therefore, scientific analyzes in clinical and/or experimental oncology play an increasingly notable role, which can help test natural compounds with possible antitumor action. Given the above, the current study aims to obtain an ethanolic extract of *R. baccifera* (EERB), perform its phytochemical characterization, evaluate its safety of use through toxicity in vitro against *Artemia salina* Leach and in vivo in mice. In addition, this study investigated the antineoplastic action of EERB in Ehrlich carcinoma-bearing mice.

2. MATERIALS AND METHODS

2.1. Preparation of ethanol extract from *Rhipsalis baccifera*

Samples of leaves of *R. baccifera* were collected from the Universidade Federal de Pernambuco (UFPE - Recife Campus), Brazil, and later identified by the botanist Dr. Marlene Barbosa. A herbarium was deposited in the UFP - Geraldo Mariz herbarium of UFPE under the record 81.116. The vegetable was dried naturally in an environment protected from the sun (average temperature 25 °C) and with air circulation for 30 days, the powder being obtained in a knife mill (model Grindomix GM 300, Retsch). In order to perform the extraction, 257 g of the powder were solubilized with 2000 mL of ethanol under constant orbital agitation at an average temperature of 25 °C for 72 h. The raw extract obtained by filtration was concentrated in a rotary evaporator at a constant temperature of 40 °C to eliminate the organic solvent.

2.2. Phytochemical analysis

The chromatographic experiment was carried out by thin-layer chromatography using silica gel 60 plates (Merk), in which 15 μL of the previously prepared extract were applied, selecting the development systems whose developers were according to the researched molecules. The chromatogram was developed with Dragendorff's reagent for alkaloid research, and scopolamine was used as the standard substance. For flavonoids, an aliquot of the extract was solubilized in 70% ethanol, added chloroform (CHCl_3) and stirred to obtain two phases, chloroform and other hydroalcoholic. A combination of ethyl acetate: formic acid:glacial acetic acid:water (100:11:11:26 v/v) was applied as mobile phase and developed with Neu reagent (Neu, 1956), with observation under UV light ($\lambda = 365 \text{ nm}$), which utilized quercetin as the standard substance. Concerning the research of phenylpropanoids, the creation of two phases (chloroformic and hydroalcoholic) was also performed, a mixture of toluene: ether (1:1 v/v) was employed as a mobile phase, developed with 10% KOH, observed by same UV light, coumarin was used as a standard substance. For the investigation of terpenes, a mixture of toluene: ethyl acetate (93:7 v/v) was utilized as the mobile phase, prepared with 1% sulfuric vanillin, followed by heating in an oven ($100 \text{ }^\circ\text{C}$), the appearance of purple/green/blue stains were used as an identifier criterion. In the analysis of the presence for triterpenes, a mixture of toluene: chloroform: ethanol (40:40:10 v/v) was applied as the mobile phase, developed with sulfuric anisaldehyde, followed by heating at $100 \text{ }^\circ\text{C}$, the identification of stains with slightly pinkish and reddish color was employed as an evidence criterion.

2.3. Toxicity in vitro with *Artemia salina* Leach

According to Meyer et al. (1982), the assay was carried out using *A. salina* cysts that were set to hatch in an incubator containing seawater at $25 \text{ }^\circ\text{C}$, under artificial lighting, constant aeration and covered with aluminium foil for 48 h. EERB in distilled water (1 mL) under different concentrations (50, 100, 500 and 1000 mg/mL) was incubated with 9 mL of seawater in the presence of 10 larvae in their nauplius phase. Salt shrimp from seawater was used as a negative control. All static microcrustaceans were classified as dead while the observation took place with slight agitation. The test was performed in triplicate, counting the live and dead microcrustaceans after 24 h of incubation.

2.4. Animals

The work was carried out with Swiss adult female albino mice (*Mus musculus*), around 60 days of age and weighing between 25 - 40 g, which were acquired in the vivarium of the Department of Antibiotics at UFPE. Controlled lighting (light/dark cycle of 12 h each) and temperature of $25 \pm 3^\circ\text{C}$ in polypropylene cages with dimensions of $20 \times 30 \times 12 \text{ cm}$, lined with shavings. The Ethics approved the experimental protocol in Experimentation Committee Animal (Committee of Ethics in Animal Experimentation - CEUA) of the Center for

Biosciences, UFPE, under registration 23076.041254 / 2016-75.

2.5. Determination of acute in vivo toxicity

Acute oral toxicity assay was performed according to the experimental protocol Guideline 423 (OECD, 2002) to determine the median lethal dose (LD_{50}) with sequential doses, following the proposal of the Brazilian Society of Science in Laboratory Animals/Brazilian College of Animal Experimentation (COBEA). The experimental groups of mice contained three animals per group. As suggested by the protocol, when there are no preliminary studies, the initial dose of 300 mg/kg should be started, where the next dose to be tested was determined from the result of the first application. All animals were kept under identical conditions. The feed was suspended for at least 6 h before the experiment, and the water was released ad libitum; the feed was reinstated after 3 h of administration. Initially, a group treated with EERB 300 mg/kg and a control group that received only 0.9% saline were used. The EERB was solubilized with distilled water, and, subsequently, the solution was administered orally to the mice, introduced into the animal's digestive tract through a metal cannula attached to a syringe (gavage). According to OECD guidelines, there were no deaths with the initial concentration, and the concentration was increased to 2000 mg/kg. The 2000 mg/kg concentration eliminated 50% of the animals and determined the $-\text{LD}_{50}$. The behavioural changes of the animals were evaluated separately after administration of the extract for 60 min. and then daily for fourteen days, with daily weighing, consumption of feed and water. The handling and care of animals followed the ethical principles of animal experimentation according to criteria established by CEUA.

2.6. Antitumor activity

2.6.1 Implantation of tumor mass

The implantation of Ehrlich's carcinoma was elaborated according to Stock et al. (1955) and Komiyama and Funayama (1922). Cells were removed from a donor animal eight days after implantation by paracentesis and introduced into recipient animals, subcutaneously in the subaxillary region, at a concentration of $25 \times 10^6 \text{ cells / mL}$ for tumor transplantation.

2.6.2 Investigation of antitumor action

Twenty-four animals were used for the in vivo experimentation divided equally between 4 groups of 6 animals each. The treatment started 48 h after inoculation and was carried out for 7 consecutive days. The solutions of each group were administered orally by intragastric tube (gavage), except for the positive control group (treated with an antineoplastic drug) that received the solution intraperitoneally. The solution used by group 1 (negative control) for the treatment of animals was 0.9% saline; animals in group 2 (positive control) were treated with the chemotherapy medication cisplatin (0.5 mg/kg), animals in group 3 were treated with 200 mg/kg of EERB while group 4 used 100 mg/kg of the extract in treatment. The last

two groups received 10% and 5% of the LD₅₀ in the oral acute toxicity assay.

One day after the treatment, the animals were anaesthetized and consecutively sacrificed to remove tumors and organs (liver, lung, kidney and spleen), which were then fixed in buffered formalin and kept in this state for 24 h. Subsequently, the organs were processed for inclusion in paraffin, and the sections were stained with hematoxylin-eosin (H.E.) for routine histopathological analysis. The technique of choice for blood removal was a cardiac puncture for the different analyses. Blood collected in anticoagulant ethylenediaminetetraacetic acid (EDTA) was used for haematological analyzes (red cell, leukocyte and haemoglobin count) as well as hematimetric indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). For biochemical dosages such as urea, creatinine, transaminases, alkaline phosphatase, blood was collected in tubes containing separating gel, centrifuged for 10 min at 3500 rpm. The tumor inhibition index was calculated by the difference between the treated and control groups, in which they were excised, dissected and weighed. The histopathological analysis was performed with Nikon Eclipse E200 LED microscope, 100× magnification with a docked camera and Motic Images Plus 2.0 Software for capture.

After weighing the tumor, inhibition was calculated compared to the control group, according to Machoń et al. (1981) Eq.

$$TWI (\%) = \frac{(C - T)}{C} \times 100$$

Where: TWI = tumor inhibition percentage, C = tumor weights mean of the control group, and T = tumor weights mean of the treated group with EERB.

2.7. Statistical analysis

SPSS software assisted in the statistical analyses. The Shapiro-Wilk test assessed normality. The Kruskal-Wallis test was used to compare the distribution of the parameter of interest between the treatment groups on occasions when normality was not indicated. Statistical significance was interpreted considering the level of 5%.

3. RESULTS AND DISCUSSION

3.1. Phytochemical profile

The phytochemical approach of *R. baccifera* performed with crude ethanolic extract indicated alkaloids, flavonoids, phenylpropanoids, terpenes and triterpenes. According to the literature, the alkaloids are the most found secondary metabolites in Cactaceae (Cassels, 2019; Santos-Díaz & Camarena-Rangel, 2019), ratifying the findings in our study. It is noted that not all species of these plants have all compounds as found in this study. For example, Santos (2018a) demonstrated that in the crude ethanolic extract of *Harrisia adscendens* (Gürke), Britton & Rose (Cactaceae), only the alkaloids were

detected. However, negative results of alkaloids in the samples do not necessarily imply their absence. Possibly the amount of them was small to be detected; moreover, the climate and the collection area can also influence the production of these metabolites.

Also belonging to the Cactaceae family, the forage palm (*Opuntia ficus-indica* (L.) Mill.), submitted to chromatographic analysis by C. Santos et al. (2018), demonstrated the presence of flavonoids. Traces of terpenes were identified, corroborating the literature that reports the presence of terpenes in the chemical composition of palm (Feugang et al., 2006). The presence of flavonoids and terpenes verified in this work agrees with those described by Zhong et al. (2010), where the analyzed phytochemical profile of the *Astrophytum myriostigma* Lem. (Cactaceae) extract resulted in positive results for triterpenes and alkaloids, corroborating the results found by Garza-Padrón et al. (2010).

3.2. Toxicity in *Artemia salina* Leach

Regularly used to qualify the toxicity of plant extracts, *A. salina* has the advantage of being cheap, reliable and reproducible (Maia-Neto et al., 2020). In general, bioactive compounds are toxic to *A. salina* larvae. So, the lethality of this microcrustacean can be used as a quick and simple preliminary test during the isolation of natural products (M.S. Oliveira et al., 2021). The evaluation of the lethality of EERB against microcrustaceans is important to be investigated since some natural compounds can show high toxicity when they are administered to organisms in high doses. Figure 1 shows the viability of the larvae with the treatment of EERB in different concentrations. It was found that the group with the highest prevalence of mortality was 1000 mg/mL (53.3%) followed by 500 mg/mL (20.0%) and 100 mg/mL (6.6%). No mortality was observed in the control group and in the group treated with 50 mg/mL de EERB, which showed that 100% of the larvae were viable (alive). The lethality of microcrustaceans increased with a concentration in a dose-dependent manner.

Our results showed less toxicity when compared to those obtained by Sánchez et al. (2016), which when assessing the toxicity of the extract of *O. ficus-indica*, also belonging to the Cactaceae family, popularly known as "forage palm", where a relatively lower concentration (100 µg/mL), was able to eliminate about 60% of microcrustaceans.

3.3. Acute *in vivo* toxicity

The raw ethanolic extract of *R. baccifera* was administered orally with a dose equivalent to 2000 mg/kg, which produced clinical signs of intoxication in the first 60 min of observation, as shown in Table 1. Thus, it was observed that the most pronounced effects occurred on the central nervous system (CNS), mainly stimulating, such as claw posture, escape reaction and vocal trembling; depressants, such as prostration; and other behaviours were seen as reflux. In this assay, one animal had a seizure episode and died after 12 min of administration of the EERB. The other animals were followed

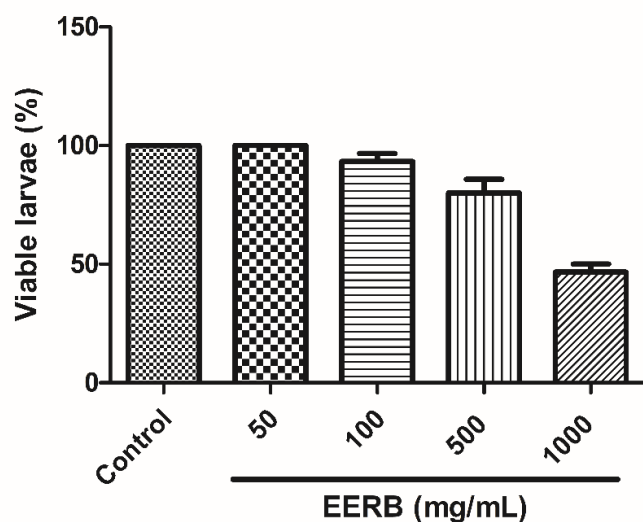


Figure 1. Percentage of viable Brine shrimp larvae exposed to EERB after 24 h.

up to the 14th day of the experiment with daily monitoring.

Table 1

Clinical signs observed in mice assessed after oral administration of *Rhipsalis baccifera* extract.

Clinical signs	EERB (200 mg/kg)
1. Central nervous system	
1.1 Stimulating signs	
Aggressiveness	-
Fine coarse/ tremors	+
Convulsions	+
Piloerection	+
Vibrissa movement	-
Claw Stance	+++
Jump	+
Vocal thrills	++
Tachycardia	-
Agitation	+
Tail erection	-
Escape reaction	+++
1.2 Depressors	
Pavilion auricle	+
Prostration	++
1.3 Other behaviours	
Stereotype	+
Reflux	+++
Snout edema	+
2. Autonomic nervous system	
Increased respiratory rate	+
Diuresis	+
Photophobia	-
3. Mortality	
	33.3%

- = no effect; + = light effect; ++ = moderate effect; +++ = accentuated effect.

According to the results obtained and analysis of toxicity by oral route, the ethanolic extract of *R. baccifera* was classified

following the criteria of the Harmonized Global System (GHS) belonging to category 5 (2000 – 5000 mg/kg) since the DL₅₀ was established at 2000 mg/kg, according to the protocol followed (OECD, 2011). **Table 2** shows the mean ± standard deviation of weight gain, organ weight and food consumption of the animals in the control and treated groups. It is observed that the highest average consumption of water and food was in the control group, being statistically (p-value = 0.046 for both). However, there is no statistical difference in animal weight gain and organ weight was observed.

Table 2

Animals' weight and organs as well as food consumption of mice after treatment with EERB.

Evaluated factor	Treatment		p-value ¹
	2000 mg/kg	Control	
Average weight gain	1.892±0.657	0.381±1.393	0.248
Liver	1.865±0.171	1.996±0.127	0.248
Spleen	0.133±0.006	0.230±0.660	0.083
Kidneys	0.386±0.011	0.377±0.031	1.000
Lung	0.191±0.065	0.236±0.025	0.564
Water	9.571±0.000	14.286±0.000	0.046
Ration	6.179±0.000	11.786±0.000	0.046

Values expressed as mean ± standard deviation of the mean. ¹ p-value of the Mann-Whitney test (if p-value <0.05, the distribution of the assessed factor differs between the assessed groups).

Verifying acute toxicity establishes the first step for the toxicological investigation of herbal medicines (Déciga-Campos et al., 2007). Commonly, animal models have a good outlook for human toxicities, around 70-80% (Kola & Landis, 2004). Acute toxicity studies aim to characterize the dose/response relationship and calculate the LD₅₀, which helps identify the toxicity of the substance. The toxic response presented by a substance is related to the dose administered or absorbed by the individual, the time and frequency of exposure, in addition to the route of administration used (Barros & Davino, 2008).

In the study by Sim et al. (2010), acute oral toxicity was performed to establish the safety parameters of crude methanol extracts of *Pereskia bleo* (Kunth) DC. and *Pereskia grandifolia* Haw. (Cactaceae) leaves using the procedure described by the OECD with some modifications. The mice did not show any change in weight, where they increased their body mass without clinical signs of toxicity at any dose, with no significant changes being observed. Abdul-Wahab et al. (2012) also assessed the possibility that *P. bleo* extracts (Cactaceae) developed a toxic effect after oral administration; the rats received a single dose of 500 mg/kg. No injuries or bleeding were observed, as well as behavioural changes. The safe indication for pharmacologically active doses can be confirmed by analyzing the *P. bleo* extract with a low toxicity profile.

No toxic mortality, signs or symptoms were observed at single doses 1250, 2500 or 5000 mg/kg referring to the extract of *H. polyrhizus*, commonly known as pitaya (Hor et al., 2012). According to Messias et al. (2010), the methanolic extract of *C. jamacaru* showed no toxicity for pregnant rats during pregnancy, with no change in the weight gain of these females. Most organs, except for the spleen, did not show significant variation

concerning weight compared to the control group, and the macroscopic examination also did not show changes in the analyzed organs.

The evaluation of the acute oral toxicity of the methanolic extract of *Opuntia joconostle* Web. (Cactaceae) was carried out following the method prescribed by the OECD by Osorio-Esquivel et al. (2012). No toxicological symptoms were shown, all animals survived, and weight gain in the treated groups was similar to the value in the control group. The acute oral toxicity of the ethanolic extract of *Pilosoreceus gounellei* presented an LD₅₀ > 2000 mg/kg of body weight without causing behavioural alterations, showing its low toxicity in Wistar rats (Dias et al., 2015) (Dias et al., 2015). Thus, as shown in the results with other species of the same family, showing toxicity at high doses.

In our histopathological analysis for acute toxicity revealed that the liver, kidneys, lung and spleen of the control group had, respectively, preserved lobular center vein, preserved hepatocyte nuclei and organized radially in rows with preservation of the hepatic sinusoids; renal glomeruli and tubules conserved, and peri-glomerular space conserved; preserved alveoli, bronchi and terminal bronchioles with preserved epithelial lining tissue; preservation of the capsule/trabecula and lymph nodes with preserved splenic sinusoids (Figure 2)

The group treated with EERB 2000 mg/kg had a preserved lobular center vein, disorganization of the hepatocyte rows with consequent disorganization of the sinusoids, increase in the volume of hepatocytes and presence of cytoplasmic vacuolations. There was also a slight increase in peri-glomerular space and glomerulus with preserved renal tubules; slight increase in the alveoli walls and decrease in the lumen; preservation of the capsule/trabecula and lymph nodes with splenic sinusoids in disorganization due to possible blood congestion (Figure 2).

The concentrations of lipids and glycogen, or the combination of toxic agents with intracytoplasmic lipids, can be explained by the presence of cytoplasmic vacuolizations, which increase the volume of hepatocytes. The accumulation of lipids and the decrease in glycogen in the cytoplasm of hepatocytes impair metabolic activities (Gayão et al., 2013).

The histopathological analysis revealed that the liver of the control group (Figure 2) had preserved lobular central vein, hepatocyte nuclei preserved and organized radially in rows with preservation of hepatic sinusoids. It was observed in the group treated with EERB 2000 mg/kg that they had preserved central lobular vein, disorganization of the hepatocyte rows with consequent disorganization of the sinusoids, increase in the volume of hepatocytes and presence of cytoplasmic vacuolations. The presence of cytoplasmic vacuolations, which increase the volume of hepatocytes, indicates the existence of regions with a probable concentration of lipids and glycogen or the combination of toxic agents with intracytoplasmic lipids (A.A. Santos et al., 2004). The accumulation of lipids and the decrease in glycogen in the cytoplasm of hepatocytes impair metabolic activities.

Histopathological analysis for acute toxicity revealed that the kidneys of the control group had conserved renal glomeruli and tubules and conserved peri-glomerular space (Figure 2). The group treated with EERB 2000 mg/kg, on the other hand, showed a slight increase in the peri-glomerular and glomerulus space with preserved renal tubules. After histopathological analysis for acute toxicity, the lung presented alveoli, bronchi, and terminal bronchioles preserved with preserved epithelial lining tissue (Figure 2). When treated with EERB 2000 mg/kg, it presented a slight increase in the alveoli walls and decreased lumen. The last organ analyzed histopathologically for acute toxicity was the spleen, wherein the control group presented preservation of the capsule/trabecula and lymph nodes with preserved splenic sinusoids.

3.4. Antitumor activity

It was found that EERB had inhibitory effects on tumor growth. Percent tumor inhibition was calculated by the mean of tumor weights 24 h after the last treatment dose. Tumor inhibition (TW%) was found against Ehrlich's carcinoma, in which the dose of 125 mg/kg presented TW% of 75.8% and the dose of 250 mg/kg, TW% of 84.1% when compared to the control group (0.9% saline solution). All analyzed and treated animals resisted until the end of the treatment. Regarding the analyzed standard drug, it was able to inhibit 74.7% of tumor growth. Figure 3 shows the histopathological analysis of the antitumor activity presented in the control group. Spleen with preserved capsule, preserved trabeculae, lymph nodes and sinusoids of the spleen; kidneys with glomeruli, as well as renal tubules in normal preservation and preserved periglomerular space; lungs with alveoli, bronchi, and terminal bronchioles with preserved epithelial lining; Liver with lobular central vein and sinusoidal capillaries with normal appearance, hepatocytes with normal morphology and regular nuclei. As for the aspect of the tumor, a necrotic center was found with infiltration of adjacent muscle and adipose tissue, presenting cells with pleomorphism.

The standard group tested with 0.5 mg/kg cisplatin, and the standard group had liver with preserved central lobular vein, smaller-calibre sinusoids, enlarged hepatocyte nuclei and condensed chromatin. Still on the treatment with cisplatin, we can highlight: lungs with alveoli, bronchi and terminal bronchioles with preserved epithelial lining, spleen with preserved capsule, trabeculas, lymph nodes and increased sinusoids of the spleen and kidneys with preserved glomeruli and renal tubules and peri- increased glomerular. The tumor treated in this group had a smaller area of necrosis, slower growth and less infiltration of adjacent muscle and adipose tissue (Figure 3).

The standard drug used can explain the severe liver and kidney damage caused to the organs of animals in the cisplatin group. Cisplatin is a standard antineoplastic drug that forms covalent bonds with plasma proteins and is widely distributed to tissues, especially the kidneys and liver. Despite having favourable pharmacokinetics, it was observed that it caused,

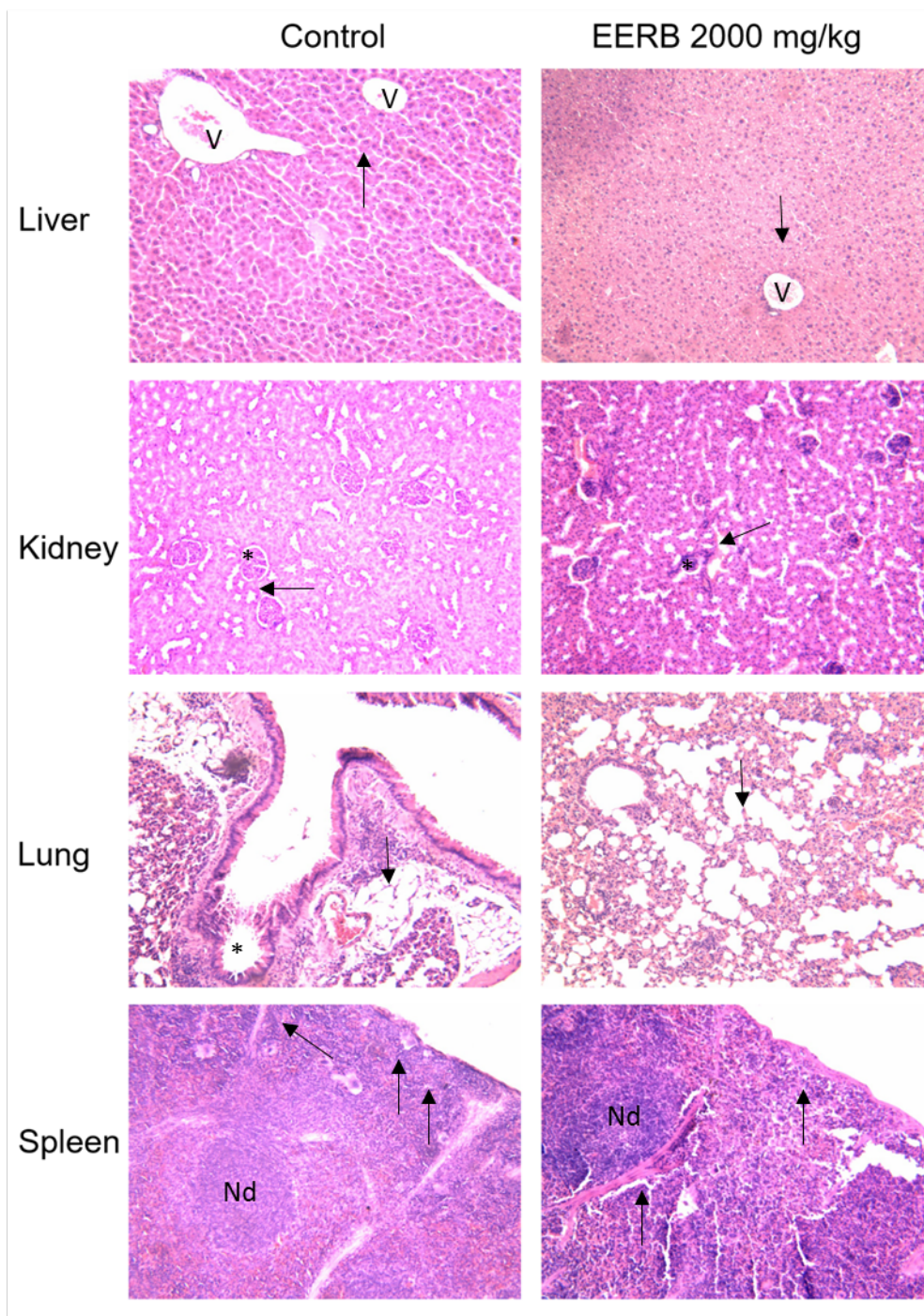


Figure 2. Representative photomicrographs of animals' liver, kidney, lung, and spleen from the control group (0.9% saline) and EERB (2000 mg/kg). **Liver:** The centrilobular vein (v) is seen in all images, preserved hepatocyte nuclei in remark cords (arrows) in treatment group animals with EERB it is possible to see disorganization of the hepatocyte rows with consequent disorganization of the sinusoids, increase in the volume of hepatocytes (arrows) and presence of cytoplasmic vacuolations. **Kidneys:** in control, it is possible to see a kidney with renal glomeruli (*) and contorted tubules (arrows) without alterations. In the treatment group, an animal with EERB slightly increased peri-glomerular space and glomerulus with preserved renal tubules (arrow). **Lungs:** in the control, preserved alveoli, bronchi and terminal bronchioles (*) with preserved epithelial lining tissue; in the treatment group animal with EERB, it is possible to see walls thickening the alveoli and decrease the alveoli in their lumen. **Spleen:** Lymphatic nodes (Nd) are well delimited in controlling and preserving the capsule/trabecula (arrows) in the treatment group. It is possible to see the preservation of the capsule/trabecula (arrows) and lymphatic nodes (Nd) with splenic sinusoids in disorganization due to possible blood congestion.

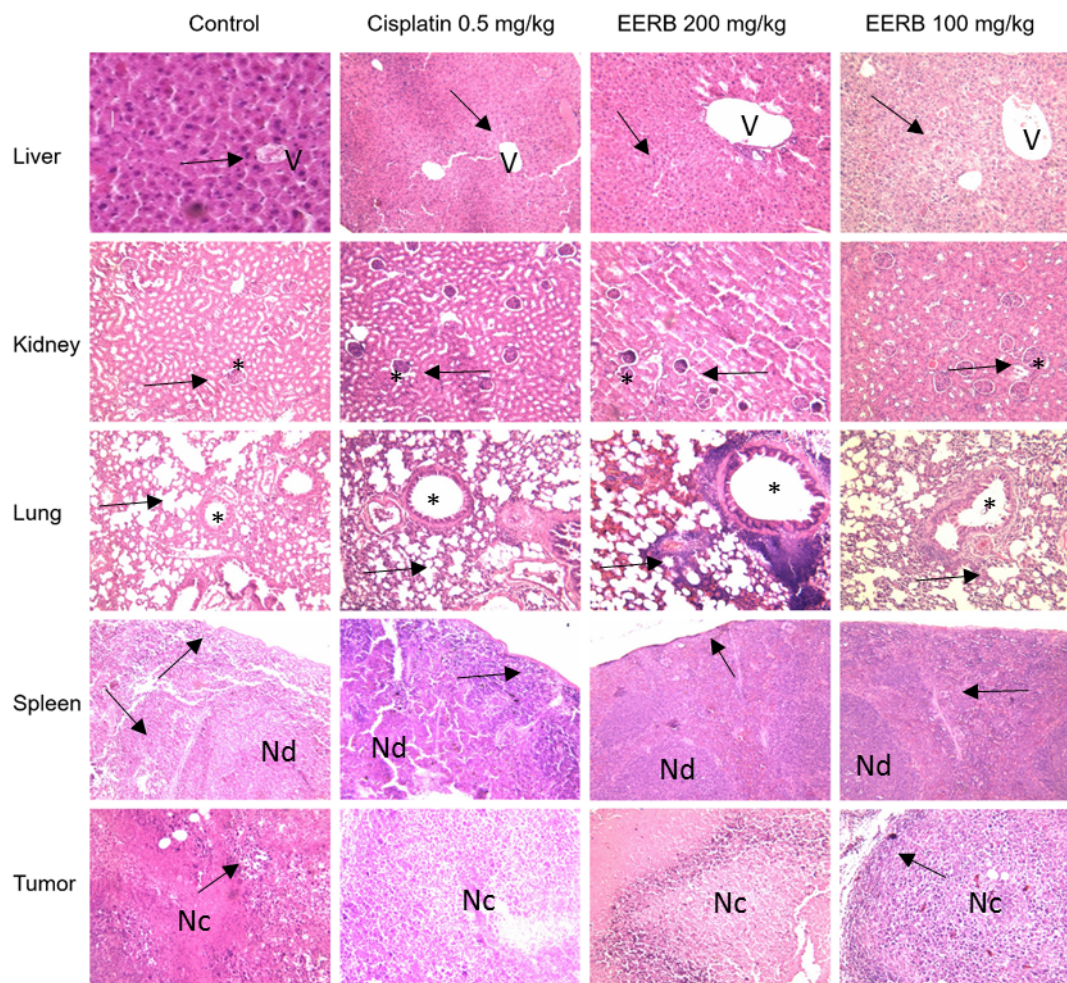


Figure 3. Representative photomicrographs of the liver, kidney, lung, spleen and tumor of animals from the control group (0,9% saline), Cisplatin (0,5 mg/kg) and EERB (100 and 200 mg/kg). Histological evaluation of the control group animals' liver, kidney, lung and spleen demonstrated the same pattern as the control group represented in figure 2 and tumor of the animals with necrotic center (Nc), cellular pleomorphism and infiltration of adjacent tissues (arrow). The standard group tested with 0.5 mg/kg cisplatin, **liver:** preserved lobular central vein (v), smaller calibre sinusoids (arrow), increased hepatocyte nuclei, and condensed chromatin. **Kidneys:** preserved glomeruli and renal tubules (arrow) and increased peri-glomerular space (*). **Lungs:** without alterations. **Spleen:** preserved capsule and trabecula (arrow), lymphatic nodes (Nd) and increased spleen sinusoids. **Tumor:** smaller area of necrosis (Nc), slower growth and less infiltration of adjacent muscle and adipose tissue. The treated group with EERB 200 mg/kg, **liver:** preserved lobular center vein (v), smaller calibre sinusoids (arrow), increased hepatocyte nuclei with disarray and condensed chromatin; **Kidneys:** preserved glomeruli and increased peri-glomerular (*) space and preserved renal tubules (arrow); **Lungs:** enlarged alveolar wall (arrow), bronchi and terminal bronchioles with more excellent calibre (*). **Spleen:** preserved capsule, trabecula (arrow) and lymph nodes (Nd). **Tumor:** extensive area of necrosis (Nc). The group that received EERB 100 mg/kg, **liver:** with the lobular central vein (v) with larger calibre, increased and disorganized hepatocytes (arrow) with the presence of vesicles in its interior and condensed chromatin. **Kidneys:** unharmed glomeruli (*), smaller calibre renal tubules (arrow) with cell nuclei of the epithelial lining tissue increased; **Lungs:** preserved. **Spleen:** preserved. **Tumor:** extensive area of necrosis (Nc), accentuated pleomorphism, presence of mitosis, absence of haemorrhage or calcification and less infiltration of adjacent adipose tissue (arrow).

as well as most antineoplastic agents, critical adverse reactions, especially nephrotoxicity and hepatotoxicity. In the kidneys, three mechanisms of action have been proposed: apoptosis and necrosis, inflammation and oxidative stress that cause a reduction in the glomerular filtration rate, proximal tubular injury and an increase in salts. In the liver, transaminases increase, alter the organ's energy metabolism, cause stiffening of the liver membrane, lipid peroxidation, oxidative damage to cardioplipin and proteins with sulfhydryl groups, and liver cell death (Visacri et al., 2019).

The treated group that received EERB at a 200 mg/kg concentration had lungs with an enlarged alveolar wall, larger-caliber bronchi and terminal bronchioles, liver with preserved central lobular vein, and smaller-calibre sinusoids disarranged enlarged hepatocyte nuclei and condensed chromatin. Spleen with preserved capsule, intact trabeculae, lymph nodes and splenic sinusoids; kidneys with preserved glomeruli and enlarged periglomerular space and preserved renal tubules. In addition, the tumor had an extensive area of necrosis, which indicates high toxicity to Ehrlich's carcinoma, corroborating the high percentage of tumor inhibition (Figure 3). The group with the lowest concentration by EERB 100 mg/kg treatment had lungs with alveoli, bronchi and terminal bronchioles with preserved epithelial lining; liver with a more prominent lobular central vein, enlarged and disorganized hepatocytes with the presence of vesicles in its interior and condensed chromatin. Spleen with preserved capsule, trabeculae, lymph nodes and sinusoids of the spleen maintained. Kidneys with uninjured glomeruli, smaller-calibre renal tubules with enlarged epithelial lining tissue cell nuclei. Also, in the tumor area treated with 100 mg/kg, there was an extensive area of necrosis, marked pleomorphism, presence of mitosis, absence of haemorrhage or calcification, and less infiltration of adjacent adipose tissue (Figure 3).

The mean and standard deviation of the animals' weight gain are shown in Table 3, organ weight and food consumption in control and treated group. It is observed that the highest average consumption of water and food was in the control group, being statistically significant (p-value = 0.046 for both), indicating that the control group had a higher level of water and food consumption during the time of the experiment. However, there is no statistical difference between animal weight gain and organ weight. The mean and standard deviation of the weight of animals, tumors and organs evaluated in the study according to the experimental groups: treated EERB 100 mg/kg, (cisplatin) EERB 200 mg/kg and control (saline solution 0.9%), pattern, can also be observed. It was higher in the standard group (mean = 2.714) and in the control group (mean = 0.048). It was also observed that the comparison test was significant (p-value = 0.047), which indicated a significant difference in weight loss between the experimental groups.

Concerning the kidneys, the lowest mean weight was in the standard group (mean = 0.434), and the highest mean weight was in the group that received EERB 200 mg/kg (mean = 0.622), the comparison test was significant for this evaluated

Table 3

Animals' weight, evaluated organs and food consumption according to the study group in mice after assessment of acute toxicity.

Evaluated factor	Dose		P-value ¹
	200 mg/kg	Saline 0.9%	
Average weight gain	1.89±0.65g	0.38±1.39g	0.248
Liver	1.86±0.17g	1.99±0.12g	0.248
Spleen	0.13±0.00g	0.23±0.66g	0.083
Kidneys	0.38±0.01g	0.37±0.03g	1.000
Lung	0.19±0.06g	0.23±0.02g	0.564
Water (mL)	9.57±0.00g	14.28±0.00g	0.046
Ration (g)	6.17±0.00g	11.78±0.00g	0.046

Values expressed as mean ± standard deviation of the mean. ¹p-value of the Mann-Whitney test (if p-value <0.05, the distribution of the assessed factor differs between the assessed groups).

organ (p-value = 0.006), showing that the evaluated groups were relevant.

When analyzing the tumor, it was found that the lowest mean weight was in the group treated with EERB 200 mg/kg (mean = 0.151). In contrast, the highest mean weight was observed in the control group (mean = 0.947). Furthermore, the mean comparison test was significant (p-value = 0.004), which confirmed the existence of a statistically significant difference concerning tumor weight.

The biochemical parameters urea, creatinine, GOT, GPT and ALP are shown in Table 4. Urea and creatinine are metabolites that are laboratory-tested to determine renal function. In seven-week-old mice, the average urea found in the serum is 20.7 mg/dL of blood (Rusyn & Threadgill, 2010), while creatinine has an average of 0.28 mg/dL (Riken, 2008).

The hepatic assessment can be measured through GOT, GPT and ALP measurements. Its origin is mostly plasma, causing it to increase rapidly after liver injury (Schumann et al., 2002). The mean dosage of the parameters mentioned above in the serum of seven-week-old mice is 41 U/L and 152 U/L, respectively (Rusyn & Threadgill, 2010). Alkaline phosphatase, also known as FAL, is composed of a group of membrane-associated isoenzymes. The mean serum dosage in seven-week-old mice is 86 U/L (Yuan & Korstanje, 2008).

Although there was a difference in the means of measures between the groups evaluated (Table 4), the comparison test was not significant in any of the measures (p-value was greater than 0.05 in all parameters), indicating no relevant difference. In urea, creatinine, GOT, GPT and ALP between the study groups showed that the treatment with EERB was not hepatotoxic.

The haematological analysis involves studying the concentration, morphological structure and functions of the elements that constitute blood and its products. We can highlight three fundamental elements, including red blood cells (erythrocytes), white blood cells (leukocytes) and platelets (thrombocytes). The absolute red cell count ranges from 7 to 11 × 10⁶ / mm³ in mice (Weiss & Wardrop, 2010). The hematimetric index (hematocrit) is measured by the relative percentage of erythrocytes in the total amount of blood, and its values

Table 4

Levels of urea, creatinine, GOT, GPT and ALP after antitumor assay in mice with Ehrlich's carcinoma.

Evaluated parameter	Evaluated group				p-value ¹
	0.9% saline	Cisplatin	EERB 200 mg/kg	EERB 100 mg/kg	
Urea	22.00±3.16	20.33±2.25	19.67±1.21	21.67±3.50	0.484
Creatinine	0.55±0.14	0.50±0.09	0.43±0.05	0.48±0.10	0.291
GOT	11.07±6.36	15.53±13.44	8.73±4.26	11.57±6.61	0.819
GPT	12.95±6.91	17.87±14.85	10.40±5.07	13.45±7.23	0.751
ALP	21.83±4.49	26.73±6.90	22.33±4.13	23.42±4.27	0.530

Values expressed as mean ± standard deviation of the mean. ¹p-value of the Kruskal-Wallis test (if p-value <0.05, the distribution of the evaluated factor differs between the evaluated groups). AST= aspartate transaminase, ALT= alanine transaminase and ALP= alkaline phosphatase.

in mice are generally between 40 and 50% (Bain, 2006). Haemoglobin is the main protein that makes up erythrocytes and is responsible for transporting oxygen (O₂) from the lungs to tissues and returning carbon dioxide (CO₂) to the lungs. Its concentration in mice is between 10 and 17 g/dL (Weiss & Wardrop, 2010). Hematimetric indices that are correlated with erythrocytes will provide additional information about red blood cells and haemoglobin. Mean corpuscular volume (MCV) measures the mean size (volume) of erythrocytes (Bain, 2006). The MCV of mice is approximately 45 to 55 fm³. The mean corpuscular haemoglobin (MCH) corresponds to the mean amount of haemoglobin in erythrocytes and mice, ranging from 13 to 17 pg. Meanwhile, the mean corpuscular haemoglobin concentration (MCHC) measures the haemoglobin concentration in each erythrocyte and is usually around 30 and 38 g/dL in this same species (Weiss & Wardrop, 2010).

White blood cells contribute to the immune and inflammatory process, and their quantification includes total and differential counts. Usually, their rodent count is between 2 and 10x10³/mm³. Among the essential types of leukocytes evaluated during the differential counting, we can mention the lymphocytes and monocytes mononucleated and the neutrophils, eosinophils and basophils polymorphonuclears (Hedrich & Bullock, 2004; Weiss & Wardrop, 2010).

Neutrophil polymorphonuclear cells perform the first line of defence against the invasion of microorganisms, tissue trauma or some inflammatory signs, such as circulating phagocytes and immune response modulators. Its count in mice is between 20 and 30% of the total white blood cell count (Hedrich & Bullock, 2004; Weiss & Wardrop, 2010). Lymphoid mononuclear cells contribute to the body's innate immune response, but their main feature mediates adaptive immunity (Turgeon, 2005). Lymphocytes comprise 70-80% of the differential leukocyte count in mice and may be above 80% in young animals.

Monocytes are cells whose main characteristic is phagocytosis and the elimination of bacteria. In mice, they represent 0 to 2% of the total white blood cell count. Eosinophils are cells that act in the pathogenesis of many inflammatory processes simultaneously with basophils, including helminth infections and allergic pathologies. Its percentage in mice represents 0 to

7% of the total white blood cell count (Brandt et al., 2003).

Thrombocytes also play an essential role in the integrity of the endothelium due to the release of growth factors and pro-angiogenic cytokines. Its reference value for mice is between 900 and 1600 × 10³/mm³ (Weiss & Wardrop, 2010).

The haematological analyzes are presented in Table 5. The test of comparison of the means of the parameters between the groups evaluated was significant only in the hematocrit factor (p-value = 0.040). In other parameters, the test was close to significance.

Ehrlich's carcinoma, as shown, showed sensitivity to EERB. This tumor has been used as an experimental model, which can be transplantable with accelerated growth and epithelial nature with very aggressive behaviour, having been widely cited in the literature in research related to the antitumor properties of new therapeutic agents (Kaneno et al., 2004; Melo et al., 2004). We certify that the most strong dosage, with more significant tumor inhibition, was (200 mg/kg), correlating that the EERB is dose-dependent.

Researches show the strong effect on the antitumor activity of plants from the same family of cacti, such as Orozco-Barocio et al. (2013), where they evaluated the feedback of *Lophocereus schottii* (Engelmann) Britton and Rose (Cactaceae) to solid lymphoma of the gastrocnemius muscle in mice, measuring the volume of the tumor. tumor progression was slower in animals treated with 100 mg/kg of the extract inoculated both intramuscularly and intratumorally, compared to the group that received 50 mg/kg and the control group. These results can be assumed from alkaloids and triterpenes present in every plant and stem (Argueta et al., 1994; Kircher, 1969). It is already understood that alkaloids have anti-cancer effects and repress tumor development (Li et al., 2009; Pettit et al., 2009; Wang et al., 2009).

Likewise, triterpenoids are favourable antineoplastic agents and show antiproliferative activity when analyzed against various neoplastic cell lines. Several studies indicate that triterpenes contribute to tumor inhibition, favour apoptosis in vitro and in vivo, and inhibit cell cycle progression (He et al., 2011; Misawa et al., 2012; Palanimuthu et al., 2012; Saleem, 2009; Siddique & Saleem, 2011). Malek et al. (2009) observed the activity of *P. bleo* against the normal MRC5 cell line, a lineage that comes from human lung fibroblast cells, where the

Table 5
Antitumor evaluation of EERB through haematological markers in Ehrlich carcinoma mice.

Evaluated parameter	Evaluated group		Cisplatin	EERB 200 mg/kg	EERB 100 mg/kg	p-value ¹
	0.9% saline	0.9% saline				
Red blood cells	4475000.00± 647047.14	4475000.00± 235457.00	5170000.00± 235457.00	5111666.67± 336239.00	4651666.67± 1047462.01	0.151
Hemoglobin	14.47±0.80	15.52±0.80	15.52±0.80	15.40±0.93	16.03±1.46	0.070
Hematocrit	42.67±1.75	46.67±2.42	46.67±2.42	46.00±2.83	48.17±4.45	0.040
MCV	90.68±1.30	91.62±1.49	91.62±1.49	90.97±0.79	91.85±2.16	0.677
MCH	29.87±0.43	30.08±0.12	30.08±0.12	30.02±0.04	29.95±0.47	0.360
MCHC	33.03±0.82	33.10±0.11	33.10±0.11	33.07±0.10	33.12±0.16	0.394
Leukocytes	6666.67 ±1505.54	6833.33± 1471.96	6833.33± 1471.96	6416.67± 917.42	6833.33± 1602.08	0.965
Typical lymphocytes	51.50%±3.02	52.33%±4.50	52.33%±4.50	52.50%±2.17	52.33%±1.86	0.956
Neutrophils	41.17%±2.48	40.50%±5.65	40.50%±5.65	41.83%±2.79	42.00%±2.68	0.919
Monocytes	4.00%±1.41	2.83%±1.33	2.83%±1.33	3.50%±2.07	3.33%±1.63	0.617
Eosinophils	3.33%±1.51	4.33%±1.86	4.33%±1.86	2.17%±0.75	2.33%±1.21	0.111
Platelets	238500.00± 75434.08	177666.67 17511.90±	177666.67 17511.90±	150833.33± 41920.96	226666.67± 62295.00	0.071

Values expressed as mean ± standard deviation of the mean. ¹ p-value of the Kruskal-Wallis test (if p-value <0.05, the distribution of the evaluated factor differs between the evaluated groups). MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, and MCHC= mean corpuscular haemoglobin concentration.

analyzed extract was not as effective as doxorubicin, used as a standard. This result supports the assertion that doxorubicin is a powerful cytostatic drug, which is widespread for treating neoplastic pathologies, but its routine use can lead to significant adverse effects (Gille et al., 2002).

Although the cytotoxicity of *P. bleo* is not as efficient as doxorubicin, it presents as an optimizing point the lower toxicity against the normal cell line MRC5 compared to doxorubicin. Thus, its use in association with cytotoxic therapeutic drugs can reduce the adverse effects of some of these drugs. Amir et al. (2007) report that, in addition to the fact that they have a high antitumor property, natural products point to potential synergy with cytotoxic therapeutic drugs stipulated in preclinical studies.

Harlev, Nevo, Solowey, and Bishayee (2013) observed and verified the magnificent anticancer preventive and curative properties in plants of the Cactaceae family, evidenced research with several cancer cell lines, providing evidence of anticancer activity. However, few studies have documented synergistic analyzes using in vivo tumor models. If it is accessible, replicate the concentrations revealed in the in vitro activity in laboratory animals' blood or serum. The same bioactive plant or component has not been studied in both in vitro and in vivo systems.

4. CONCLUSION

Phytochemical analysis of the EERB revealed the presence of alkaloids, flavonoids, terpenes, triterpenes and phenylpropanoids. EERB was not toxic in the *A. salina* bioassay using 100 mg/mL; however, there was also an increase in toxicity with the increase in concentration. On the other hand, the EERB in vivo test showed low toxicity. EERB showed significant tumor inhibition compared to the control group for Ehrlich carcinoma since the percentage of tumor growth inhibition of 84.1% and 75.8% at doses of 200 mg/kg and 100 mg/kg of weight, respectively, in Swiss albino mice (*Mus musculus*). Thus, the study shows a significant tumor activity of the EERB against Erlich's carcinoma and reinforces the importance of developing effective and low-cost alternatives that increasingly seek to act in diseases considered severe and familiar, such as cancer, increasing positive effects and minimizing any side effects.

CONFLICTS OF INTEREST

The authros declares that there is no conflict of interest.

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ETHICAL APPROVAL

The Ethics approved the experimental protocol in Experimentation Committee Animal (Committee of Ethics in Animal Experimentation - CEUA) of the Center for Biosciences, UFPE, under registration 23076.041254 / 2016-75.

AUTHOR CONTRIBUTIONS

This work was performed in collaboration among all authors. JAC: term, conceptualization, methodology, formal analysis, resources, project administration, funding acquisition. LSMN: methodology, formal analysis, resources. WFO and JRPCS: methodology, writing - original draft, writing - review & editing. ALCF, CCS, PMBR, VSA, LADMMR, AAS, AFMO and IAS: writing - original draft, writing - review & editing, visualization, supervision. RCDS, RJOC, JMLM, STP and CRFN: methodology. All authors read and approved the final version of the manuscript.

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