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ORIGINAL ARTICLE

Microemulsion based on methyl 3,4,5-trihydroxybenzoate for the topical treatment of cutaneous leishmaniasis: an *in vivo* assay

Thiago de Jesus BACHA[1,2]*, Bruno Bezerra JENSEN[2], Daniele de Carvalho RODRIGUES[1], Messe Elmer Torres da SILVA[2], Amanda Oliveira ARAÚJO[2], Katia Solange Cardoso Rodrigues dos Santos GERALDI[1], Robson Cabral VALADÃO[2], Francimeire Gomes PINHEIRO[2], Antonia Maria Ramos FRANCO[2]

[1] Universidade Federal do Amazonas, Manaus, Amazonas, Brazil

[2] Instituto Nacional de Pesquisas da Amazônia – INPA, Manaus, Amazonas, Brazil

* Corresponding author: thiagobacha@live.com; https://orcid.org/0000-0003-1314-5172

ABSTRACT

Cutaneous leishmaniasis, caused by protozoa of the genus *Leishmania*, presents diverse clinical manifestations, and current therapeutic options have limitations, including long treatment periods, potential hospitalization, and excessive pain during treatment. Methyl gallate, a phenolic compound found in plants such as *Libidibia ferrea*, presents promising antileishmanial activity. Combining this compound with existing leishmaniasis medications could lead to reduced dosages and the minimization of side effects. This study aimed to assess the efficacy of a microemulsion containing methyl gallate, either on its own or in combination with Glucantime®, for the experimental treatment of cutaneous leishmaniasis in a 30-day *in vivo* assay using golden hamsters infected with *Leishmania (Leishmania) amazonensis*. The control groups included an untreated positive control and an uninfected, untreated negative control. After treatment, we evaluated clinical, parasitological, and biochemical parameters. While none of the treatments achieved clinical or parasitological cure, notable improvements were observed in the combined group, with significant reductions in snout skin lesions and parasite load when compared to the control. Biochemical parameters such as creatinine, CK-MB, GOT, and GPT remained unchanged, but urea and CPK levels significantly increased in all the experimental groups relative to the control. In conclusion, the integration of a topical methyl gallate microemulsion with intralesional Glucantime® showed potential as an effective treatment for cutaneous leishmaniasis. Further investigations into optimal dosages and therapeutic schemes are warranted in order to enhance treatment outcomes.

KEYWORDS: natural product, gallic acid derivatives, biological assay, neglected diseases

Microemulsão à base de 3,4,5-trihidroxibenzoato de metila no tratamento tópico da leishmaniose cutânea: um ensaio *in vivo*

RESUMO

A leishmaniose cutânea, causada por protozoários do gênero *Leishmania*, apresenta diversas manifestações clínicas e as opções terapêuticas atuais têm limitações, incluindo longos períodos de tratamento, possível hospitalização e dor excessiva durante o tratamento. O galato de metila, um composto fenólico encontrado em plantas como *Libidibia ferrea*, mostra atividade antileishmania promissora. A combinação deste composto com os medicamentos existentes para leishmaniose pode levar a dosagens reduzidas e à minimização dos efeitos colaterais. Este estudo teve como objetivo avaliar a eficácia de uma microemulsão contendo galato de metila, isoladamente ou em combinação com Glucantime®, no tratamento experimental da leishmaniose cutânea em um ensaio *in vivo* de 30 dias em hamsters sírios infectados com *Leishmania (Leishmania) amazonensis*. Os grupos de controle incluíram um controle positivo não tratado e um controle negativo não infectado e não tratado. Após o tratamento, avaliamos parâmetros clínicos, parasitológicos e bioquímicos. Embora nenhum dos tratamentos tenha alcançado cura clínica ou parasitológica, melhorias notáveis foram observadas no grupo combinado, com redução significativa nas lesões cutâneas do focinho e na carga parasitária em comparação com o controle. Parâmetros bioquímicos como creatinina, CK-MB, TGO e TGP permaneceram inalterados, os níveis de ureia e CPK aumentaram significativamente em todos os grupos experimentais em relação ao controle. Em conclusão, a integração da microemulsão tópica de galato de metila com Glucantime® intralesional mostrou potencial como um tratamento eficaz para a leishmaniose cutânea. Mais investigações sobre dosagens ideais e esquemas terapêuticos são necessárias para melhorar os resultados do tratamento.

PALAVRAS-CHAVE: produto natural, derivados do ácido gálico, ensaio biológico, doenças negligenciadas

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INTRODUCTION

Cutaneous leishmaniasis (CL) is caused by the protozoa of the genus *Leishmania,* which are transmitted by the bites of sandflies and cause different clinical manifestations in humans. Some of the main species from the New World are *Leishmania mexicana* Biagi, 1953*, Leishmania amazonensis* Lainson & Shaw, 1972 and *Leishmania venezuelensis* Bonfante-Garrido, 1980 (Bailey and Lockwood 2007). According to the latest epidemiological survey, CL has been reported in 92 countries (WHO 2021). In Brazil, CL has significant importance, and is regarded as a crucial public health issue. Due to its tropical climate and the presence of suitable vectors, such as sandflies, the Amazon region provides favorable conditions for the transmission of the parasite responsible for the disease. Moreover, the expansion of human activities, such as deforestation and disorderly occupation, contributes to the rise in the number of cases of cutaneous leishmaniasis in the region (Souza et al. 2021).

Due to the diversity of *Leishmania* species and the associated clinical manifestations of CL, the Brazilian Ministry of Health recommends different drugs (meglumine antimoniate, pentamidine isethionate or amphotericin B) and different therapeutic schemes that can be used in the treatment of this disease (Brasil 2017). However, despite the different protocols, there are reports of parasite resistance and difficulties in carrying out the treatment due to the longevity and toxicity of the indicated drugs (Bastos et al. 2016). An alternative medication for the treatment of CL is miltefosine (hexadecylphosphocholine), which is administered orally, but can cause harm to the fetus, including fetal death in *in vivo* models, and therefore should not be used by pregnant women (CONITEC 2018).

Considering these negative aspects of existing treatments, it is necessary to search for new active ingredients and different, less invasive, pharmaceutical formulations as an alternative to the currently available treatments for CL. A potential source for new treatments are bioactive molecules extracted from medicinal plants and microorganisms (Jensen et al. 2017; Garzon et al. 2021). Methyl gallate is a phenolic compound that shows promising antileishmanial activity and is found in plants such as *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz (Fabaceae) (Comandolli-Wyrepkowski et al. 2017), *Moringa oleifera* Lam. (Moringaceae) (Kaur et al. 2014), and *Margaritaria nobilis* Lf (Phyllanthaceae) (Moraes et al. 2015).

Methyl gallate extracted from *L. ferrea* was evaluated *in vitro* for antileishmanial activity by Jensen (2020), and demonstrated leishmanicidal activity in promastigotes and amastigotes of *Leishmania (Leishmania) amazonensis* and the absence of a cytotoxic profile. Commercially purchased methyl gallate effectively inhibited the growth of promastigotes *in vitro* and reduced the rate of infection of macrophages by amastigotes of *L. (L.) amazonensis* (Dias et al. 2020).

There are no reports in the literature regarding the evaluation of methyl gallate on its own or in combination with Glucantime® for the experimental *in vivo* treatment of CL, but the topical use of a microemulsion containing the dichloromethane fraction of *L. ferrea* containing methyl gallate showed *in vivo* antileishmanial activity in hamsters, with control of the evolution of the lesions, presence of a moderate inflammatory profile and reduction of the parasitic load (Jensen 2020).

Regarding pharmaceutical formulations, microemulsified systems have advantages such as an increased stability of compounds, bioavailability, and the improved permeation of bioactive molecules and their release, in addition to being less painful, easy to apply, and quickly absorbed by the skin (Callender et al. 2017; Ghorbanzadeh et al. 2019). Acknowledging the challenges posed by current treatments in terms of difficulty, discomfort and cytotoxicity, this study pursued the primary objective of formulating a novel microemulsified solution that incorporates methyl gallate for the experimental *in vivo* treatment of cutaneous leishmaniasis. We determined the microemulsion's particle size, pH, and zeta potential was determined, and evaluated its efficacy when administered on its own or in combination with Glucantime® at various concentrations and biochemical dosages.

MATERIAL AND METHODS

Origin and maintenance of parasites

The strain used in this study was *Leishmania (Leishmania) amazonensis* (MHO-BR-2009-IM5584). The sample used was cryopreserved in the strain cryobank of the Leishmaniosis and Chagas Disease Laboratory at Instituto Nacional de Pesquisas da Amazônia (INPA).

Preparation and characterization of microemulsions

The active ingredient used in the study was methyl gallate (gallic acid methyl ester, methyl 3,4,5-trihydroxybenzoate), linear formula (HO)3C6H2CO2CH3, CAS 99-24-1, purchased from Sigma-Aldrich (St. Louis, MO, USA).

Two microemulsions were prepared in May 2022, one with 2% methyl gallate (MMG) and a placebo microemulsion (MPL) without the active ingredient. Both were developed based on isopropyl alcohol, Tween® 20, distilled water, oleic acid and 2% methyl 3,4,5-trihydroxybenzoate (Sigma-Aldrich®) as described by Jensen (2020). The reagents were stirred for about 2 minutes, until a homogeneous, transparent and monophasic solution was obtained. The two microemulsions were stored at room temperature in amber tubes in the Leishmaniasis and Chagas Disease Laboratory at INPA.

Particle size, viscosity and zeta potential were assessed with a light-scattering particle size analyzer (Zetasizer, Malvern

Panalytical®). The pH was measured using a pH and ORP meter (pH 21, Hanna®).

In vivo experiment

The treatment protocol was developed following Comandolli-Wyrepkowski et al. (2017), using golden hamsters (*Mesocricetus auratus* Waterhouse, 1839) as an experimental model for this specific disease. *Leishmania* (*Leishmania*) *amazonensis* was chosen due to its widespread occurrence in Brazil, making it a relevant and representative target. The dosage of the topical microemulsion that was applied to the golden hamsters was calculated based on preliminary studies conducted by Jensen (2020), while the dosage of Glucantime® followed the therapeutic recommendation of 10 to 20 mg kg^{-1} day^{-1} by the Brazilian Ministry of Health (Brasil 2017).

We used 48 male, adult golden hamsters, with an average weight of 120 ± 6 g and aged 60 days, were obtained from and kept in the Central Animal Facility at INPA in air-conditioned rooms (photoperiod 12-12), with *ad libitum* feeding conditions and free of pathogens. The animals were divided into eight experimental groups containing six animals per group; seven groups were inoculated in the snout with a volume of 0.1 mL of stationary phase promastigotes (10^6 parasites mL^{-1}) of *L. (L.) amazonensis* and one group was not inoculated. Fifteen days after inoculation, when leishmaniasis lesions appeared, the treatment of the animals was started according to the following experimental design:

I) Group MMG (methyl gallate microemulsion): infected and treated with the methyl gallate microemulsion (10 mg kg^{-1} day^{-1}) topically;

II) Group MPL (placebo microemulsion): infected and treated with topical placebo microemulsion;

III) Group GIL10 (intralesional Glucantime® 10 mg Sb^V kg^{-1} day^{-1}): infected and treated with Glucantime® (10 mg Sb^V kg^{-1} day^{-1}) intralesionally (IL);

IV) Group GIL20 (intralesional Glucantime® 20 mg Sb^V kg^{-1} day^{-1}): infected and treated with Glucantime® (20 mg Sb^V kg^{-1} day^{-1}) via IL;

V) Group MMG+GIL10: infected and treated with Glucantime® (10 mg Sb^V kg^{-1} day^{-1}) IL and with methyl gallate (10 mg kg^{-1} day^{-1}) topically;

VI) Group MMG**+**GIL20**:** infected and treated with Glucantime® (20 mg Sb^V kg^{-1} day^{-1}) IL and with methyl gallate (10 mg kg^{-1} day^{-1}) topically;

VII) Group IST: infected and untreated (positive control);

VIII) Group NIST: uninfected and untreated (negative control).

The microemulsions (MMG and MPL) and Glucantime® were administered to the animals in a volume of 0.15 mL over the epidermis. The animals treated topically with the methyl gallate microemulsion received daily treatment for a period of 30 days. The animals that were treated intralesionally with Glucantime® received a dose on the 1st, 15th and 30th day (total of three applications). Subsequently, all animals remained under observation for another 15 days (without any treatment), totaling 30 days of treatment and 15 days of follow-up.

During the treatment period, the total volume of the lesion (length, width and height) was measured on alternate days using a digital caliper (Zaas® Precision) to analyze the progression of the lesion, and photodocumentation of the clinical evolution of the lesions was also performed.

After completing the treatment, the animals were euthanized with Vetnil® (ketamine hydrochloride 10%) and Syntec® (xylazine 2%), as recommended by the committee on research with animals at INPA, and biological material was collected for parasitological and biochemical evaluation. For the parasitological evaluation, a tissue sample extracted from the lesion area of each animal was used for imprinting onto glass slides. Subsequently, the biological material present on the slides underwent fixation using methyl alcohol and staining with Giemsa solution (Sigma). The infection rate was evaluated as the count of amastigotes and macrophages present in the 25 fields of each rectangular glass slide under an optical microscope (100x objective) (Comandolli-Wyrepkowski et al. 2017).

Parasite viability was assessed by sowing another part of the lesion sample in Novy-MacNeal-Nicolle (NNN) medium and left for seven days at 25 ºC, after which the presence or absence of parasites was determined in the cultures. A culture was considered negative for the presence of parasites only when there was total absence of parasites. Parasites were quantified under an optical microscope (100x magnification) and classified into the following score scale: 0 (no parasites present), 1 (1 to 9 parasites), 2 (10 to 100 parasites), and 3 (> 100 parasites) (Comandolli-Wyrepkowski et al. 2017).

The biochemical response was analyzed by collecting 2 mL of blood from each animal. Indicators of kidney function (urea and creatinine), the cardiac markers creatine phosphokinase (CPK) and creatine phosphokinase fraction MB (CK-MB), and the liver markers glutamic-oxaloacetic transaminase (GOT) and glutamate-pyruvic transaminase (GPT) were analyzed with an automated biochemistry analyzer (URIT 8031, MHLab®). CPK and CK-MB indicate potential cardiac or skeletal muscle damage, and creatinine and urea indicate renal impairment as adverse reactions to drug toxicity (Thrall et al. 2015).

Statistical analysis

We used analysis of variance (ANOVA) to compare the experimental groups, and the Kruskal-Wallis test when the data violated the assumptions of normality and variance homogeneity for parametrical analysis (lesion volume). For

paired comparisons between a treatment and its control (parasite viability, biochemical variables and infection rate), the t-test was used, or the non-parametric Mann-Whitney test in cases of non-normality. The significance level used was 5% in all statistical comparisons. All analyzes were performed in the R program (R Core Team 2020) version 4.1.1 and Microsoft Excel Professional Plus 2019.

Ethical aspects

This study was submitted to and approved by the Animal Research ethics committee on research with animals (CEUA) at INPA (approved under protocol # 023-2021 CEUA-INPA).

RESULTS

Microemulasion characterization

The microemulsions were optically clear, odorless, both had a mean particle size of ~5 nm. MMG had a viscosity of 0.88 cP, magnitude of zeta potential -10.7 mV and pH of 5.5, while MPL had a viscosity of 0.78 cP, magnitude of zeta potential -11.6 mV and pH of 5.8.

In vivo experiment

At the end of the experimental period (45 days), all treatments and the IST control showed a 94% increase in the volume of leishmaniasis lesions when compared to the NIST control. In the treatment groups, there was an average reduction of 42.2% in lesion volume in MMG ($p < 0.0001$), 56.1% in MMG+GLI10 ($p < 0.0001$), 56.1% in MMG+GLI20 ($p < 0.0001$), 45.9% in GLI10 ($p = 0.0004$) and 31.3% in GLI20 ($p = 0.0103$), when compared to the IST control (Figure 1).

There was no clinical and parasitological cure in any of the treatment groups and all groups of infected animals had snouts with edema, redness and crust formation during and after the completion of the experimental treatments (Figure 2). We did not observe any reaction that caused irritation on the skin of the animals when topically treated with the microemulsion.

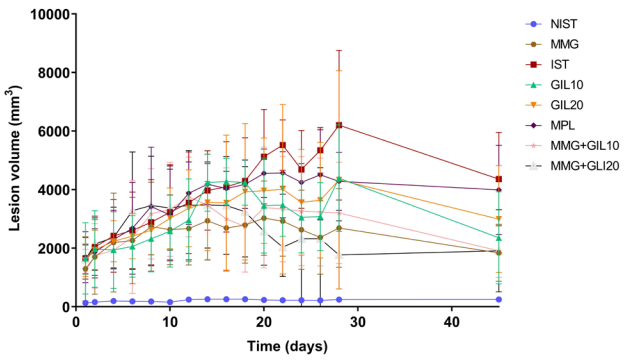

Figure 1. Evolution of the lesion volume (mm^3) of the snout of treated experimental animals and of the control groups not infected and infected with *Leishmania* (*Leishmania*) *amazonensis*. NIST: uninfected and untreated; IST: infected and untreated; MPL: infected and treated with topical placebo microemulsion; GIL10: infected and treated with Glucantime® (10 mg SbV kg^{-1} day^{-1}) intralesionally; GIL20: infected and treated with Glucantime® (20 mg SbV kg^{-1} day^{-1}) intralesionally; MMG: infected and treated with microemulsion incorporated with methyl gallate (10 mg kg^{-1} day^{-1}) topically; MMG+GIL10: infected and treated with Glucantime® (10 mg SbV kg^{-1} day^{-1}) intralesionally and with a microemulsion of methyl gallate (10 mg kg^{-1} day^{-1}) topically; MMG+GIL20: infected and treated with Glucantime® (20 mg SbV kg^{-1} day^{-1}) intralesionally and with a microemulsion of methyl gallate (10 mg kg^{-1} day^{-1}) topically. This figure is in color in the electronic version.

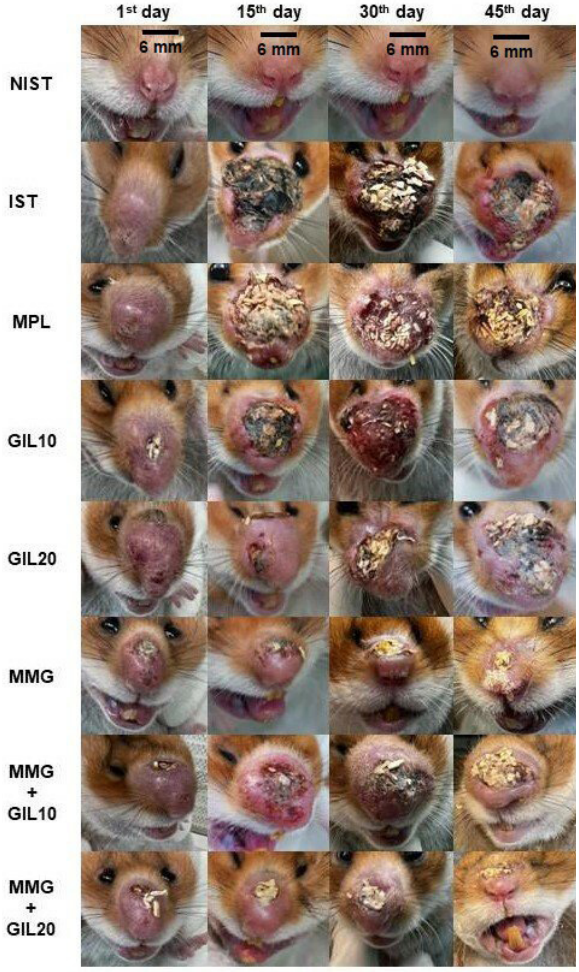

Figure 2. Evolution of lesions caused by cutaneous leishmaniasis in golden hamsters (*Mesocricetus auratus*) infected with *Leishmania* (*Leishmania*) *amazonensis*. The lack of standardization in image capture may have affected the accuracy of comparing lesion sizes in different images and no individual scale bars are available. A reference scale bar of 6 mm is provided for the NIST group (NIST group mean muzzle width). NIST: uninfected and untreated; IST: infected and untreated; MPL: infected and treated with topical placebo microemulsion; GIL10: infected and treated with Glucantime® (10 mg SbV kg^{-1} day^{-1}) intralesionally; GIL20: infected and treated with Glucantime® (20 mg SbV kg^{-1} day^{-1}) intralesionally; MMG: infected and treated with microemulsion incorporated with methyl gallate (10 mg kg^{-1} day^{-1}) topically; MMG+GIL10: infected and treated with Glucantime® (10 mg SbV kg^{-1} day^{-1}) intralesionally and with a microemulsion of methyl gallate (10 mg kg^{-1} day^{-1}) topically; MMG+GIL20: infected and treated with Glucantime® (20 mg SbV kg^{-1} day^{-1}) intralesionally and with a microemulsion of methyl gallate (10 mg kg^{-1} day^{-1}) topically. This figure is in color in the electronic version.

After 30 days of treatment and 15 days of post-treatment, all experimental groups presented viable parasites after re-isolation of promastigote forms in biphasic NNN medium, with the lowest parasite viability (score 2) for MMG+GIL20. However, there was no significant difference in viability scores between the treatments and the positive control (IST) (Figure 3a). Regarding the infection rate, MMG+GIL20 and MMG+GIL10 had the lowest values, differing significantly from the IST positive control ($p < 0.0001$) (Figure 3b).

Regarding the biochemical indicators of cardiac function, CK-MB (Figure 4b) did not vary significantly among groups, while the levels of CPK did not differ significantly from NIST only for MPL and MMG (Figure 4a). There was no significant difference in the levels of GOT and GPT among the experimental groups (Figure 4c,d). Likewise, no significant difference was observed in the levels of creatinine among the groups (Figure 4e), while urea was significantly higher in NIST and significantly lower in IST compared to all treatment groups (Figure 4f).

DISCUSSION

Our results indicate that the dimensions of the particles obtained in the microemulsions tested in here are aligned with the criteria established for microemulsions (less than 100 nm) by Tartaro et al. (2020). The stability of microemulsions is considered strong when the zeta potential is between 30 mV to -30 mV (Honary et al. 2013). In the context of this study, the magnitude of the zeta potential in both microemulsions is within the stability range. According to Auston (2015), the ideal pH of a formulation is standardized according to the stability pH of the active components used and the biological tolerance for skin products, with a pH of 5.0 to 8.0 as a

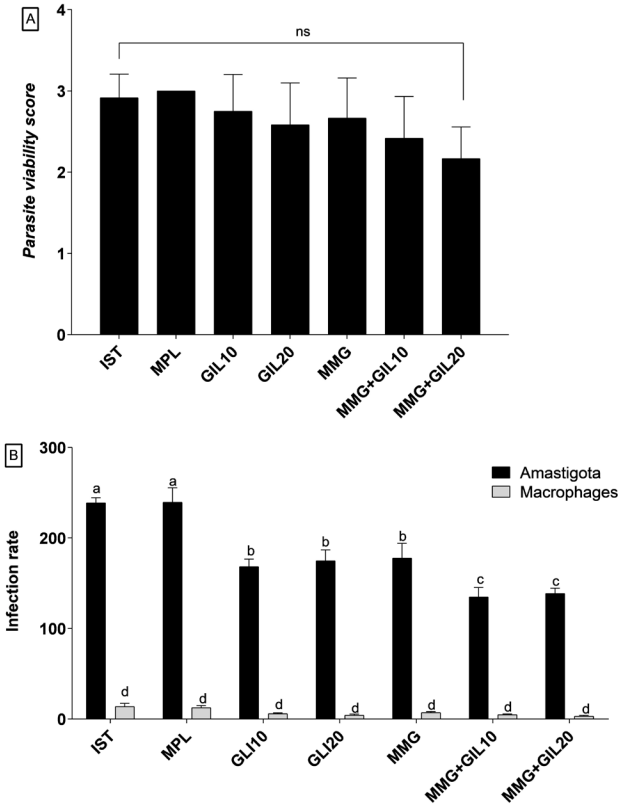

Figure 3. Parasite viability score (A) and infection rate (B) in hamsters (*Mesocricetus auratus*) infected with *Leishmania* (*Leishmania*) *amazonensis* after treatment. IST: infected and untreated; MPL: infected and treated with topical placebo microemulsion; GIL10: infected and treated with Glucantime® (10 mg SbV kg^{-1} day^{-1}) intralesionally; GIL20: infected and treated with Glucantime® (20 mg SbV kg^{-1} day^{-1}) intralesionally; MMG: infected and treated with microemulsion incorporated with methyl gallate (10 mg kg^{-1} day^{-1}) topically; MMG+GIL10: infected and treated with Glucantime® (10 mg SbV kg^{-1} day^{-1}) intralesionally and with methyl gallate (10 mg kg^{-1} day^{-1}) topically; MMG+GIL20: infected and treated with Glucantime® (20 mg SbV kg^{-1} day^{-1}) intralesionally and with methyl gallate (10 mg kg^{-1} day^{-1}) topically.

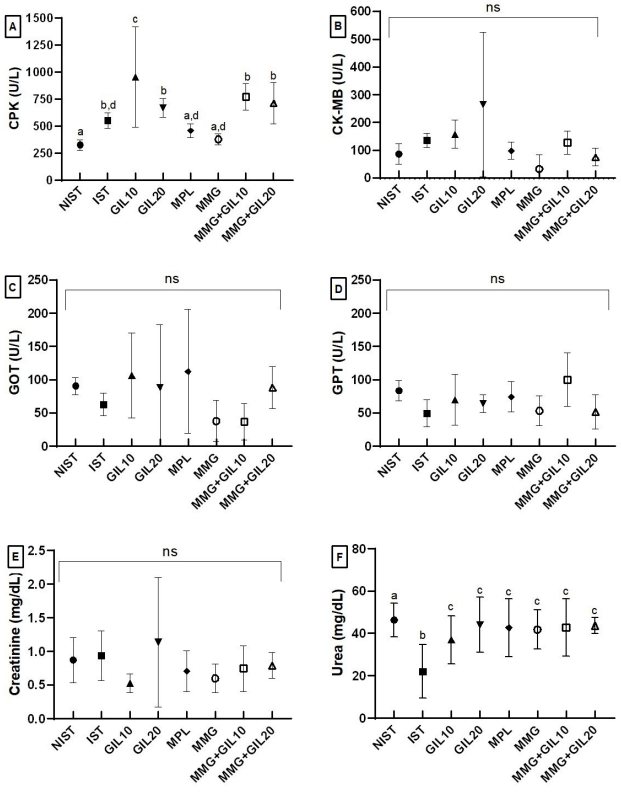

Figure 4. Biochemical indicators in hamsters (*Mesocricetus auratus*) infected with *Leishmania* (*Leishmania*) *amazonensis* after treatment. A – CPK; B – CK-MB; C – GOT; D – GPT; E – creatine; F – urea. NIST: uninfected and untreated; IST: infected and untreated; MPL: infected and treated with topical placebo microemulsion; GIL10: infected and treated with Glucantime® (10 mg SbV kg^{-1} day^{-1}) intralesionally; GIL20: infected and treated with Glucantime® (20 mg SbV kg^{-1} day^{-1}) intralesionally; MMG: infected and treated with microemulsion incorporated with methyl gallate (10 mg kg^{-1} day^{-1}) topically; MMG+GIL10: infected and treated with Glucantime® (10 mg SbV kg^{-1} day^{-1}) intralesionally and with a microemulsion of methyl gallate (10 mg kg^{-1} day^{-1}) topically; MMG+GIL20: infected and treated with Glucantime® (20 mg SbV kg^{-1} day^{-1}) intralesionally and with a microemulsion of methyl gallate (10 mg kg^{-1} day^{-1}) topically.

reference. The pH of our formulations was compatible with the skin tissue. The results of these tests indicate a characteristic profile of a stable microemulsion.

The *in silico* binding affinity mechanism involves the inhibition of *Leishmania* pteridine reductase (PTR1) and oligopeptidase B (OPB) targets by an isopropyl gallate derivative, which had a high power of comparison with amphotericin B (Melo et al. 2022). This evidence suggests that it is a promising alternative for the treatment of leishmaniasis, as it was selectively more toxic to the parasite than to mammal cells.

We observed that the experimental groups with the associated treatment (MMG+GLI 10 and MMG+GLI 20) had a lower infection rate when compared to the other infected groups, and that both groups and the treatment with only methyl gallate (MMG) presented a lower lesion volume when compared to the other groups, corroborating the studies by Dias et al. (2020) and Jensen (2020), who demonstrated that methyl gallate has *in vitro* efficacy in reducing the viability of promastigote and amastigote forms of *L. (L.) amazonensis*. Dias et al. (2020) also inferred that methyl gallate presented a higher selectivity index when compared to meglumine antimonate and amphotericin B and stimulation of TNF-α, IL-12, ROS and NO production, in addition to showing a decrease in IL-10 and macrophage activation, with an impact on the infection rate. In addition, Jensen (2020) demonstrated that methyl gallate did not show cytotoxicity in murine peritoneal macrophages and J774 lineage cells.

The mechanism of action of Glucantime®, as well as other medications used in the treatment of cutaneous leishmaniasis is still unknown; however, several studies have sought to investigate possible therapeutic targets (Yang et al. 2007). In BALB-c mice, opsonized amastigotes of *L. (L.) amazonensis* induce the activation of MAPK-ERK and a consequent superinduction of IL-10 in infected macrophages, contributing to the exacerbation of the disease, and when these pathways are deactivated, there is a reduction in the lesion and parasitic load in (Yang et al. 2007).

Another route with significant importance in the control of parasitic load is reactive oxygen species (ROS), which has no effect on the death of *L. (L.) amazonensis,* but may be associated with the control of lesion size in the early stages of infection, regulating the inflammatory response and the amount of neutrophils in the lesions (Roma et al. 2016). Other studies report that quercetin, a molecule from natural products, has antileishmanial activity against promastigotes and amastigotes due to its ability to induce the production of ROS and the disruption of the mitochondrial function of the parasite (Fonseca-Silva et al. 2011; Fonseca-Silva et al. 2013).

Methyl gallate can exert pharmacological effects through multiple pathways and targets, such as PI3K-Akt, ERK1-2, Caspase, AMPK-NFkB, Wnt-β-catenin, TLR4-NF-kB, MAPK, p53, NLRP3, ROS, EMT (Liang et al. 2023). It is thus possible that the reduction of the leishmaniotic lesion and the parasitic load in our study in the golden hamsters treated with the methyl gallate microemulsion on its own or in combination with Glucantime® may be related to activation or deactivation of any of these pathways. Further studies should aim to elucidate the mechanisms of action of this molecule against *L. (L.) amazonensis*.

Despite being a traditional model for studying various *Leishmania* species, the golden hamster also has drawbacks, such as its high susceptibility to *L. (L.) amazonensis* infection, which hampers lesion control for parasitological cure. Additionally, each of the traditionally chosen inoculation sites (snout and paw) has disadvantages. Lesion size can be difficult to measure on the paw and can hinder animal mobility, while lesions on the snout can hinder feeding and breathing, and can lead to inconsistency in the results in both cases. Hence, while these studies should persist for comparative reasons, alternative experimental models should also be explored (Robledo et al. 2012).

The reference values for renal function indicators in hamsters are 0.4 -1.0 (mg $d\ell^{-1}$) for creatinine and 12-26 (mg $d\ell^{-1}$) for urea, both dosed in blood serum (Carpenter and Marion 2018). Although creatinine values were within the normal range in all our treatments, mean urea values were above the acceptable limit in all experimental groups, including the NIST control, suggesting that some interference may have occurred and that urea should be dosed in subsequent studies. For the CPK index, as a reference, we used the normal range of 20-220 ($U\ L^{-1}$) determined for mice (Melo et al. 2008), as there is no description for the golden hamster. Therefore, the fact that all experimental groups presented values above the reference (> 250 $U\ L^{-1}$) may be related to physiological differences between the species. However, significant difference in CPK levels between the NIST control and all teatments containing Glucantime® indicates the need for further studies to investigate possible skeletal muscle injuries caused by the treatment.

Overall the biochemical changes observed, especially in the groups treated with Glucantime® on its own or in combination with methyl gallate are in accordance with what is described in the Glucantime® package insert, which states that rare alterations in kidney function (elevated creatinine and urea levels), pancreatitis (inflammation in the pancreas) and severe arrhythmias may occur (Sanofi-Aventis 2011).

Our results regarding the synergistic activity of methyl gallate and Glucantime® suggest that future studies be carried out aiming at the use of methyl gallate as an adjuvant drug in the treatment of CL. The Brazilian Ministry of Health currently provides recommendations regarding drugs that can be administered concomitantly with Glucantime®, such as pentoxifylline for the treatment of CL (Brasil 2017). Methyl

gallate can be used in topical form, is painless, can be used outside the hospital environment, and enhances the effect of traditional treatments, potentially reducing the treatment period. Regarding the clinical evolution of the disease and the parasitological and biochemical indicators of animals treated with methyl gallate, our results suggest a potential for the use of this compound as an adjuvant in the treatment of cutaneous leishmaniasis. Further studies should evaluate different dosages and therapeutic regimens using the methyl gallate microemulsion in association with Glucantime®, as well as different therapeutic regimens.

CONCLUSIONS

Our results suggest that the microemulsion formulation containing methyl gallate (10 mg kg^{-1} day^{-1}) has antileishmanial activity in golden hamsters infected with *L. (L.) amazonensis*, and its combined use with Glucantime® (20 mg Sb^{V} kg^{-1} day^{-1}) via IL is more effective in controlling the progression of leishmanial lesions, while also reducing parasite load when compared to groups receiving individual drug treatments. We conclude that the combination of the methyl gallate microemulsion and Glucantime® shows potential as a complementary treatment of CL. Further *in vitro* and *in vivo* studies are needed to explore the antileishmanial activity of this drug in other *Leishmania* species, its *in vitro* cytotoxicity, *in vivo* toxicity, biochemical effects, new therapeutic approaches and to determine its potential for clinical and parasitological cure.

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DATA AVAILABILITY

The data that support the findings of this study are available, upon reasonable request, from the corresponding author.