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Evaluation of the efficacy of *Chlorella vulgaris* extract against cutaneous leishmaniasis in white laboratory mice of Balb/c

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Abstract---The current study aimed to evaluate the effectiveness of *Chlorella vulgaris* extract against the parasite *Leishmania major* that causes cutaneous leishmaniasis and compare it with ivermectin drug *in vivo*. This study was conducted in the animal house of the Department of Biology / Faculty of Education for Girls / University of Kufa, and the study period extended from 1/11/2021/ to 1/4/2022. The current study included evaluating the effectiveness of the crude alcoholic extract of *C. vulgaris* compared with Ivermectin drug *in vivo* (white mice) where the current study included the use of two concentrations of alcoholic algae extract (500, 1000) mg/ml and one concentration of the drug 2%. The results of the current study showed a decrease in the size of the pest in the group treated with alcoholic extract of the moss. With an increase in the concentration compared to the drug and the positive control group, as the size of the pest treated with the extract became 1000 mg/ml after 15 days of treatment, 0.5 cm, while the size of the pest in the group treated with the drug was 1.2 cm. The current study showed *in vivo* that the crude alcoholic extract of *chlorella* alga with a concentration of 1000 mg/ml achieved the highest relative therapeutic efficacy of 84%, while the therapeutic efficacy of ivermectin drug *in vivo* reached 64.7%. We conclude from this study that the alcoholic extract of *chlorella* alga is effective in treating cutaneous leishmaniasis ulcers compared to the drug.

Keywords---alcoholic extract of chlorella vulgaris, cutaneous leishmaniasis, ivermectin drug.

Introduction

Leishmaniasis is a parasitic disease caused by different types of parasitic leishmaniasis. It has three main clinical forms: cutaneous, mucosal and visceral leishmaniasis (Reithinger and Dujaden, 2007; Karimi and Nabipour, 2015; Alemayehu and Alemayehu, 2017). The disease is transmitted to humans through the bite of a female sandfly of the genus Phlebotomus. Injecting the parasitic fly into exposed areas of the body and ulcers appear in different places on the skin of the infected person (face, head, arms, lower extremities....etc). The size and distribution of ulcers depends on the immune factors and skin sensitivity of the affected person (Al-Hucheimi, 2005; Goto and Lindoso, 2010).

The Leishmania parasite has two stages in its life cycle and needs two hosts to complete its life cycle. The first host is the sandfly (the invertebrate host) and the stage is called the promastigote stage, and the second host is the vertebrate host (human) and this stage is called unflattering phase (Hojjat, 2015). Cutaneous leishmaniasis in the ancient world is represented by two manifestations, one of which is Anthroponotic Cutaneous Leishmaniasis (ACL) caused by the parasite tropica.L, The second is Zoonotic Cutaneou Leishmaniasis (ZCL), which is caused by the parasite *L. major* (Acha and Szyfers, 1980; WHO, 2003 Al-Obaidi, and Al-Samarai 2009). At present, approved vaccines against leishmaniasis are not used in humans. As a result, it became difficult to control vectors and other factors, and this leads to failure to control the spread of the disease (Farias-Junior et al., 2012).

Researchers have resorted to using medicinal plants in an attempt to find alternative treatments to reduce the side effects or without effects, due to the negative effects of chemotherapy on liver functions, diarrhea, nausea and abdominal pain, and it is possible that the infection will return in certain cases and the parasite's resistance to some therapeutic substances, and because the treatment Medicinal plants are inexpensive and available, so they are used in large parts of the world (Al-Mubarak 2006, Koshki et al., 2017;). Several types of algae have also been used that can be sources of many new important compounds with biological activity (Athbi, 2017), and one of these algae is *Chlorella vulgaris*. It is one of the green algae that is abundant in fresh water such as lake and river water (Liani & Katoch, 2017), and it is a rich source of natural chemicals that can be extracted through simple methods (Pacheco et al., 2015; Verma & Khan, 2016). The current study aims to evaluate the effectiveness of the alcoholic extract of *Chlorell vulgaris* on the parasite cutaneous leishmaniasis (CL) and its comparison with Ivermectin drug *in vivo.*

Materials and methods Experimental animals

Swiss white mice Mus musculus of Balb/c were used in the current study. They were 3-4 weeks old and weighing 2-3 kg, which were obtained from the animal house of the Faculty of Science / University of Kufa. These mice were transferred directly to the animal house of the Department of Life Sciences / Faculty of Education for Girls / University of Kufa, they were raised under the appropriate environmental conditions in terms of temperature, ventilation, provision of

drinking water and the appropriate diet And they were placed in cages, with a rate of 6 mice per cage, and 5 cages, with a total number of 30 mice.

Preparation of the alcoholic extract of Chlorella vulgaris

The extract was prepared according to the method of Rois et al. (1987), where 200 gm of dry algae powder was weighed and placed in a conical flask, then 250 ml of Absolute methanol was added to it. The mixture was mixed using a magnetic stirrer for 24 hours. Filter the mixture using filter paper. The filtrate was transferred to a glass Petri dish. Leave the filtrate at laboratory temperature until the alcohol has evaporated and the dry algal extract is obtained.

In vivo study
Preparation of the ointment

Concentrations of (1000, 500) mg/ml of alcoholic extract were prepared using Vaseline, adding 1 and 0.5g weight to 1g of Vaseline to prepare the ointment used in treating infected animals. The concentrations were kept in glass bottles until use.

Injection the animals

Mice were injected into the tail and foot area with (1) ml of the anterior flagella instar of L. major at a parasitic density (1 x 10^6)after sterilizing the injection area with 70% ethyl alcohol (Wangoo et al., 1989). To determine the effect of the alcoholic extract of C. vulgaris against Leishmania parasite The study was designed into five groups and as follows: -

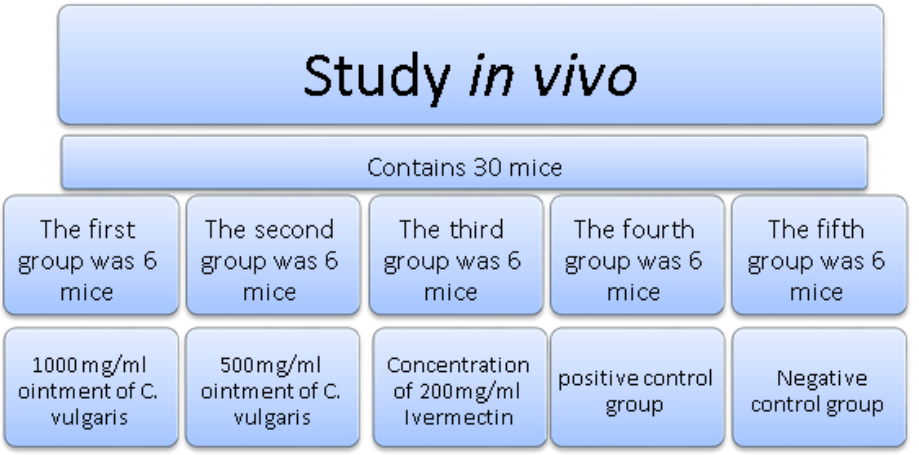

Figure 1. The treated aggregates used in the in vivo study

Experience Design

A 30 mice (BALB/c) of Mus musculus were injected with 1 ml of L. major parasite, and 96 hours after the onset of infection they were treated with the ointment prepared from the alcoholic extract of C. vulgaris with Ivermectin drug.

Treating Infected Animals

A 15-day experiment was conducted on 30 mice infected with cutaneous leishmaniasis. The mice were divided into five groups, each group comprising six mice. After it was confirmed that they were infected with cutaneous leishmaniasis, the first group was treated with 1000 mg/ml ointment and the second group was treated with 500 mg/ml ointment that was prepared. From the alcoholic extract of C. vulgaris, while the third group was injected with Ivermectin drug, and the fourth group was an infected and untreated group, the control group was considered positive, and the fifth group was uninfected and untreated with any treatment, and the control group was considered negative. The affected areas were applied with the ointment using cotton for each concentration, where the ointment forms a thin layer on the affected area, where groups of infected animals were treated daily for a period of 15 days, except for the group that was injected with Ivermectin at a concentration of 200 mg/ml where it was injected subcutaneously only once on the first day, while the positive control group was left untreated for the purpose of comparison, the response to treatment was followed up by taking measurements with a ruler of the size of the pest before treatment and after periods of treatment with the ointment (Tabassam et al., 2008).

Determination of Treatment Efficacy

The therapeutic efficacy of the extract was calculated in vivo. Measurements were taken with a ruler for the size of the pest before treatment and after periods of treatment with the ointment to determine the therapeutic efficacy of each treatment group, according to the equation (Tabassam et al., 2008).
In vivo treatment efficiency % = size of injury before treatment - size of injury after treatment / size of injury before treatment x 100

Statistical Analysis

The Chi-square test and Least significant difference (LSD) test were carried out to show which concentration is more effective using SPSS system (Al-Rawi, 1984).

Results

Effect of alcoholic extracts of algae C. vulgaris compared to ivermectin in the vitality of the amastigote phase in vivo. Table (1) shows the rate of response of infected animals to the alcoholic extract of C. vulgaris compared to ivermectin drug. The results of the statistical analysis showed the effect of treatments on the size of infection with cutaneous leishmaniasis. Where it clearly appeared after the fifth day of treatment, where a clear superiority of the chlorella algae methanol extract appeared over the traditional treatment during the treatment periods, leading to complete recovery from the disease. The highest rate of response and high recovery was recorded in the treated group with a concentration of 1000 mg/ml of the methanolic extract of chlorella alga at a rate of 0.5 cm after 15 days of treatment, followed by the group with ivermectin drug at a concentration of 2% at a rate of 1.2 cm, while the group treated with a concentration of 500 mg/ml of

chlorella algae showed an average rate of healing 1.8 cm after 15 days of treatment.

Table 1
The size of the injury (cm) before and after treatment in laboratory mice infected and treated with different concentrations for periods of time

Treatment type	Concentration mg/ml	Injury size (cm)			
		before treatment	After 5 days	After 10 days	After 15 days
The control		3.5	3.5	3.5	3.5
C. vulgaris	500	3.5	3	2.5	1.8
	1000	3.2	2.8	1.5	0.5
Ivermectin	2	3.4	2.9	2	1.2
LSD P < 0.05		Non sign	0.7	1.5	0.9

Determination of the percentage of efficacy of treatment in vivo

The therapeutic efficacy of the alcoholic extract of chlorella algae compared to ivermectin drug was determined after 5, 10 and 15 days of treatment. As we note that there is a clear significant effect on the therapeutic efficacy as shown in Table (2), where the alcoholic extract of chlorella algae recorded the highest therapeutic efficacy after the fifth day of treatment. The treatment was 14.7 the superiority of the drug. While after ten days, the metholic extract of chlorella algae showed a concentration of 1000 mg/ml, a clear superiority over the drug by 53.12, and this superiority continued until the 15th day of the treatment. The chlorella extract recorded the highest rate of therapeutic efficiency and pump it by 84.37.

Table 2
The therapeutic efficacy of alcoholic extracts of chlorella compared to the traditional treatment with ivermectin drug in vivo

Treatment type	Concentration mg/ml	therapeutic efficacy %		
		The fifth day	Day ten	fifteenth day
C. vulgaris	500	14.28	28.57	48.57
	1000	14.7	53.12	84.37
Ivermectin	2	12.5	41.17	64.7
chi-square arithmetic		4.4	6.8	9.7
tabular chi-square P < 0.05		1.15	1.15	1.15

Discussion

Leishmaniasis is a parasitic disease caused by Primary parasites of the genus Leishmania. It is a neglected disease and there are several factors that contribute to the spread of the disease, especially the difficulty in eradicating the vector flies,

as well as the lack of a vaccine (Freitas-Junior et al. 2012). It is transmitted to humans through the female sandfly vector (de Vrieset al. 2009; Makdisi & Homsi 2015). There are many Medicines used in the treatment of cutaneous leishmaniasis, including sodium stibogluconate (Pentostam) as well as Glucantime (Camacho et al, 2003), and these drugs have many side effects, which are muscle pain, a decrease in white blood cells, inflammation in the pancreas and joints, and an enlarged heart (Desjeux et al. .,2013) ,Medicinal plants have been used by many researchers to find an alternative treatment with fewer or no side effects in addition to being inexpensive and widely used in the world (Al-Mubarak 2006, Koshki et al., 2017;).

Algae have been used as natural sources of compounds with biological activity (Athbi, 2017). Algae are characterized by containing many active substances, including microalgae, which are of medical and commercial importance because they contain active and biologically active substances and molecules in the treatment of diseases, as well as their use as antimicrobials for many microorganisms from including parasites (Munoz & Fernandez, 2017), The results of the in vivo study showed that the use of the methanolic extract of C. vulgaris showed a clear efficacy in the treatment of the cutaneous leishmaniasis parasite when used as an ointment on the skin pests that gradually decreased in size after a period of use of the algal extract. This is a good indicator of the efficiency of the algal extract in the treatment of cutaneous leishmaniasis, especially the high concentrations of the extract during the study period, where the concentration of 500 mg / ml showed a clear decrease in the size of the infection after 15 days of treatment by 1.8 cm, while the size of the infection using a concentration of 1000 mg / ml reached 0.5 cm . Where the results of this study agreed with the results of the study of Spavieri et al. (2010) who demonstrated the effectiveness of extracts of the green alga Cladophora rupestris against the parasite Leishmania donovani, as well as agree with the results of (Goiris et al, 2014.)

This effect is attributed to the active chemical compounds found in the alcoholic extract of algae, which act on the mitochondrial membrane, which leads to a decrease in protein and loss of movement, and this leads to the death of the parasite (Sousa, 2017), The algae extract also contains pcoumaric acid and ferulic acid, which have antioxidant properties (Ruela-deSousa et al., 2010). Ivermectin drug also showed effectiveness in eliminating Leishmania parasite, which showed a decrease in infection size of 1.2 cm. These results are in agreement with the findings of (Opara & Ameh, 2005) and this effect comes through the effect of the drug on the nervous system (Clark, et al 1995).

Relative therapeutic efficacy of alcoholic extract of *C. vulgaris* and ivermectin *in vivo*

The alcoholic extract of chlorella algae, at a concentration of 1000 mg/ml, showed a clear therapeutic efficacy in vivo, reaching 84.37, compared with other concentrations of the alcoholic extract of chlorella alga and the drug. As these extracts inhibited the growth of *Leishmania* parasite, this is due to the active chemicals contained in the alcoholic extract of algae, which are considered highly biologically efficient materials such as vitamins, proteins, long chain unsaturated

fatty acids, hydrocarbons, heterocyclic compounds, phenols and terpenes. In addition, they contain a high percentage of fats, which makes them of high nutritional value, as these fatty acids are not manufactured by humans and therefore it is important to obtain them from food (Andrade et al., 2018).The algae's content of such substances explains its activity against the parasite and killing it through its effect on carbohydrate metabolism, mitochondria and respiration (Delorenzi et al., 2001), in addition to the action of the fatty acids present in these algae as antimicrobials and antiparasitics. As mentioned by Rayan et al. (2005) in his study of the important role played by these acids in the resistance of the parasite Giardia sp. In particular, Dodecanoic acid through its effect on the active phase, where these acids lead to the death of the parasite through its accumulation in the cytoplasm of the cell, leading to the rupture of the cell membrane.

Conclusions

We conclude from the current study that the ointment of the alcoholic extract of *C. vulgaris* had a significant and clear effect with increasing the concentration in the treatment of skin pests of animals infected with cutaneous leishmaniasis *in vivo*, and the alcoholic extract of *chlorella* at a concentration of 1000 mg/ml showed a clear therapeutic efficacy *in vivo*.

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