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Isopentyl caffeate as a promising drug for the treatment of leishmaniasis: An *in silico* and *in vivo* study

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ABSTRACT

Leishmaniasis is recognised as the second largest parasitic disease worldwide and yet a neglected disease. The current pharmacological treatments are associated with significant challenges, including high toxicity, high cost and parasitic resistance. Considering the potential of isopentyl caffeate (ICaf) as an anti-leishmanial agent, the present work evaluated the *in vivo* toxicity of ICaf and the absorption, distribution, metabolism, and excretion (ADME) properties *in silico*, aiming at the treatment of *Leishmania amazonensis*. For the *in vivo* toxicity testing, Swiss mice (*Mus musculus*) were treated with a single dose of ICaf. During the 14-day evaluation period, the animals underwent assessments including hippocratic screening, weight measurement, as well as histological and hematological evaluations. Analysis of ADME properties of ICaf was conducted to evaluate its pharmacokinetic characteristics and bioavailability. Characteristics, such as molar refractivity through Lipinski's Rule of Five, were identified. The *in silico* results showed that ICaf is considered to have good oral bioavailability and has potential to be considered as a new drug. From the *in vivo* toxicity testing, none of the evaluated parameters revealed toxicity of ICaf to the animals when treated intraperitoneally. The *in vivo* treatment reduced the lesion and the parasite load at the tested doses, corroborating the assumption that ICaf may be a potential pharmacological alternative against *L. amazonensis*.

1. Introduction

Leishmaniasis, a well-known neglected disease transmitted by protozoan parasites belonging to the *Leishmania* genus, poses a significant global socioeconomic and public health burden (Gimeno-Pitarch et al., 2024). It predominantly affects impoverished populations in regions of Africa, Asia, and Latin America, putting millions of people at risk of infection (Narsimulu et al., 2024). Presently, leishmaniasis affects about 12 million people worldwide. The World Health Organization (WHO) reports that this parasitic condition is endemic in 98 countries, with 9 countries experiencing endemicity exclusively for visceral leishmaniasis (VL), 21 countries are endemic for cutaneous leishmaniasis (CL) alone, and 68 countries have endemicity for both forms of the disease (Ornellas-Garcia et al., 2023).

More than 20 species of the genus *Leishmania* are known to cause the disease. These species can cause various clinical forms, such as visceral

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leishmaniasis (VL), mucocutaneous leishmaniasis (MCL), and cutaneous leishmaniasis (CL) (Nuwangi et al., 2023). *Leishmania* species responsible for human diseases include *L. infantum* and *L. donovani*, which cause visceral leishmaniasis (VL), *L. braziliensis* and *L. peruviana* cause the cutaneous-mucosal form, and *L. amazonensis, L. Mexicana, L. tropica*, *L. major*, *L. aethiopica*, which are responsible for causing the cutaneous form (Kaushal et al., 2023). VL is the most severe one, characterized by pronounced symptoms including weight loss, fever, hepatosplenomegaly, hypergammaglobulinemia, and pancytopenia. If left untreated, VL can ultimately lead to a fatal outcome (Singh et al., 2024). CL represents a clinical variant of leishmaniasis distinguished by lesions capable of partially or completely damaging the mucous membranes, leading to disfigurement and social ostracism of the affected individuals. MCL, the prevalent form of the disease, is characterized by painless and persistent symptoms. Lesions typically manifest in regions where sand fly bites occur, including the arms, legs, and face (de Vries and Schallig, 2022; Pederiva et al., 2023).

The classical drugs for treating VL and CL are pentavalent antimonials, which are commercially available as sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®). Other drugs, such as amphotericin B deoxycholate (Fungizone®), miltefosine and pentamidine, have also been commonly used in the treatment of leishmaniasis (Verdan et al., 2023). However, the currently available drugs cannot be regarded as ideal due to their association with low therapeutic efficacy rates, significant side effects and the emergence of parasites with drug-resistant phenotype (Kumar et al., 2024). Unfortunately, limited progress has been made in terms improvement of the therapeutic options. Therefore, the search for new strategies and the discovery of novel compounds with anti-leishmanial activity is of paramount importance.

Isopentyl caffeate is a phenylpropanoid ester of plant origin, derived from caffeic acid, and has interesting pharmacological properties, due to its antioxidant and antimicrobial activity (Steverding et al., 2016). The activity of isopentyl caffeate against *Trypanosoma brucei* was described as having a growth inhibition value of GI50 < 0.2 μg/mL and selectivity index > 100, with inhibition action of the lysosomal protease cathepsin L of *T. brucei* (Steverding et al., 2016).

Our research group has previously reported the potent anti-leishmanial action of isopentyl caffeate (ICaf) (Marques et al., 2020; Oliveira et al., 2021), showing IC_{50} values of 0.39 μg/mL (1.56 μM) for *L. amazonensis* and 0.43 μg/mL (1.71 μM) for *L. infantum* (Marques et al., 2020; Oliveira et al., 2021). ICaf induced relevant morphological changes in *Leishmania* promastigote forms, such as modification of the parasite body by reducing the size of the flagellum, blebs on the plasma membrane, and cellular aggregation, which corroborate its leishmanicidal action. Experiments deciphering the ICaf mechanisms of action revealed that it targets the parasite mitochondrion, leading to a significant downregulation of its metabolic activity and electric membrane potential, which results in the production of reactive oxygen species (ROS), that induce loss of plasma membrane integrity and parasitic death. Furthermore, a potent anti-amastigote activity was also identified for ICaf, with IC_{50} values of 1.52 μM and 1.60 μM calculated for intracellular amastigotes of *L. amazonensis* and *L. infantum*, respectively. Importantly, ICaf was well tolerated by THP-1 macrophages, resulting in excellent selectivity indices, namely, 90.5 after 24 h, 132.4 after 48 h and 120.1 after 72 h for *L. amazonensis* and 119.3 after 24 h, 115.1 after 48 h and 114.1 after 72 h for *L. infantum*. However, against promastigote forms, it was 117.1 for *L. amazonensis* and 106.2 for *L. infantum* after 72 h of treatment with ICaf (Oliveira et al., 2021).

To identify a potential drug candidate, it is necessary to comprehend its pharmacokinetic and pharmacodynamic properties, typically accomplished through a series of *in vivo* experiments aimed at assessing toxicity. Additionally, *in silico* analysis utilizing ADME (Absorption, Distribution, Metabolism, and Excretion) tools can provide valuable insights into new compounds. In this context, *in silico* strategies, such as computational modeling techniques, optimize the time and expense required to develop a drug. To this end, it seeks to recognize the similarity of drugs through their chemical structures and physicochemical properties to improve the chances of safety and desirable results (Jia et al., 2020; Sakyi et al., 2023), and predict pharmacokinetic profiles (ADME). Subsequently, further *in vivo* experiments are conducted to evaluate the efficacy of the treatment (Huang et al., 2020; Mirzaei, 2020).

Considering the rationale presented above, the aim of our work was to assess the absorption, distribution, metabolism and excretion (ADME) properties of this promising and potent antileishmanial compound (ICaf), by initially applying an *in silico* approach. As a logical progression of these studies, we investigated the drug toxicity profile using mice as an *in vivo* model. Finally, we evaluated the capacity of ICaf to control the *in vivo* infection in mice.

2. Material and methods

2.1. Materials

The ICaf was synthesized by Professor Damião P. de Souza from the Department of Pharmaceutical Sciences of the Federal University of Paraíba (PB, Brazil), according to the methodology described by Araújo et al. (2019). If not otherwise stated, all other reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Home supplied ultra-pure water (MilliQ, Merk Millipore, Burlington, MO, USA) was used for the performed experiments.

2.2. In silico experiments - ADME property analysis

The ADME properties of the compound (pharmacokinetic, bioavailability and druglikeness characteristics) were evaluated using the *in silico* Swiss ADME tool (http://www.swissadme.ch/) from the Swiss Institute of Bioinformatics. Using SMILES (simplified molecular input line entry specification) code, a line notation used to analyze the molecular structure of ICaf was used and then the prediction of bioavailability parameters was performed: polarity, lipophilicity, molecule size, flexibility, solubility and saturation. Together, the blood–brain barrier (BBB) permeation and the human intestinal absorption patterns, were evaluated using the predictive model BOILED-Egg (Brain or IntestinaL Estimate D Permeation Method), which allows the calculation of the lipophilicity and polarity of molecules. The activity of ICaf in the central nervous system (CNS) was assessed by observing the affinity of the efflux transporter P-glycoprotein (P-gp) (Daina and Zoete, 2016). Lipinski's rule of five was implemented to investigate the logP, molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors, and molar refractivity of ICaf (Lipinski, 2004). By definition, to satisfy the rule, the compound must have a logP ≤ 5 ICaf, a number of hydrogen bond acceptors ≤ 10, a number of hydrogen bond donors ≤ 5, and a molecular weight ≤ 500 Da (Lipinski, 2004).

2.3. In vivo toxicity assay

For the *in vivo* toxicity assay, fifty-six male Swiss mice (*Mus musculus*) aged two months and weighing between 20 and 25 g, from the vivarium at Universidade Tiradentes were used. The present study respected the ethical principles in research with laboratory animals established by the Brazilian Guideline for the Care and Use of Animals for Scientific and Didactic Purposes and, by the CONCEA Guidelines for the Practice of Euthanasia It is also in accordance with the Law 11.794/2008 and was approved by the Ethics Committee on the use of animals of the institution: Tiradentes University CEUA 011020A 030219. The study was carried out according to the guidelines for the care of laboratory animals (ANVISA, 2013; ISO, 2022).

For the toxicity tests in the acute phase, the animals were split into four experimental groups, each comprising six animals. These groups were administered a single oral dose of the ICaf suspension at varying

doses: 0.5, 5, and 50 mg/kg, alongside the control group which received the vehicle (PBS with 10 % DMSO and 0.1 % Tween 80). The animals were monitored over a period of 14 days, following administration on day 1. Individual filming sessions were conducted at specific time-points, namely, 15 min, 30 min, 1 h, 2 h, 4 h and 8 h (da Silva Bortoleti et al., 2019). The behaviour of animals was video-recorded and analysing, until the 14th day, hippocratic screening parameters, such as irritability, tremors, convulsions, piloerection, respiration, and mortality. Water and feed consumption were monitored throughout the 14-days period. At the end of the study, the animals were euthanized, and their organs (heart, lungs, spleen, liver, and kidneys) were extracted for microscopic evaluation (Cunha-Júnior et al., 2016).

Blood collection from the animals was performed at three different times, before the start of treatment (day 0), after treatment (day 2) and at the end of the experiment (on the 14th day). It is worth noting that the animals were fasted for 13 to 16 h before blood collection.

Blood samples were taken from the tail vein for creatinine, urea, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) tests. The concentrations in the serum were determined by colorimetric enzymatic tests, with spectrophotometric identification in an automatic biochemical analyzer, using commercial Labtest® kits, following the manufacturer's instructions. At the end of the *in vivo* efficacy study (after 14 days), the taken blood samples were kept at room temperature for 30 min followed by centrifugation for 10 min at 3500 × g. To assess renal and hepatic toxicity, serum was collected from each blood sample. The serum levels of treated rats were compared with untreated rats. Data were expressed as mean ± SD (n = 4/group). For the toxicity tests in the acute phase, the animals were split into four experimental groups, each comprising six animals. The test kits were used on Labmax Plenno equipment. All results were obtained by automated biochemical testing in triplicate. All results were obtained by automated biochemical testing in triplicate (Calvo et al., 2020).

Animals were euthanized after sampling the blood. under CO_2 inhalation, and the brain, heart, kidneys, liver, lungs, spleen, testes and ovaries were removed, weighed, and examined macroscopically. The weight of the organs was calculated as the percentage of relative weight:

$$\frac{organweight}{bodyweight} \times 100$$

Organswere fixed in formalin, dehydrated, diaphanized, embedded in paraffin, and 5-μm thick sections were obtained, stained with hematoxylin/eosin for histological examination. The microscopic analysis of all tissue samples was carried out as a blind study, i.e., by analyzing the tissues randomly to minimize possible interferences such as to which group of animals the tissue under observation belongs. The architectural and cellular features of the parenchymal and stromal components of the organs, as well as the presence of necrosis, or degenerative changes and architectural features of the tissue samples were evaluated (da Silva et al., 2015).

The toxicity results are represented as mean together with standard deviation of tests performed in duplicates. Differences between distinct groups were assessed using two-way ANOVA for repeated measures [factor 1: treatment (Control; Dose 0.5; Dose 5 and Dose 50) and factor 2: time (day 1, day 6 and day 14)], followed by a posteriori Tukey test, when appropriate. All tests were two-tailed and with a significance level of 5 %.

2.4. In vivo infection and treatment

2.4.1. Parasites and cultivation

Leishmania amazonensis (MHOM/BR75/Josefa strain) was obtained from the Leishmania Collectio of the Fundação Oswaldo Cruz (FIOCRUZ; Leishmania Type Culture Collection-LTTC-WDCM 731, RJ, Brazil). Promastigotes were maintained in 199 medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10 % fetal bovine serum (FBS) (Cultilab, São Paulo, SP, Brazil), 100 U/mL penicillin, 100 μg/mL streptomycin and 5 mg/mL hemin (Sigma-Aldrich, St Louis, MO, USA) at 26 °C in a BOD incubator (MOD. 347CD; FANEM, São Paulo, Brazil).

2.4.2. Animals' infection

For the *in vivo* infection assays, female mice (*Mus musculus*/C57BL/6; BALB/c) with a body weight of approximately 20 g (6–8 weeks), from the vivarium of the Federal University of Rio de Janeiro (RJ, Brazil), were intradermally infected with 2×10^6 *L. amazonensis* promastigotes in stationary phase, in the right paw of the animals. The infection was monitored over time by measuring the increase of the paw thickness using a dial caliper (Mitutoyo 7301, Kanagawa, Japan). The present study respected the ethical principles in research with laboratory animals established by the Brazilian Guideline for the Care and Use of Animals for Scientific and Teaching Purposes and, by the CONCEA Guidelines for the Practice of Euthanasia, it is also in accordance with the precepts of the Law 11.794/2008 and was approved by the Ethics Committee on the use of animals of the respective institution: Health Sciences Center of the Federal University of Rio de Janeiro CEUA A11/19–080-18 (ANVISA, 2013; ISO, 2022).

2.4.3. Animals' treatment

The treatments were started from the seventeen-day post-infection by intraperitoneal injections of 30 and 60 mg/kg of ICaf with a total of 15 doses, while the control animal group received the vehicle (PBS with 10 % DMSO and 0.1 % Tween 80). In the final of treatment, the infected paw was excised and disinfected by placing it for 1 min in 70 % alcohol. Spleen and liver were also removed and placed in Eppendorfs containing 1 mL of 199 medium with 10 % FBS. These organs were then homogenized. A volume of 50 μL of the homogenates was diluted four-fold in 150 μL of 199 medium per well, in 96-well plates, which were then-placed in a BOD incubator for 7 to 14 days at 26 ∘C. By the end of this timeframe, plates were checed under an optical microscope and, for the determination of the parasite load, the last well in which parasites were seen was considered. For the calculation, the following equation was used:

$$Numberofparasites = \frac{4X}{Massoforgans(g)}$$

where X is the number of the last well in which parasites were observed. Statistical analyses were performed using GraphPad Prism 8 software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical differences between values were evaluated by the parametric One-Way ANOVA (two-tailed) or t-Student test. Differences between the control and treated groups were considered statistically significant at $p \leq 0.05$.

2.4.4. Statistical analysis

All experiments were carried out in triplicate, using three independent experimental sets. Unidirectional ANOVA was used for the statistical analysis of data in a GraphPad Prism 4.0 software. To evaluate numerical data, descriptive analysis, including mean and standard deviation, was used.

3. Results and discussion

3.1. In silico experiments

ICaf exhibited bioavailability levels that fall within the optimal and desirable range, indicating potential use for oral administration and effective systemic absorption. In Fig. 1, the molecular structure of ICaf is shown for a more in-depth analysis of the bioavailability (Fig. 2).

The evaluation of ICaf bioavailability encompassed various key characteristics, including solubility, lipophilicity, polarity, molecule size, flexibility, and saturation (Fig. 2). The grey area depicted in the

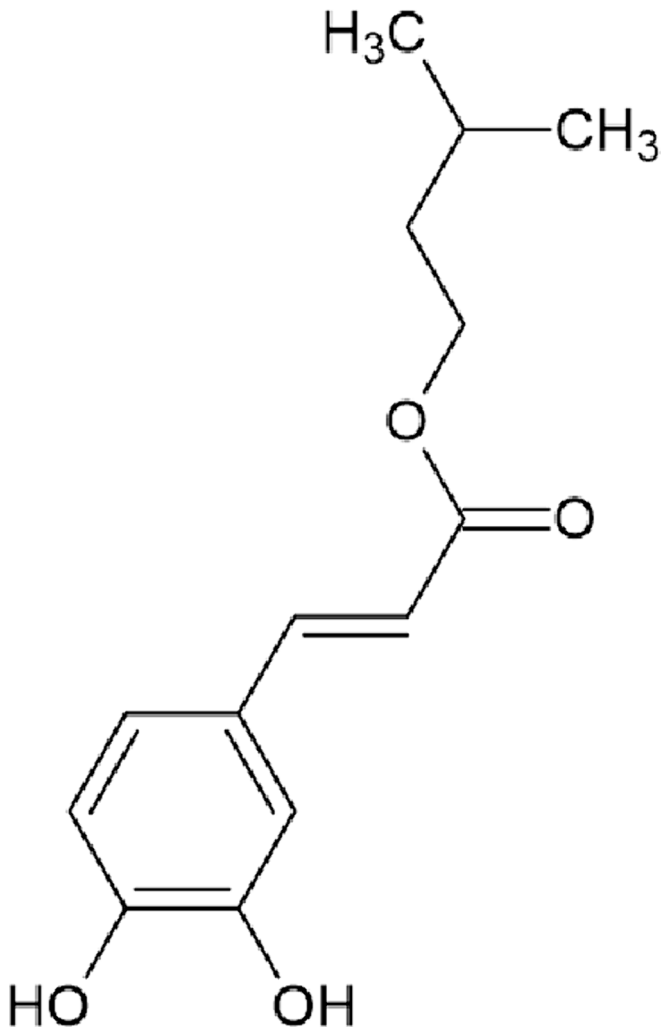

Fig. 1. Molecular structure of isopentyl caffeate, (E)-isopentyl 3-(3,4-dihydroxyphenyl) acrylate.

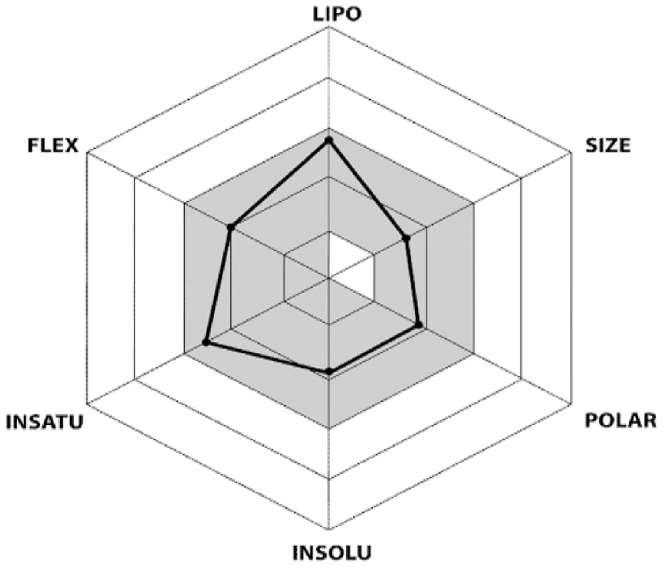

Fig. 2. Bioavailability radar of isopentyl caffeate. Gray area stands for the bioavailability limits of the compound, and bold lines refer to the values reached by the compound. (Captions: LIPO, lipophilicity; SIZE, molecular size; POLAR, polarity; INSOL, solubility; INSATU, saturation; FLEX, flexibility).

graph represents the bioavailability limits of the compound, while the bold line indicates the real values achieved by ICaf. Before formulating a candidate drug, knowledge of the human intestinal absorption is essential and computational screening is important for solving problems related to absorption before synthesis (Ahmad et al., 2024).

Solubility stands to the maximum amount that a substance is dissolved in a specific volume of solvent at a certain temperature and pressure. In terms of lipophilicity, drugs exhibiting a higher partition coefficient generally demonstrate enhanced permeability across hydrophobic biomembranes. This characteristic can lead to increased pharmacological effects and a more favorable bioavailability profile (Soares et al., 2022).

In general, the ICaf drug exhibited a favorable lipophilicity profile, a critical factor for passive absorption of substances, ensuring its effectiveness. It is well-known that an imbalance in lipophilicity can result in strong binding of the drug to plasma proteins, leading to a decrease in the free drug concentration and consequently reducing its potency. Furthermore, substances that are highly hydrophilic or polar often exhibit decreased bioavailability due to their low permeability through cell membranes. Utilizing computational modeling, we found that ICaf possesses an appropriate polarity, indicating no difficulties in penetrating the cell membrane and thus ensuring uncompromised bioavailability (Severino et al., 2022). Additionally, it is important to mention that the absorption of a substance is determined by several physicochemical properties that also involve the size of the molecule. Thus, the diffusion gradient is determined by a concentration gradient, where the movement occurs from a region of high concentration to one of low concentration. In this sense, the drug was also suitable, since it was able to penetrate membranes. As seen in the literature, smaller molecules are prone to penetrate through membranes much faster than those of larger size (Jain, 2020).

According to the obtained computational model, ICaf has adequate flexibility and is within the optimal limits to be considered a pharmacological drug. These characteristics have been extensively explored in biochemical systems, since they are associated with processes, such as molecular recognition and catalytic activity (Stanzione et al., 2021). Studies describe that the factors related to the flexibility of molecules and the distribution of polar groups are good predictors of oral bioavailability. In addition, drugs must meet the criteria of ten or fewer rotational bonds and polar surface area $\leq$ 140 Å2 for a high probability of appropriate oral bioavailability, in mouse experiments, regardless the molecular weight (Maliar et al., 2023).

The saturation is an important requirement for organic molecules, because the presence of unsaturated bonds in the molecule can mean the possibility of reactions taking place (Pedersen-Bjergaard et al., 2019).

In the study by Melo et al. (2022), isopropyl gallate showed acceptable *in silico* pharmacokinetic parameters, with the ADMET results showing appropriate lipophilicity to ensure a good oral bioavailability. The *in vitro* analysis demonstrated that the compound could reduce the infectivity in parasitized macrophages, attributed to the activation of macrophage pathways, followed by inhibition of *Leishmania major* (Melo et al., 2022).

In silico pharmacokinetic properties were also used by Santos et al. (2023), who synthesized and characterized a range of thiosemicarbazones and thiazoles, showing their appropriate oral bioavailability and permeability, according to Lipinski's rules. The *in vitro* antiparasitic activity of such compounds confirmed their potential against different forms of *T. cruzi* and *L. amazonensis* (Santos et al., 2023).

In silico analysis carried out using SwissADME tools also showed that the physicochemical properties and bioavailability of amentoflavone favour the use of this biflavonoid for oral administration. Based on this result, an *in vitro* analysis was carried out in which the compound was recommended as a promising candidate for advanced studies to develop new drugs against leishmaniasis (Rizk et al., 2022).

The prediction of ICaf to permeate brain and gut was performed

using the BOILED-Egg predictive model (Daina and Zoete, 2016). This model performs predictive calculations that allows to estimate the ability of molecules to cross physiological barriers. The evaluation is of utmost importance, because these are among the most relevant pharmacokinetic parameters for drugs. These predictions are made from correlation calculations between polar surface area (PSA) and partition coefficient (LogP) values, which are compared to a database of hundreds of molecules already investigated. Thus, according to this predictive model, i.e. the position of the drug (×) within both radars, ICaf was shown to have adequate absorption characteristics through the gastrointestinal tract (⇀ continuous line) and permeation capacity through blood–brain barrier (BBB) (—, discontinuous line) (Fig. 3). This result complements those observed in the bioavailability radar of ICaf (Fig. 2), but sheds light on the possible central impacts that ICaf would bring, since the treatment of leishmaniasis is systemic and/or topical.

Table 1 shows the calculated physicochemical properties of ICaf. When observing the ADME properties, ICaf was shown to be hydrophobic (WLOGP = 2.59), poorly water-soluble, and of high molecular weight, which may compromise its biological capacity, suggesting the need for complexation with another molecule. This approach was described Marques et al. (2020), when proposing the development of an inclusion complex between ICaf and β-cyclodextrin to increase the solubility and bioavailability of the compound.

Regarding the pharmacokinetic characteristics (Table 2), ICaf showed high gastrointestinal absorption and BBB permeation capacity. Brain bioaccumulation capacity identified by assessing the potential substrate for P-gp was also observed. P-glycoprotein (P-gp) is an important transporter that influences how drugs penetrate the brain. To act effectively in the CNS, a drug needs to cross the brain capillary endothelial cells (BCEC) composing the BBB. P-gp is a well-known efflux transporter of the BBB; it has the function of precisely regulating drug membrane permeability between blood and brain by BCEC, astrocytes and pericytes, since it maintains a low concentration of unbound molecules in the brain compared to plasma (Watanabe et al., 2021). Generally, the ability of a substance to cross BBB depends on several factors, including the molecular size, electrical charge, lipophilicity, among others, requirements that enabled ICaf to cross BBB, as confirmed by the data shown in Table 1 and Figs. 2 and 3.

Table 1
Calculated physicochemical properties of ICaf.

Physicochemical Properties	
Formula	$C_{14}H_{18}O_4$
Molecular Weight	250.29 g/mol
N°. of heavy atoms	18
N°. of heavy aromatic atoms	6
Fraction Csp3	0.36
N°. Revolving connections	6
Number of H-bond donors	2
Number of H-bond acceptors	4
Molar refractivity	70.71
TPSA	66.76 Å^2
Log Po/w (WLOGP)	2.59

Table 2
Calculated pharmacokinetic parameters of ICaf.

Pharmacokinetic Properties	
Gastrointestinal Absorption	High
BBB Permeant	Yes
Substrate P-gp	No
Inhibitor CYP1A2	Yes
Inhibitor CYP2C19	No
Inhibitor CYP2C9	No
Inhibitor CYP2D6	No
Inhibitor CYP3A4	No
Log Kp (skin permeation)	−5.01 cm/s

Besides the evaluation of these properties, analysis regarding the interaction of ICaf with liver enzymes, mainly, the isoforms of the cytochrome *P*-450 oxidase enzyme, CYP, which are responsible for metabolizing xenobiotics (Manikandan and Nagini, 2018), was also performed. Among the five evaluated isoforms, ICaf showed inhibitory action only for CYP1A2. The CYP1A2 enzymes play an important role in the oxidative metabolism of 90 % of the active ingredients of drugs in clinical use. The inhibitory action on CYP1A2 can determine an increase in plasma concentration and a reduction in its metabolites, and influence the clinical outcome and pharmacological effects (Gunes and Dahl,

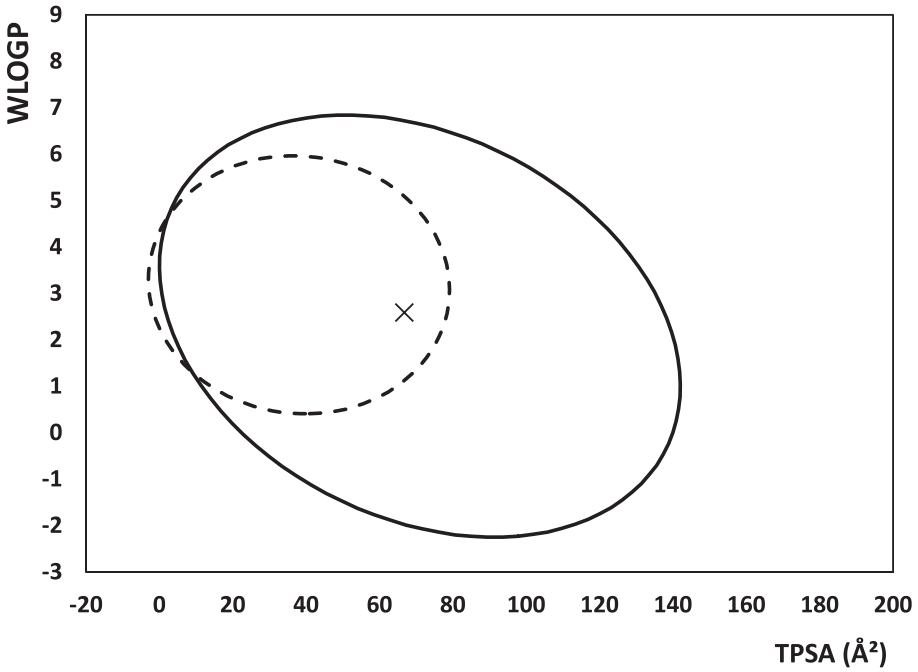

Fig. 3. Prediction of the brain and intestinal permeability of isopentyl caffeate using the BOILED-Egg predictive model. Caption: (×) isopentyl caffeate, (–) human intestinal absorption (HIA) and (– – –) blood–brain barrier (BBB).

2008). In other words, when an enzyme is inhibited by a specific compound, there is a modification in the biotransformation conditions, leading to interactions that can either potentiate or inactivate the drug.

Overall, the results observed in the present study corroborate the assumption that ICaf could be easily metabolized by the liver and excreted in its biotransformed form, despite this inhibition. Regarding skin permeation capacity (Log Kp), it was shown to be favorable for topical use (-5.01 cm/s). Penetration through the stratum corneum occurs mainly via two pathways: the intercellular route, where the drug diffuses around the corneocytes through the intercellular lipid matrix, and the transcellular route, where the drug crosses the corneocytes and the lipid matrix (Phatale et al., 2022). The skin is a barrier to drug penetration, and the search for molecules with characteristics favoring permeation is fundamental, as well as the development of methodologies that ensure an efficient penetration through the layer. In this sense, for the development of a topical drug, aspects such as the nature and concentration of the active ingredients, the excipients and the type of system used to transport the drug should be considered.

Regarding the druglikeness parameters (Table 3), the compound satisfied all the criteria established by Lipinski's rule of five, which states that a substance must present adequate values for 4 parameters (in multiples of 5) to be a good drug: molecular mass less than or equal to 500, Log P greater than or equal to 5, hydrogen bond acceptors less than or equal to 10 and hydrogen bond donors less than or equal to 5 (Lipinski, 2004). Furthermore, ICaf has met the principles established by the Ghose, Veber, Egan, and Muegge models. Ghose's model defines that the compound should have the LogP value between −0.4 to 5.6, molecular weight from 160 to 480 Da, and the total number of atoms in the compound from 20 to 70 (Ghose et al., 1999). Veber's rule confirms that the compound has rotational bonds less than or equal to 10 and a polar surface area less than or equal to 140 Å2 (Veber et al., 2002). Muegge's model, on the other hand, states that the compound should have a LogP less than or equal to 5, amolecular weight between 200 and 600 Da, the number of hydrogen bond acceptors less than or equal to 10, the number of hydrogen bond donors less than or equal to 5, the number of rotational bonds less than or equal to 15 and the polar surface area less than or equal to 150 Å2 (Muegge, 2003). In case of ICaf, the LogP was found to be 2.59, the molecular weight of 250.29 g/mol, with 2 hydrogel bond donors, 6 the rotational bonds and 66.76 Å2 the polar surface area (Table 1), thus satisfying the criteria of all models (Table 3).

Egan et al. (2000) used data from the literature of compounds that are well absorbed and others that are poorly absorbed in humans, and built a statistical model for recognizing passive intestinal absorption patterns. Based on the physical processes involved in membrane permeability and the interrelationships between the available descriptors, the descriptors chosen for inclusion in the model were AlogP98 and PSA. These rules enable the prediction of the oral bioavailability profile for novel substances, which subsequently determines the adequacy of the time and extent of absorption of the active ingredient at the intended site.

The bioavailability calculation of the compound was greater than 50 %. Thus, the studied compound presented potential for its use as a pharmacological drug. According to the study by da Silva Bortoleti et al. (2019), ICaf was able to cause signs of rounding and shrinkage in the membrane, and loss of membrane integrity as a result of the appearance of blisters. The membrane is known to maintain the integrity of the cell, and any damage of this structure can result in intracellular imbalance and impact the survival of the parasite. Similarly, in our previous study, ICaf induced several severe morphological alterations in *Leishmania* cells, including irreversible plasma membrane damage that resulted in parasite death (Oliveira et al., 2021).

Table 3
Calculated parameters of druglikeness of ICaf.

Druglikeness	
Lipinski	Yes
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

3.2. *In vivo toxicity assay*

Acute toxicity tests provide valuable insights into the potential acute toxicity and lethality of a given compound. These tests consist in the administration of the compound to an experimental model, followed by monitoring the model for a period of 14 days post-administration. One notable advantage of these tests is their ability to yield informative results while utilizing a reduced number of animals, in contrast to the traditional LD_{50} test (Erhirhie et al., 2018).

To assess the potential activity of the tested compound in the CNS and its impact on animal behavior, an initial behavioral screening was conducted. None of the animals displayed any of the assessed parameters, such as irritability, tremors, convulsions, piloerection, breathing abnormalities, or mortality. The hippocratic screening analysis provided a comprehensive assessment of the toxicological nature of the administered doses, taking into account the animals' level of consciousness, disposition, reflexes, and CNS-related activities.

The amount of water (Fig. 4) and feed (Fig. 5) consumed for 14 days were also evaluated. For this analysis, the average consumption of both water and feed was performed on days 1–5, 6–9 and 10–14, while the animals were allocated into four experimental groups (Control; Dose 0.5; Dose 5, 0 and Dose 50). The weight evaluation is amongst the parameters commonly used in toxicological assessment to inform toxicological risks of a certain. Statistical analysis (two-way ANOVA) showed no significant interaction between the factors neither for water consumed ($p > 0.05$) (Fig. 4) nor for food consumed ($p > 0.05$) (Fig. 5).

To date, there is limited knowledge regarding the acute toxic effects of ICaf administration. Nevertheless, previous studies investigated the toxicity of caffeic acid derivatives, including isopropyl caffeineate. Lira et al. (2018) explored the *in vivo* antioxidant potential and toxicity of isopropyl caffeineate in female Balb/c mice. Upon administration of 300 mg/kg, the authors observed signs of CNS depression, such as sedation, loss of atrial reflex, reduced hyperactivity, diminished response to touch, as well as constipation and decreased muscle tone. It is important to note that although isopropyl caffeineate differs in potency and efficacy from ICaf, the lowest administered dose in the study was six times higher than that of ICaf, while the highest dose was 40 times greater.

Considering that systemic toxicity often manifests as a reduction in

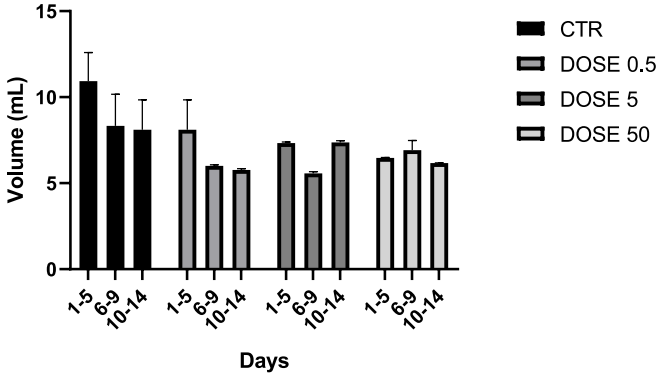

Fig. 4. Water consumption (mL) during 14 days after challenging mice with ICaf. 1–5: mean consumption on days 1 to 5; 6–9: mean consumption on days 6 to 9; 10–14: mean consumption on days 10 to 14. CTR: control group. Dose 0.5: 0.5 mg/kg of ICaf; Dose 5: 5.0 mg/kg of ICaf. Dose 50: 50 mg/kg of ICaf.

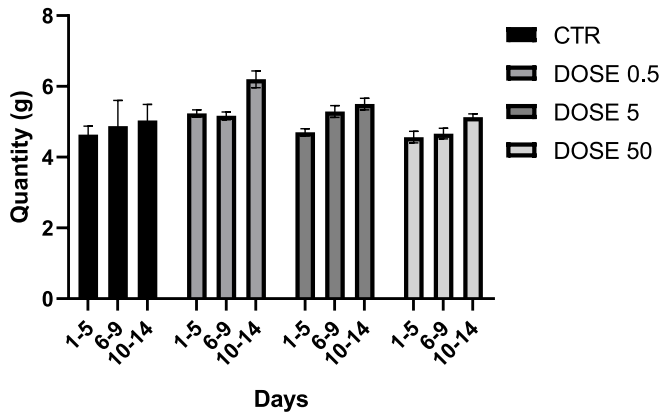

Fig. 5. Food consumption (grams; g) during 14 days after challenging mice with ICaf. 1–5: mean consumption on days 1 to 5; 6–9: mean consumption on days 6 to 9; 10–14: mean consumption on days 10 to 14. CTR: control group. Dose 0.5: 0.5 mg/kg of ICaf; Dose 5: 5.0 mg/kg of ICaf. Dose 50: 50 mg/kg of ICaf.

the evaluated parameters, it is noteworthy that no alterations were observed in the analysis. Therefore, the results of our study indicate that the acute administration of ICaf at doses of 0.5, 5, and 50 mg/kg did not induce toxicity in the animals based on these parameters. The animal organs (heart, lungs, spleen, liver, and kidneys) were subsequently collected and further analyzed. These organs are frequently susceptible to metabolic reactions caused by toxic agents (Roy et al., 2015). However, the histological analysis revealed no tissue alterations associated with the administration of any of the doses of ICaf (Fig. 6).

It is worth mentioning that these selected organs (heart, lungs, spleen, liver, and kidneys) were chosen because they also present alterations in case of *Leishmania* infections. For example, the spleen is an initial site where the specific immune response against the leishmaniasis parasite occurs, besides being involved in systemic inflammation, increasing its size (Poulaki et al., 2021). Leishmaniasis auses morphological and functional disorders in the liver, such as hyperplasia of Kupfer cells, formation of granulomas in the portal region, fibrosis and hepatitis.

The blood of the animals was also collected to investigate toxicological biomarkers. No significant differences ($p > 0.05$) were observed between untreated and ICaf-treated animals regarding the evaluated biochemical parameters studied, including glucose, urea, creatinine, alkaline phosphatase, AST and ALT levels (Fig. 7).

Using isopropyl caffeate ester, Lira et al. (2018) reported lower levels of AST and ALT when comparing the untreated control animals with those given 300 and 2000 mg/kg. In addition, urea levels decreased at the dose of 300 mg/kg, which justifies the high dosage. According to the observed physiological effects, such as the hippocratic screening and weight data, ICaf did not show high toxicity (corroborating the *in silico* data) and also demonstrated possible renal and hepatic protection. Based on the results of this study, it is clear that acute administration of ICaf at doses of 0.5, 5.0, and 50 mg/kg did not induce any observable toxicity in the animals, which is a promising indication for the potential use of the low dose.

3.3. *Icaf treatment controls in vivo lesion growth and parasite load*

While Swiss mice (*Mus musculus*) were used for toxicity analysis, female mice (*Mus musculus*/C57BL/6; BALB/c) were selected to evaluate the effect of ICaf treatment of *L. amazonensis*-infected animals. These differences are not relevant for the objectives of the present study and by including both sexes in the studies, the results may be applicable to different population groups. As presented in Fig. 8, the control groups comprised female mice treated intraperitoneally with vehicle control group (PBS with 10 % DMSO and 0.1 % Tween 80) or treated with 30 or 60 mg/kg for 15 days. In the treatment using 60 mg/kg, we observed a reduction of lesion size (Fig. 8**A**) and a 95.5 % reduction in the parasite load at the site of infection (Fig. 8**B**). However, no difference was observed when used 30 mg/kg (Fig. 8**A, 8B**). No differences were also observed in both treatments in the parasite load of draining lymph nodes (Fig. 8**C**). In addition, the mice treated with 60 mg/kg also showed a reduction in parasite loads in the spleen (Fig. 8**D**) compared to infected

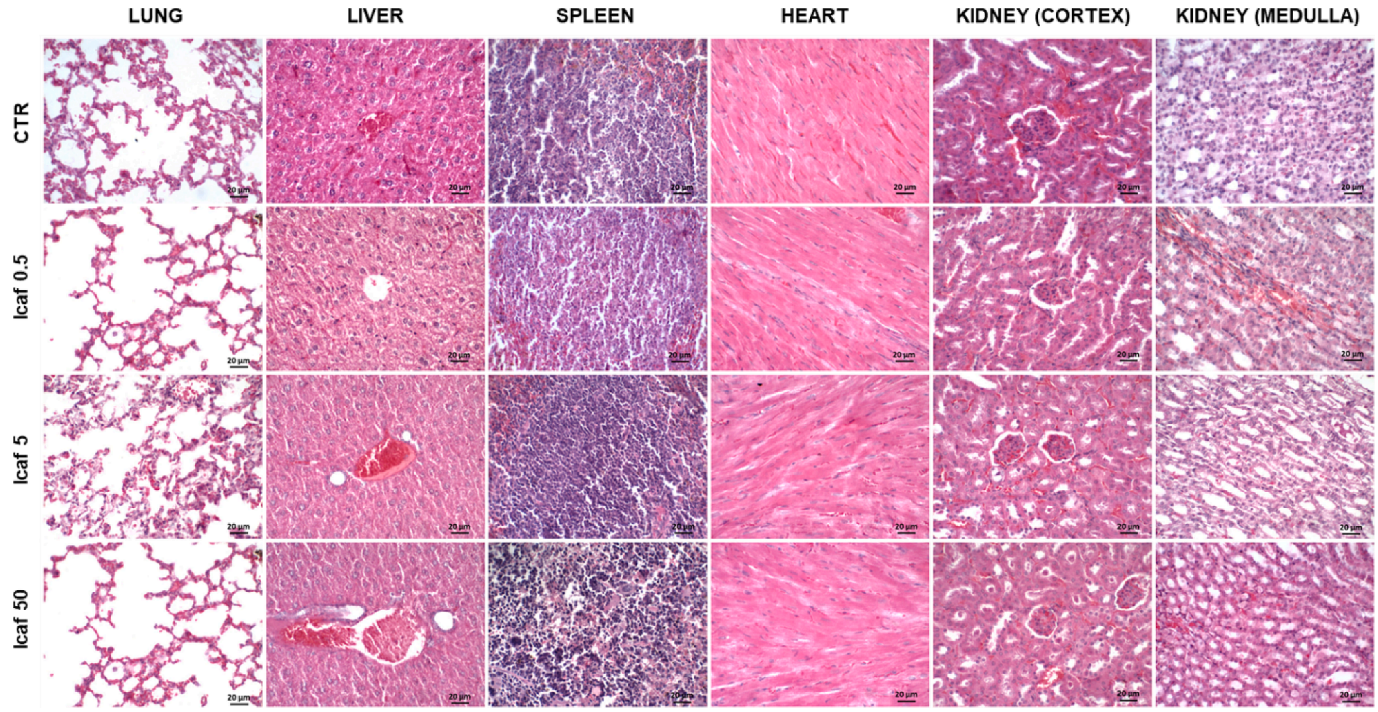

Fig. 6. Histological analysis of key organs after challenged of mice with ICaf. CTR: control group. ICaf 0.5: 0.5 mg/kg of ICaf. ICaf 5: 5 mg/kg ICaf. ICaf 50: 50 mg/kg of ICaf.

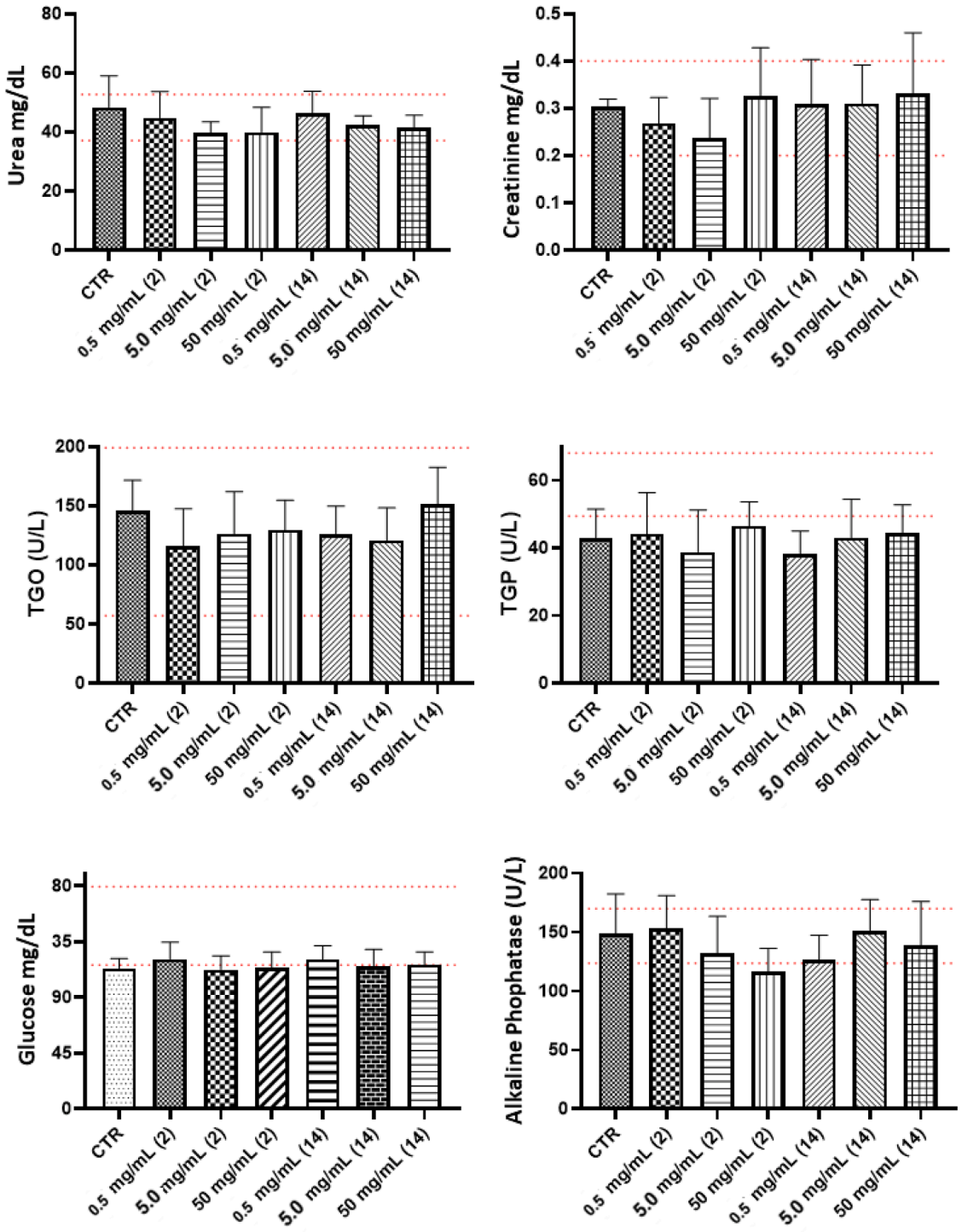

Fig. 7. Biochemical profile in untreated and ICaf-treated mice. The blood was collected on days 2 and 14, and the samples were analyzed to measure the following parameters: urea, creatinine, AST, ALT, glucose and alkaline phosphatase. Non-treated animals (control; CTR) and animals treated with different ICaf concentrations (0.5, 5.0 and 50 mg/kg) were evaluated. Dashed in red represents the reference values used. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mice treated with a vehicle; the group treated with 30 mg/kg also showed a difference in the parasite load in the spleen, with a reduction of 98.87 % (Fig. 8**D**), demonstrating that at this concentration there was already an impediment in the visceralization. These results indicate that ICaf at 60 mg/kg controls lesion growth and parasite load in the paw and spleen, without showing toxicity as documented in the supplementary material. BALB/c mice were infected on the right paw with 2 × 106 stationary-phase *L. amazonensis* promastigotes (Josefa strain) by intradermic route. Seventeen days after infection, the animals were treated with ICaf with 30 mg/kg or 60 mg/kg intraperitoneally with fifteen doses. Mean values of the enzyme markers of hepatocellular damage (ALT and AST) in the control group that received the vehicle (PBS with 10 % DMSO and 0.1 % Tween 80) were 17.2 U/L (ALT) and 37.6 U/L (AST), while the groups treated with 30 mg/kg showed similar values of 13.2 U/L (ALT) and of 28.9 U/L (AST). The groups treated with 60 mg/kg also showed similar values of 11.8 U/L (ALT) and 27.3 U/L

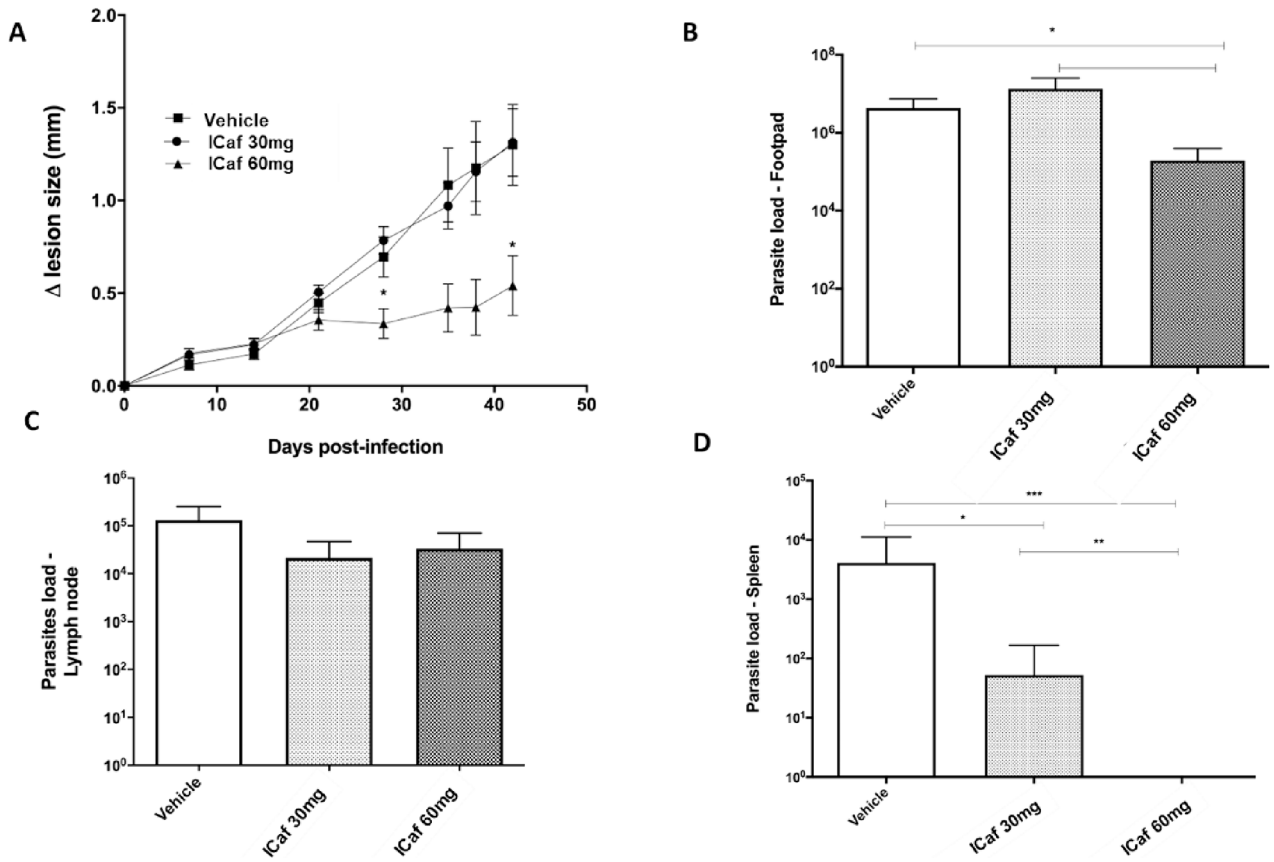

Fig. 8. *In vivo* effect of ICaf. BALB/c mice were infected in the right paw with 2×10^6 stationary-phase *L. amazonensis* promastigotes (Josefa strain) by intradermic route. Seventeen days after infection, the animals were treated with ICaf with 30 mg/kg or 60 mg/kg intraperitoneally with fifteen doses (15 days). (A) Lesion size (mm) were measured once weekly; control group received vehicle (PBS with 10 % DMSO and 0.1 % Tween 80) or treated with 30 or 60 mg/kg/day. (B) Parasite load were determined by LDA at footpad. (C, D) Draining lymph node and spleen. Statistical difference between means values were evaluated by One-way ANOVA with Tukey post-test (N = 4–5 mice per group).

(AST), which suggest the absence of ICaf-induced liver toxicity (Supplementary Material S1 and S2).

Intraperitoneal administration was chosen in studies involving induction of infections or administration of infectious agents because it allows effective distribution of the infectious agent into the peritoneal cavity. Intraperitoneal administration ensures rapid absorption of the substance directly into the bloodstream, which is an advantageous method for quickly achieving effective therapeutic levels. We could demonstrate through experimental studies, that intraperitoneal administration ensures effective treatment delivery in animal models that mimic the conditions of leishmaniasis. Collectively, our results suggest that ICaf has potential therapeutic effects against *L. amazonensis* infection. However, further studies are required to disclose the underlying mechanisms and assess the long-term safety and efficacy of this treatment.

A study by Rottini et al. (2015) confirmed the antileishmanial activity of (-)α-bisabolol against *L. amazonensis*, reporting that this drug was capable of inhibiting the growth of promastigotes by 50 % at a concentration of 8.07 μg/mL (IC50). When tested against amastigote forms, (-)α-bisabolol inhibited growth IC50 was reduced down to 4.15 μg/mL. These results show that the promastigotes were more sensitive to treatment with ICaf when compared to (-)α-bisabolol.

4. Conclusions

Leishmaniasis is a neglected parasitic disease associated with high toxicity, cost and parasitic resistance when treated with currently available pharmacological options. This study aimed to evaluate the properties of ICaf for the treatment of *L. amazonensis*. Evaluation of *in vivo* toxicity in mice showed no adverse effects on multiple parameters over a 14-day period. *In silico* analysis indicated favorable pharmacokinetic characteristics and bioavailability for ICaf. Intraperitoneal treatment with ICaf showed a reduction in lesion size and parasite burden. Therefore, ICaf can be considered a potential alternative treatment against *L. amazonensis*, offering promising pharmacological perspectives for the treatment of this disease.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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UIDP/00102/2020 (Programmatic funding).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crbiot.2024.100209.

References

Ahmad, I., Rabbi, F., Nisar, A., Ul-Haq, Z., Khan, A., 2024. In vitro-in silico pharmacology and chemistry of Stercularin, isolated from Sterculia diversifolia. Comput. Biol. Chem. 109, 108008.

ANVISA, 2013. Guia para a condução de estudos não clínicos de toxicologia e segurança farmacológica necessários ao desenvolvimento de medicamentos. Agência Nacional de Vigilância Sanitária, Brasília, Brazil, pp. 970–975.

Araújo, M.O., Freire Pessoa, H.L., Lira, A.B., Castillo, Y.P., de Sousa, D.P., 2019. Synthesis, antibacterial evaluation, and QSAR of caffeic acid derivatives. J. Chem. 2019, 3408315.

Calvo, A., Moreno, E., Larrea, E., Sanmartín, C., Irache, J.M., Espuelas, S., 2020. Berberine-loaded liposomes for the treatment of Leishmania infantum-infected BALB/c mice. Pharmaceutics 12 (9), 858.

Cunha-Júnior, E.F., Martins, T.M., Canto-Cavalheiro, M.M., Marques, P.R., Portari, E.A., Coelho, M.G.P., Netto, C.D., Costa, P.R.R., Sabino, K.C.d.C., Torres-Santos, E.C., 2016. Preclinical studies evaluating subacute toxicity and therapeutic efficacy of LQB-118 in experimental visceral leishmaniasis. Antimicrob. Agents Chemother. 60 (6), 3794–3801.

da Silva, R.O., Andrade, V.M., Rêgo, E.S.B., Dória, G.A.A., dos Santos Lima, B., da Silva, F.A., de Souza Araújo, A.A., de Albuquerque Júnior, R.L.C., Cardoso, J.C., Gomes, M.Z., 2015. Acute and sub-acute oral toxicity of Brazilian red propolis in rats. J. Ethnopharmacol. 170, 66–71.

da Silva Bortoleti, B.T., Tomiotto-Pellissier, F., Gonçalves, M.D., Miranda-Sapla, M.M., Assolini, J.P., Carloto, A.C., Lima, D.M., Silveira, G.F., Almeida, R.S., Costa, I.N., 2019. Caffeic acid has antipromastigote activity by apoptosis-like process; and anti-amastigote by TNF-α/ROS/NO production and decreased of iron availability. Phytomedicine 57, 262–270.

Daina, A., Zoete, V., 2016. A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. ChemMedChem 11 (11), 1117–1121.

de Vries, H.J., Schallig, H.D., 2022. Cutaneous leishmaniasis: A 2022 updated narrative review into diagnosis and management developments. Am. J. Clin. Dermatol. 23 (6), 823–840.

Egan, W.J., Merz, K.M., Baldwin, J.J., 2000. Prediction of drug absorption using multivariate statistics. J. Med. Chem. 43 (21), 3867–3877.

Erhirhie, E.O., Ihekwereme, C.P., Ilodigwe, E.E., 2018. Advances in acute toxicity testing: Strengths, weaknesses and regulatory acceptance. Interdiscip Toxicol 11 (1), 5–12.

Ghose, A.K., Viswanadhan, V.N., Wendoloski, J.J., 1999. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. J. Comb. Chem. 1 (1), 55–68.

Gimeno-Pitarch, L., Almela, P., Nos, P., 2024. Leishmania infection in patients with inflammatory bowel disease: Case series and literature review. Gastroenterol Hepatol 47 (1), 82–92.

Gunes, A., Dahl, M.-L., 2008. Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms. Pharmacogenomics 9 (5), 625–637.

Huang, W., Percie du Sert, N., Vollert, J., Rice, A.S., 2020. General principles of preclinical study design, Good research practice in non-clinical pharmacology and biomedicine. Handb Exp Pharmacol. 257, 55–69.

ISO. 2022. ISO 10993-2:2022(en): Biological evaluation of medical devices — Part 2: Animal welfare requirements, https://www.iso.org/obp/ui/#iso:std:iso:10993:-2:ed-3:v1:en (accessed on February 1st 2023).

Jain, K.K., 2020. An overview of drug delivery systems. Methods Mol. Biol. 2059, 1–54.

Jia, C.-Y., Li, J.-Y., Hao, G.-F., Yang, G.-F., 2020. A drug-likeness toolbox facilitates ADMET study in drug discovery. Drug Discov. Today 25 (1), 248–258.

Kaushal, R.S., Naik, N., Prajapati, M., Rane, S., Raulji, H., Afu, N.F., Upadhyay, T.K., Saeed, M., 2023. Leishmania species: A narrative review on surface proteins with structural aspects involved in host-pathogen interaction. Chem Biol Drug Des 102 (2), 332–356.

Kumar, S., Bodhale, N., Patra, S.K., Sarode, A., Zhao, L., Sarkar, A., Saha, B., 2024. Interleukin-7 potentiates MAPK10-elicited host-protective vaccine against Leishmania donovani. Cytokine 174, 156475.

Lipinski, C.A., 2004. Lead-and drug-like compounds: the rule-of-five revolution. Drug Discov. Today Technol. 1 (4), 337–341.

Lira, A.B., Montenegro, C.A., de Oliveira, K.M., de Oliveira Filho, A.A., da Paz, A.R., de Araújo, M.O., de Sousa, D.P., de Almeida, C.L.F., da Silva, T.G., Lima, C., Diniz, M., Pessôa, H.L.F., 2018. Isopropyl caffeate: A caffeic acid derivative-antioxidant potential and toxicity. Oxid Med Cell Longev 2018, 6179427.

Maliar, T., Maliarová, M., Purdešová, A., Jankech, T., Gerhardtová, I., Beňovič, P., Dvořáček, V., Jágr, M., Viskupičová, J., 2023. The adapted POM analysis of avenanthramides in silico. Pharmaceuticals 16 (5), 717.

Manikandan, P., Nagini, S., 2018. Cytochrome P450 structure, function and clinical significance: A review. Curr Drug Targets 19 (1), 38–54.

Marques, C.S., Barreto, N.S., Oliveira, S.S.d., Santos, A.L., Branquinha, M.H., Sousa, D.P. d., Castro, M., Andrade, L.N., Pereira, M.M., Silva, C.F.d., 2020. β-cyclodextrin/isopentyl caffeate inclusion complex: Synthesis, characterization and antileishmanial activity. Molecules 25 (18), 4181.

Melo, D.S., Nery Neto, J.A.O., Santos, M.S.D., Pimentel, V.D., Carvalho, R.C.V., Sousa, V. C., Sousa, R.G.C., Nascimento, L.G.D., Alves, M.M.M., Arcanjo, D.D.R., Sousa, D.P., Carvalho, F.A.A., 2022. Isopropyl gallate, a gallic acid derivative. In silico and in vitro investigation of its effects on leishmania major. Pharmaceutics 14 (12).

Mirzaei, M., 2020. Science and engineering in silico. Advanced Journal of Science and Engineering 1 (1), 1–2.

Muegge, I., 2003. Selection criteria for drug-like compounds. Med. Res. Rev. 23 (3), 302–321.

Narsimulu, B., Jakkula, P., Qureshi, R., Nasim, F., Qureshi, I.A., 2024. Inhibition and structural insights of leishmanial glutamyl-tRNA synthetase for designing potent therapeutics. Int. J. Biol. Macromol. 254, 127756.

Nuwangi, H., Agampodi, T.C., Price, H.P., Shepherd, T., Weerakoon, K.G., Agampodi, S. B., 2023. The stigma associated with cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL): A protocol for a systematic review. PLoS One 18 (5), e0285663.

Oliveira, S.S., Marques, C.S., de Sousa, D.P., Andrade, L.N., Fricks, A.T., Jain, S., Branquinha, M.H., Souto, E.B., Santos, A.L., Severino, P., 2021. Analysis of the mechanisms of action of isopentenyl caffeate against Leishmania. Biochimie 189, 158–167.

Ornellas-Garcia, U., Cuervo, P., Ribeiro-Gomes, F.L., 2023. Malaria and leishmaniasis: Updates on co-infection. Front. Immunol. 14, 1122411.

Pederiva, M.M.C., Santos, S.M.D., Rivarola, L.G.S., Guerreiro, V.J., Lopes, K.S., Lima Junior, M., Neitzke-Abreu, H.C., 2023. Asymptomatic leishmania infection in humans: A systematic review. J Infect Public Health 16 (2), 286–294.

Pedersen-Bjergaard, S., Gammelgaard, B., Halvorsen, T.G., 2019. Introduction to pharmaceutical analytical chemistry, 2nd Edition,. John Wiley & Sons, John Wiley & Sons. ISBN: 978-1-119-36275-3:

Phatale, V., Vaiphei, K.K., Jha, S., Patil, D., Agrawal, M., Alexander, A., 2022. Overcoming skin barriers through advanced transdermal drug delivery approaches. J. Control. Release 351, 361–380.

Poulaki, A., Piperaki, E.T., Voulgarelis, M., 2021. Effects of visceralising leishmania on the spleen, liver, and bone marrow: A pathophysiological perspective. Microorganisms 9 (4).

Rizk, Y.S., Hardoim, D.J., Santos, K.B.A., Zaverucha-do-Valle, T., Taniwaki, N.N., Almeida-Souza, F., Carollo, C.A., Vannier-Santos, M.A., Arruda, C.C.P., Calabrese, K. D.S., 2022. Amentoflavone isolated from Selaginella sellowii Hieron induces mitochondrial dysfunction in Leishmania amazonensis promastigotes. Parasitol Int 86, 102458.

Rottini, M.M., Amaral, A.C.F., Ferreira, J.L.P., Silva, J.R.d.A., Taniwaki, N.N., Souza, C.d. S.F.d., d'Escoffier, L.N., Almeida-Souza, F., Hardoim, D.d.J., Gonçalves da Costa, S. C., Calabrese, K.d.S., 2015. In vitro evaluation of (−)α-bisabolol as a promising agent against Leishmania amazonensis. Exp. Parasitol. 148, 66–72.

Roy, S., Majumdar, S., Singh, A.K., Ghosh, B., Ghosh, N., Manna, S., Chakraborty, T., Mallick, S., 2015. Synthesis, characterization, antioxidant status, and toxicity study of vanadium–rutin complex in Balb/c mice. Biol. Trace Elem. Res. 166, 183–200.

Sakyi, P.O., Kwofie, S.K., Tuekpe, J.K., Gwira, T.M., Broni, E., Miller 3rd, W.A., Wilson, M.D., Amewu, R.K., 2023. Inhibiting Leishmania donovani Sterol Methyltransferase to Identify Lead Compounds Using Molecular Modelling. Pharmaceuticals (Basel) 16 (3).

Santos, N.F.N., Junior, N., de Oliveira, J.F., Duarte, D., Dos Santos Soares, J.C., Clara Marques, D.S., da Silva Santos, A.C., Nogueira, F., Alves Pereira, V.R., Alves de Lima, M.C., da Cruz Filho, I.J., 2023. Synthesis, characterization, antioxidant and antiparasitic activities new naphthyl-thiazole derivatives. Exp Parasitol 248, 108498.

Severino, P., Santana, W., Lisboa, E.S., Santos, V.L.d., Lima, E.T.d.S., Cardoso, J.C., Albuquerque-Junior, R.L.d., Naveros, B.C., Santini, A., Souto, E.B., 2022. Cutaneous/mucocutaneous leishmaniasis treatment for wound healing: classical versus new treatment approaches. Microbiol. Res. 13 (4), 836–852.

Singh, A., Mahapatra, B., Banerjee, A., Singh, S., Singh, S., Dubey, V.K., Das, P., Singh, R. K., 2024. Leishmania antigens activated CD4(+) T cells expressing CD200R receptors are the prime IL-10 producing phenotype and an important determinant of visceral leishmaniasis pathogenesis. Cytokine 173, 156435.

Soares, J.X., Santos, Á., Fernandes, C., Pinto, M.M., 2022. Liquid chromatography on the different methods for the determination of lipophilicity: An essential analytical tool in medicinal chemistry. Chemosensors 10 (8), 340.

Stanzione, F., Giangreco, I., Cole, J.C., 2021. Use of molecular docking computational tools in drug discovery. Prog. Med. Chem. 60, 273–343.

Steverding, D., da Nóbrega, F.R., Rushworth, S.A., de Sousa, D.P., 2016. Trypanocidal and cysteine protease inhibitory activity of isopentyl caffeate is not linked in Trypanosoma brucei. Parasitol Res 115 (11), 4397–4403.

Veber, D.F., Johnson, S.R., Cheng, H.-Y., Smith, B.R., Ward, K.W., Kopple, K.D., 2002. Molecular properties that influence the oral bioavailability of drug candidates. J. Med. Chem. 45 (12), 2615–2623.

Verdan, M., Taveira, I., Lima, F., Abreu, F., Nico, D., 2023. Drugs and nanoformulations for the management of Leishmania infection: a patent and literature review (2015–2022). Expert Opin. Ther. Pat. 33 (3), 137–150.

Watanabe, R., Esaki, T., Ohashi, R., Kuroda, M., Kawashima, H., Komura, H., Natsume-Kitatani, Y., Mizuguchi, K., 2021. Development of an in silico prediction model for p-glycoprotein efflux potential in brain capillary endothelial cells toward the prediction of brain penetration. J Med Chem 64 (5), 2725–2738.