

## Comparing the Effectiveness of Pistachio Gum and Glucantime against Cutaneous Leishmaniasis *in vitro*

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### Abstract

Cutaneous leishmaniasis is one of the most prevalent parasitic diseases. Glucantime is one of the most common drugs used for its treatment, but it has serious side effects, especially on the liver and kidney. As pistachio gum is used locally on a limited scale for Cutaneous Leishmaniasis, this article aimed to compare the effectiveness of pistachio gum and Glucantime against cutaneous leishmaniasis *in vitro*. The survival of *Leishmania* parasites that cause the cutaneous form of the disease was estimated for pistachio gum extract at 5% and 10% concentrations, and it was compared with Glucantime at different concentrations (100, 200, and 300 mg/ml), after one, 24, and 48 hours of incubation. Pistachio gum at 10% concentration surpassed all Glucantime concentrations during all tested periods. Glucantime at 300 mg/ml activity was equal to the pistachio gum extract 5% only after one hour of incubation. Glucantime (200 mg/ml and 100 mg/ml) was less effective than pistachio gum extract at all periods tested in this research. It was found that Pistachio gum is highly effective on *Leishmania* parasites.

**Keywords:** cutaneous leishmaniasis, glucantime, pistachio gum

### Резюме

Кожната лайшманиоза е едно от най-разпространените паразитни заболявания. Глюкантимът е едно от най-често използваните лекарства за лечението му, но има сериозни странични ефекти, особено върху черния дроб и бъбреците. Тъй като дъвката от шамфъстък се използва на местно ниво в ограничен мащаб за лечение на кожна лайшманиоза, целта на тази статия е да се сравни нейната ефективност с тази на глюкантим срещу кожна лайшманиоза *in vitro*. Оценена е преживяемостта на паразитите на *Leishmania*, които причиняват кожната форма на заболяването, при третиране с екстракт от дъвка от шамфъстък в концентрация 5 и 10%. Резултатите са сравнени с тези при третиране с глюкантим в различни концентрации (100, 200 и 300 mg/ml), за период от 1, 24 и 48 часа инкубация. Екстрактът от дъвка от шамфъстък с концентрация 10% превъзхожда всички концентрации на глюкантим през всички тествани периоди. Активността на глюкантим при 300 mg/ml е равна на тази при въздействие с 5% екстракт от шамфъстък само след един час инкубация. Глюкантим (200 mg/ml и 100 mg/ml) е по-малко ефективен от екстракта от екстракт от шамфъстък през всички периоди, тествани в това изследване. Установено е, че екстракт от шамфъстък е високо ефективен по отношение на паразитите на *Leishmania*.

### Introduction

Cutaneous leishmaniasis is a neglected tropical disease caused by species of the genus *Leishmania*, which includes *L. major*, *L. tropica*, and *L. aethiopica* (Abbas *et al.*, 2022), it is transmitted to humans by the bite of the sand fly, especially *Phlebotomus sergenti* (Madusanka *et al.*, 2022). Cutaneous leishmaniasis is associated with a wide range of clinical manifestations and epidemiological diversity. It has different degrees of severity that

depend on the presence of the parasite inside the cells and the immune response of the host. The infection with cutaneous leishmaniasis can be associated with occasional scars and deformities (Alghamdi, 2020). There is still no safe and effective treatment for cutaneous leishmaniasis. The first-line drug of choice in the treatment of cutaneous leishmaniasis is the intra-lesional injection of penta antimony derivatives such as meglumine antimoniate (MGA),

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Acta Microbiol. Bulg. 2024; 40(01). <https://doi.org/10.59393/amb24400113>

which is commercially known as glucantime (Brayfield 2014; Iranpour *et al.*, 2019). In addition, about 10 to 15% of patients with cutaneous leishmaniasis did not respond to glucantime. MGA inactivity could be due to the varying efficacy and emergence of glucantime resistance (Brustolin *et al.*, 2022). Serious side effects were also observed on important organs in the body (liver - pancreas - blood system). It also has side effects on heart rhythms, muscular and joint pain, fever, and headache. The high cost of treatment is a problem in poor countries (Iranpour *et al.*, 2019). To solve this problem there is an increased scientific interest in searching for more effective and less harmful treatment methods (Brustolin *et al.*, 2022).

Medicinal plants are considered a good choice because they contain many effective phytochemicals that have synergistic effects in general. One of these plants is pistachio (*Pistacia vera*) a native plant to a number of temperate countries and grows in all central, western, and eastern regions (Zarei *et al.*, 2022). The genus *Pistacia* belongs to the Anacardiaceae family (Abdullah and Rajab Agha, 2019), from which an excreted gum is produced by making longitudinal incisions in the bark. It is dried by the sun to become solid or semi-solid pieces, crispy with a pale color, aromatic smell, and acceptable taste (Bozorgi *et al.*, 2013), it was used in ancient Greece. It has been used to treat intestinal disorders and peptic ulcers for more than 2500 years (Nezami and Gallego, 2023). Pistachio gum (pistachio oleoresin) consists of two main groups of phytochemicals. The first one is essential oil. It is prepared by hydro distillation and contains  $\alpha$ - Pinene as the main phytochemical,  $\beta$ -pinene, camphene, (bicyclic monoterpenes) (Alma *et al.* 2004),  $\beta$ -caryophyllene (a bicyclic sesquiterpene with extensive biological activities), (Bozorgi, *et al.*, 2013) and limonene -a monocyclic monoterpene (Napoli *et al.*, 2021). The second one is triterpenes such as isomasticadienonic, masticadienonic, masticadienolic oleanolic, ursolic, and acids which are pentacyclic triterpenoid acids (Moeini *et al.* 2019, (Bozorgi, *et al.*, 2013). The remaining phytochemicals are phytosterols, flavonoids, saponins, and hydrotannins, (Bagheri *et al.*, 2021), and (Parvardeh *et al.*, 2002). Pistachio gum is used in many countries as an antibacterial, antifungal, and antiparasitic agent (Alghamdi, 2020).

## Materials and Method

### *Preparation of the culture medium Novy-MacNeal-Nicolle-Medium (N.N.N.)*

The glass tubes were covered with aluminum

foil and sterilized in an autoclave for 15 minutes at 121°C and 1.5 bar. The NNN culture medium was prepared by dissolving 1.4 g of agar-agar with 0.6 g of NaCl in 90 ml distilled water, heating with stirring, adding about 15 ml of defibrinized blood, and then 5 ml streptomycin, carefully to avoid the formation of bubbles, then transferring the medium to sterile glass tubes, which were placed in slanted position until the agar solidified, kept at 4°C until using the culture (Ladopoulos, *et al.*, 2015)

### *Collecting cutaneous leishmaniasis samples*

Cutaneous leishmaniasis samples were collected from the lesion of patients attending the Leishmaniasis Control Centre in Al-Furqan in Aleppo from 1/1/2022 to 15/6/2022, after cleaning the skin lesion with 70% alcohol. Then an incision was made with a sterile scalpel at the base of the skin lesion and 20  $\mu$ l of physiological buffer solution (PBS) was injected using a micropipette after which the serous fluid and blood droplets were withdrawn and cultured on culture media (Ibarra-Meneses, *et al.* 2022).

### *Collecting pistachio gum*

The pistachio gum was collected from pistachio trees during the period of proliferation of the tree fruits by making an incision in the tree trunk or branches, the dried gum was obtained in the form of secretions collected in glass containers and kept at  $\pm 4^\circ\text{C}$  until use.

### *Preparing pistachio gum as an anti-parasite of cutaneous leishmaniasis in glass*

Pistachio gum (10 g) was weighed and placed in a sterile tube, 10 g Span 80 and 10 ml DMSO were added, and the volume was adjusted to 100 ml by distilled water. The mixture was placed in a water bath at 40°C until dissolution, the sample was filtered, and the filtrate was kept at  $\pm 4^\circ\text{C}$  till use and two concentrations of pistachio gum 10% and 5% were prepared (Samimi *et al.*, 2022).

### *Effectiveness of different concentrations of pistachio gum on cutaneous leishmaniasis in synthetic medium*

Glucantime ampoules of 1500 mg/5 ml, from Sanofi Aventis (France) company, were diluted to prepare two concentrations (200 mg/ml and 100 mg/ml). After *Leishmania* parasites in the flagellate were detected in the culture, the average number of cells in the logarithmic phase was calculated. 600  $\mu$ l was prepared from 10 NNN media cultures from each sample, were performed for each concentration of pistachio gum and glucantime. Fifty  $\mu$ L

culture medium containing *Leishmania* parasites and 50 µL of each concentration of pistachio gum extract or glucantime were placed into a 1.5 mL sterile Eppendorf tube, as a tube was designated for the control sample. The experiment was performed at intervals of one, 24, and 48 hours, by incubation of ten replicates for each concentration of the studied subjects (Madusanka *et al.*, 2022; Iranpour *et al.*, 2019).

*The survival of Leishmania parasites after incubation with pistachio gum and glucantime using trypan blue dye*

After incubation for 1, 24, and 48 h, 50 µl of the medium was drawn from each of the Eppendorf tubes into another clean Eppendorf tube, and 50 µl of trypan blue was added to it for 10 minutes. Each test was done in ten replications, and the average was calculated.

*The statistical study*

The statistical analysis was carried out using the SPSS.16, and Two Way ANOVA test.

**Results**

*The survival of parasites that cause cutaneous leishmaniasis after treating them with different concentrations of pistachio gum at different time periods*

The percentage of dead *Leishmania* parasites incubated with a 10% concentration of pistachio gum extract was 22.6% after one hour, 47.4% after

24 hours of incubation, and 80.4% after 48 hours of incubation. As for 5% of pistachio gum extract, its effectiveness was lower. The percentage of dead *Leishmania* parasites was 12.7% after one hour of incubation, 40% after 24 hours, and 72.8% after 48 hours, compared to the control sample, as shown in Table 1.

*The survival of parasites that cause cutaneous leishmaniasis after treating them with different concentrations of the glucantime for different periods*

The results of the laboratory examination, after treating *Leishmania* parasites with glucantime in different concentrations (100-200-300 mg/ml) after 1, 24, and 48 hours of incubation, showed that the highest killing rate of glucantime was for the concentration of 300 mg/ml, as the percentage of dead parasites was 12.7%. After an hour of incubation 39.1% after 24 hours and 55.3% after 48 hours, then for 200 mg/ml, the percentage of dead *Leishmania* parasites was 10.5% after an hour, 28.6% after 24 hours, and 41.2% after 48 hours, while for the concentration of 100 mg/ml, the *Leishmania* dead parasites did not exceed 37.3% after 48 hours of incubation, as shown in Table 2.

*Comparing the efficacy between pistachio gum and the glucantime*

By comparing the biological activity of pistachio gum extract with glucantime for the treatment of cutaneous leishmaniasis *in vitro*, shown in Table 3.

**Table 1.** Average of dead *Leishmania* parasites by different concentrations of pistachio gum after different time periods

| Number of parasites in 100 µl of culture medium | The average of the dead leishmania parasites after different time periods |                         |      |             |                          |      |             |                          |      |             |
|---|---|-------------------------|------|-------------|--------------------------|------|-------------|--------------------------|------|-------------|
|   | Pistachio gum concentration   | After 1 h of incubation | %    | Moral Score | After 24 h of incubation | %    | Moral Score | After 48 h of incubation | %    | Moral Score |
| 17310 parasites in 100 µl                       | 10%   | 3920                    | 22.6 | 0.00        | 8215                     | 47.4 | 0.00        | 13920                    | 80.4 | 0.00        |
|   | 5%  | 2210                    | 12.7 | 0.00        | 7012                     | 40   | 0.00        | 12615                    | 72.8 | 0.00        |

**Table 2.** Percentage of dead *Leishmania* parasites by glucantime different concentrations at different time periods

| Parasites (number in 100 µl culture medium) | The average of the dead leishmania parasites |               |      |             |                |      |             |                |      |             |
|---|--|---------------|------|-------------|----------------|------|-------------|----------------|------|-------------|
|   | Glucantime concentration                     | After an hour | %    | Moral Score | After 24 hours | %    | Moral Score | After 48 hours | %    | Moral Score |
| 20016 cells100 µl                           | 300 mg/ml                                    | 2560          | 12.7 | 0.00        | 7840           | 39.1 | 0.00        | 11080          | 55.3 | 0.00        |
|   | 200 mg/ml                                    | 2120          | 10.5 | 0.00        | 5740           | 28.6 | 0.00        | 8260           | 41.2 | 0.00        |
|   | 100 mg/ml                                    | 1940          | 9.6  | 0.004       | 5120           | 25.5 | 0.00        | 7560           | 37.7 | 0.00        |

**Table 3.** Comparing the efficacy between pistachio gum and the glucantime

| Time [h] | Glucantime 300 mg/ml | Glucantime 200 mg/ml | Glucantime 100 mg/ml | Pistachio Gum 10% | Pistachio Gum 5% |
|----------|----------------------|----------------------|----------------------|-------------------|------------------|
| 1        | 12.7                 | 10.5                 | 9.6                  | 22.6              | 12.7             |
| 24       | 39.1                 | 28.6                 | 25.5                 | 47.4              | 40.0             |
| 48       | 55.3                 | 41.2                 | 37.7                 | 80.4              | 72.8             |

**Table 4.** Significant differences (P) for the mean killing of Leishmania parasites during different time periods

|                     | Glucantime 100 mg/ml | Glucantime 200 mg/ml | Glucantime 300 mg/ml | Pistachio Gum 5% | Pistachio Gum 10% |
|---------------------|----------------------|----------------------|----------------------|------------------|-------------------|
| Glucantime 100 g/ml | -                    | 0.72                 | 0.07                 | 0.013            | 0.002             |
| Glucantime 200 g/ml | 0.72                 | -                    | 0.15                 | 0.03             | 0.006             |
| Glucantime 300 g/ml | 0.78                 | 0.15                 | -                    | 0.43             | 0.15              |
| Pistachio Gum 5%    | 0.01                 | 0.03                 | 0.43                 | -                | 0.57              |
| Pistachio Gum 10%   | 0.002                | 0.006                | 0.15                 | 0.57             | -                 |

It was found that pistachio gum at both 5 and 10% concentration was superior to glucantime at all concentrations, and tested periods, except that pistachio gum at 5% showed the same activity as glucantime 300 mg/ml only after one hour of incubation. As Table 4 showed significant differences (P) between the concentrations used to determine the most effective between pistachio gum and glucantime compound during different periods, as the degree of significance between the experimental groups reached (0.008).

## Discussion

Syria is one of the areas endemic for cutaneous leishmaniasis, as we have noticed an increase of cutaneous leishmaniasis in various Syrian governorates in general and Aleppo in particular (Youssef *et al.*, 2021), which made cutaneous leishmaniasis an important health problem that calls for study, and despite the tremendous progress in understanding the clinical results of various drugs experimental treatment and the development of the molecular biology of leishmaniasis, there still a long way to get safe effective drugs for different types of cutaneous leishmaniasis. In this article, the effect of both glucantime and Pistachio gum at different concentrations on the proliferation of the flagellated parasite forms of *leishmania* cultured in medium NNN was studied. The results of this study showed that all flagellated parasite forms of leishmania are sensitive to both glucantime and pistachio gum. It was also found that the viability of cutaneous leishmaniasis parasites decreased in direct proportion to the increase in the concentration of both glucantime and pistachio gum (Iranpour *et*

*al.*, 2019), and the superiority of pistachio gum over glucantime was so remarkable. It has an effective and significant biological activity against cutaneous leishmaniasis (Madusanka *et al.*, 2022), and contains  $\lambda$ -pinene, limonene, and  $\lambda$ -phellandrene – as well as  $\alpha$ -pinene,  $\alpha$ -myrcene, 3-carene, epoxy-pinene, triterpenes, oleanolic acid, moronic acid, ticcadienonic acid, and 24 Z-masticadienonic acids. Several studies have shown that these substances have an anti-cutaneous leishmaniasis effect (Bizri *et al.*, 2021), and many studies have shown that the presence of such substances in pistachio gum plays a role in eliminating germs (Nezami and Gallego, 2023). It also contains phenolic compounds, which increase the effectiveness of the pentavalent antimony compounds, including the glucantime compound used in this study (Ibarra-Meneses *et al.*, 2022).

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