The effect of *salvia officinalis* aqueous extract on generation number and time of *leishmania tropica*

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Abstract

The present study examined the toxic effect of aqueous extracts, ethanol and petroleum ether, *Salvia Officinalis*, on the promastigote of *L. tropica* parasites growing in Tobies medium with human blood added (15%). The results showed that the aqueous and alcoholic extract and petroleum ether at different concentrations used concentrations (0.05, 0.1, 0.15, 0.2) mg / cm³ clear toxic effect on these parasites through a gradual decrease in their number with an increase in the concentration of each extract for an inverse relationship during growth periods (96, 72, 48, and 24 hours. It also showed that the high concentration of extracts (0.2) mg / cm³ led to an activation of the anterior flagellar growth with rates (80.6, 84.5, 71.9%) of the aqueous extract, ethanol alcohol and petroleum ether respectively. Exposing the frontal flagellum of *L. tropica* to ICSO concentration of both aqueous, alcoholic and petroleum extract of sage and nettle plants during the 96-hour period led to a decrease in DNA by (49.5, 55.2, 13.6), respectively, and also to RNA by (74.7, 73.9, 23.3).

Keywords: salvia officinalis; leishmania; pentostam.

1. Introduction

Leishmaniasis is one of the most important complex global diseases, Parastic protozoa is among the most common organisms that cause disease in the world and which cause serious problems in the field of health of human societies and one of the most important tropical diseases that afflict humans such as malaria, amebiasis, toxoplasmosis and disease Leishmaniasis [1-3]. Leishmaniasis (Family: *Trypanosomatidae*) has six clinical forms, which are identified by the location of parasite in the infected tissues: Visceral leishmaniasis (VL) also called kalazar and affects the liver and spleen, mucocutaneous leishmaniasis (MCL) affects the mucous tissues in the nose, mouth and throat. Mucosal leishmaniasis (ML) affects the mucosal tissues of the intestine, cutaneous leishmaniasis (CL), diffuse cutaneous leishmaniasis (DCL), post-kala-azar dermal leishmaniasis (PKDL). Visceral leishmaniasis constitutes about (70-75)% of all cases [4-7]. Leishmaniasis is a unicellular heamoflagellate
that is obligate parasitism that lives in the macrophages of the vertebral hosts [8]. Leishmaniasis is transmitted from one host to another by the bite of a female sand fly which leads to leishmaniasis and includes Cutaneous leishmaniasis, mucous cutaneous leishmaniasis or/and visceral leishmaniasis [9]. Medicinal plants, including the active compounds they contain, are an important agent for human against disease after some medicines losing their efficacy, so these plants became a source for treating diseases. *S. officinalis*, one of the most important of these plants, belong Lamiaceae [10]. *S. officinalis* contain a mixture of phenolic compounds, which are important secondary metabolites and are classified as antioxidants due to their contribution to protecting the cell and its components from free radicals that causes severe damage of genetic material and cellular functions [11].

2. Materials & methods

**Preparing plant extract**

After *S. officinalis* leaves were dried, they were ground and powdered by an electric grinder. Ethanol, Petroleum ether and aqueous extract were prepared according to the method of [12].

**Parasite**

The anterior flagellum leishmaniasis of the cutaneous Leishmaniasis parasite used in the study and obtained from Al-Nahrain University / College of Medicine / Research Center and enzymatically diagnosed by the method of isoenzymes that used by [13].

**Culture media**

Cutaneous Leishmaniasis parasite was developed and cultured on a special medium called Tobie media [14] that consist of two phases, a solid phase and a liquid phase.

**Parasite growth**

The inoculation of parasite (2x10^5 cells/ml) in culture media was done by adding 0.1ml of positive culture which has lifespan of (4) days, during the logarithmic phase of growth to glass bottles containing a new culture media and 1.9ml of sterile Luke solution, then the bottles is incubated at 26 °C.
The effect of different concentrations on the growth and number of the parasite

Several concentrations (0.05; 0.1; 0.15 and 0.5 mg/ml) of aqueous extract of S. officinalis was used, where these concentrations were added to the container glass bottles that contain culture media inoculated with Leishmania in the logarithmic stage, then incubated in the incubator at 27 for 4 days to observe the extent of its impact on the growth of generations, number and generation time of the cutaneous Leishmaniasis parasite and compare it with parasites not treated with plant extracts (control) as well as compare it with Parasites treated with Pentostam, where the number of cells in different stages of growth was counted for 24, 48, 72, 96 hours of growth. The generation time and the number of generations were calculated according to the following equations:

\[ G = \frac{t}{n} \]

G: generation time; t: time of exposure; n: number of generations.

\[ n = \frac{LogN - LogN_0}{Log2} \]

\[ n = \frac{LogN - LogN_0}{0.301} \]

n: number of generations, \( N_0 \): Number of cells added when starting the experiment

Estimation of total nucleic acids

Nucleic acids were extracted and measured in untreated (pure) Leishmania cells, treated with drugs and treated with plant extracts, to show the effect of these extracts on them, and Schneider's method was followed in (1957), wherein this method was based on separating nucleic acids from the components of the cell by dissolving them in Trichloroacetic acid, and then the quantities of these acids were quantified by other reactions depending on the nucleic acid content of the Pentoses.

3. Results

The effect of S. officinalis extracts on cutaneous leishmaniasis parasite

Leishmania tropica was counted for 5 consecutive days by counting slide to observe if ethanolic and aqueous extracts had a inhibitory toxic effect on number of L. tropica, as well as to know which concentration has the value of LC50, meaning which concentration kills
half of the leishmaniasis cells by 50% Comparison with the control samples, and also comparing it with the drug used in the treatment of cutaneous leishmaniasis and measuring the effect of these extracts and the drug on the generation time and number of generations compared to the control samples as shown in the following tables.

**Table (1): Effect of aqueous extract of S. officinalis on L. tropica for different growth periods**

| Treatment mg/cm³ | Exposure time (hr.) | Control | 0.05   | 0.1    | 0.15   | 0.2    | Pentostam |
|------------------|---------------------|---------|--------|--------|--------|--------|-----------|
|                  | Mean±SD             | 58.8 ±  0.04 | 58.4±0.008 | 50.4±0.017 | 53.6±0.008 | 46.4±0.008 | 50.4±0.017 |
| Growth percentage|                     | 100     | 99.3   | 85.7   | 91.1   | 78.9   | 85.7     |
| Inhibition percentage |                 | 0       | 0.7    | 14.3   | 8.9    | 21.1   | 14.3     |
|                  | Mean±SD             | 63.2±0.02 | 50.4±0.014 | 42.4±0.012 | 38.4±0.012 | 34.4±0.012 | 43.2±0.024 |
| Growth percentage|                     | 100     | 79.7   | 67     | 60.7   | 54.4   | 68.3     |
| Inhibition percentage |                 | 0       | 20.3   | 33.0   | 39.3   | 45.6   | 31.7     |
|                  | Mean±SD             | 75.2±0.024 | 41.6±0.008 | 36.8±0.008 | 34.4±0.012 | 30.4±0.014 | 36±0.021   |
| Growth percentage|                     | 100     | 55.3   | 48.9   | 35.1   | 40.4   | 47.8     |
| Inhibition percentage |                 | 0       | 44.7   | 51.1   | 64.9   | 59.6   | 59.6     |
|                  | Mean±SD             | 82.4±0.024 | 40.0±0.014 | 34.4±0.014 | 29.6±0.016 | 23.2±0.016 | 33.6±0.021 |
| Growth percentage|                     | 100     | 48.5   | 41.7   | 35.9   | 28.1   | 40.7     |
| Inhibition percentage |                 | 0       | 51.5   | 58.3   | 64.1   | 71.9   | 59.3     |
Table (2): Effect of ethanolic extract of *S. officinalis* on *L. tropica* for different growth periods

| Treatment mg/cm³ | Exposure time (hr.) | Control | 0.05   | 0.1    | 0.15    | 0.2    |
|-----------------|---------------------|---------|--------|--------|---------|--------|
| Mean±SD         | 24                  | 58.8 ± 0.04 | 40.8 ± 0.016 | 37.6 ± 0.012 | 37.6 ± 0.012 | 38.4 ± 0.012 |
| Growth percentage | 100                | 69.3    | 85.7   | 63.9   | 63.9    |
| Inhibition percentage | 0            | 30.7    | 36.1   | 36.1   | 34.7    |
| Mean±SD         | 48                  | 63.2 ± 0.02 | 32.0 ± 0.016 | 28.8 ± 0.012 | 30.4 ± 0.021 | 28.8 ± 0.012 |
| Growth percentage | 100                | 50.6    | 45.5   | 48.1   | 45.5    |
| Inhibition percentage | 0            | 49.4    | 54.5   | 51.9   | 54.5    |
| Mean±SD         | 72                  | 75.2 ± 0.024 | 25.6 ± 0.014 | 21.6 ± 0.021 | 19.2 ± 0.012 | 18.2 ± 0.017 |
| Growth percentage | 100                | 34.0    | 28.7   | 25.5   | 24.2    |
| Inhibition percentage | 0            | 66.0    | 71.3   | 74.5   | 75.8    |
| Mean±SD         | 96                  | 82.4 ±0.028 | 17.6 ± 0.012 | 15.2 ± 0.016 | 14.4 ± 0.012 | 12.8 ± 0.014 |
| Growth percentage | 100                | 21.3    | 41.7   | 35.9   | 28.1    |
| Inhibition percentage | 0            | 78.7    | 81.6   | 82.6   | 84.5    |
Table (3): Effect of Petroleum ether extract of *S. officinalis* on *L. tropica* for different growth periods

| Treatment mg/cm³ | Exposure time (hr.) | Control | 0.05 | 0.1  | 0.15 | 0.2  |
|------------------|---------------------|---------|------|------|------|------|
|                  | Mean±SD             |         |      |      |      |      |
| 24               | Mean±SD             | 58.8 ± 0.04 | 40.0 ± 0.014 | 37.6 ± 0.012 | 41.6 ± 0.021 | 36.8 ± 0.014 |
|                  | Growth percentage   | 100     | 68   | 63.9 | 70.7 | 62.5 |
|                  | Inhibition percentage| 0      | 32   | 36.1 | 29.3 | 37.5 |
| 48               | Mean±SD             | 63.2 ± 0.02 | 36.0 ± 0.018 | 34.4 ± 0.012 | 37.6 ± 0.012 | 28.8 ± 0.012 |
|                  | Growth percentage   | 100     | 56.9 | 54.4 | 59.4 | 45.5 |
|                  | Inhibition percentage| 0     | 43.1 | 45.6 | 40.6 | 54.5 |
| 72               | Mean±SD             | 75.2 ± 0.024 | 32.0 ± 0.018 | 29.6 ± 0.016 | 29.6 ± 0.016 | 21.6 ± 0.016 |
|                  | Growth percentage   | 100     | 44.4 | 39.3 | 39.3 | 28.7 |
|                  | Inhibition percentage| 0    | 55.6 | 60.7 | 60.7 | 71.3 |
| 96               | Mean±SD             | 82.4 ±0.028 | 31.2 ± 0.018 | 26.4 ± 0.014 | 18.4 ± 0.014 | 16.0 ± 0.014 |
|                  | Growth percentage   | 100     | 37.8 | 32.0 | 22.3 | 19.4 |
|                  | Inhibition percentage| 0     | 62.2 | 68.0 | 77.7 | 80.6 |

The effect of extracts on total nucleic acid intake

Table (4) indicates that the concentration of IC50 from the extracts of both aqueous, alcoholic, and petroluem ether led to a decrease in the amount of nucleic acids of DNA and RNA compared to the control sample during a period of (96) hours of growth by (13.6, 55.2,
49.5%) and (23.3, 73.9, 74.7%), respectively, for RNA. As the results of the statistical analysis showed that there were significant differences at the probability level (P ≤ 0.05) between the amount of RNA and DNA in front of the treated and non-treated flagellum.

Table (4): The effect of IC50 concentration of aqueous, alcoholic and petroleum ether extracts on the total nucleic acid amount (ug/cm³) for anterior flagellum of L. tropica at the logarithmic phase

| Treatment            | Conc. IC50 (mg/cm³) | DNA (ug/cm³) | Inhibition (%) | RNA (ug/cm³) | Inhibition (%) |
|----------------------|---------------------|--------------|----------------|--------------|----------------|
| Control              | ---                 | 0.281        | ---            | 0.481        | ---            |
| aqueous extract      | 0.05                | 0.243        | 13.6           | 0.321        | 23.3           |
| ethanolic extract    | 0.03                | 0.126        | 55.2           | 0.126        | 73.9           |
| Petroleum ether extract | 0.04              | 0.142        | 49.5           | 0.122        | 74.7           |

4. Discussion

Plants are an important source for the manufacture of medicinal drugs due to the presence of some chemicals with biological effectiveness, so it was adopted in the preparation of many medicines and medicinal drugs. Of medicines and medicinal drugs for their biological pharmacological effectiveness, speed of their therapeutic effect, and their limited negative side effects caused by chemically manufactured drugs, so they have become the best treatment methods for many diseases that afflict the human race [15].

The findings of current study that showed after treating cultures which containing L. tropica with extracts at concentrations (0.05, 0.1, 0.15, 0.2 ml/cm³) indicate a clear inhibitory toxic effect of the extract with a positive relationship on The number of cutaneous leishmaniasis and an opposite relation with the time required to obtain IC50, that lead to killing half the number of cells in the media treated with the extract compared with the pure culture media containing L. tropica without any addition (control), where the increased the
concentration of extract added to the culture containing *L. tropica* lead to increased inhibition rate of extract. This inhibitory effect of *S. officinalis* leaves extracts indicates that the various concentrations of extract have an effect on the number of cells and inhibit their growth. The reason may be that the active components affect on biological activity *L. tropica* cells and the production of proteins in the cell that leading to a defect in growth of cells. the effect of *S. officinalis* leaves extracts on Leishmania is agree with results of [15] that refeered that the *S. officinalis* leaves extracts have inhibitory effect on cutaneous leishmaniasis, also a clear inhibitory effect on the number of cutaneous leishmaniasis. Saeed et al. [17] led to a gradual decrease in the number of Leishmania tropica by using concentrations of 0.5 to 2.5 μg / ml of E. Arvense extract, and the effect of the extracts on the number and time of generation, an inverse relationship between the concentration of the extract and the growth average of the parasite could be established. The inhibitory concentration of 50% promastigotes was IC50 = 1.5 μg / mL after 96 hours and this agrees with the results of the current study.

In present study, The effect of extracts on of nucleic acids may be due to the active compounds present in sage and pineapple, which may affect the synthesis of nucleic acids or affect the enzymes that help in their manufacturing processes. [18] confirmed that the glycosidic compound Amarogentin isolated from the Swertia plant chirata works to inhibit the activity of topoisomerase I enzymes of *L. donovani* parasites at a concentration of (60) micromol / cm3, as the compound Amarogentin binds with the enzymes Topoisomerase I and thus prevents its binding with DNA to form the DNA-topoisomerase I double complex as well as the Diospyrin complex (one of the compounds derivatives). Conjunia) isolated from Diopsyros montana plant, it has anti-activity against the action of topoisomerase I enzymes in the visceral leishmaniasis parasites and prevents its attachment to the DNA and thus the DNA loses its ability to perform its cellular functions, and the effectiveness of some compounds derived from Diospyrin in affecting the effectiveness of topoisomerase enzymes for parasites have been tested. *L. major*, such as Diospyrin dimethylether, which inhibited the activity of these enzymes by (98%) when used at a concentration of (2.5) micrograms M / cm3 [19].

5. Reference

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