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Experimental Study on the Efficacy of Syzygium Aromaticum Against Cutaneous Leishmaniasis in Comparison with Pentostam

Zahra Abdullah Alamri
College of Science || King Khalid University || KSA

Abstract: Cutaneous leishmaniasis is a parasitic disease caused by a kind of Flagellate (Mastigophora) that belongs to the Leishmania. This disease is transferred by sand flies. This research was conducted to study the effect of Syzygium aromaticum on the evolution of cutaneous leishmaniasis blister in mice infected with this disease. To study the effects of this herb on the evolution of cutaneous blister caused by Leishmania, the white mice of BALB/ c species were injected by a dose of 107 parasite\ml from the parasite farm of LON-4 Leishmania major in the sacrum. After three weeks, the blister begins to appear in the injected place like a small tumor (ulceration). It begins to grow into blister then to sore which is often accompanied with bacterial and fungal infection. Experiments were conducted into two parts. The first part includes experiments conducted on mice, while the second part includes the tissue test of cutaneous blister and making sure that it is parasite free. The study covered three groups: the negative control group which is formed from eight mice; positive control group (Pentostam group) and it is formed from eight mice injected by Pentostam drug, 120 mg\kg; Syzygium aromaticum group that was administrated different doses of 5, 10, 40, 60 mg\kg orally before treated directly by ointment of Syzygium aromaticum herb on the blister place. It was found that the infected mice treated by Syzygium aromaticum orally by a dose of 40 mg\kg and also treated by Syzygium aromaticum ointment directly on the blister place gives high percent of improvement reached 72%. Then it is followed by the group treated by Syzygium aromaticum orally by a dose of 60 mg\kg as the percent of improvement reached 52%. The results showed that the Syzygium aromaticum is the best to treat the cutaneous leishmaniasis amongst other herbs.

Keywords: Cutaneous leishmaniasis, Leishmania, Flagellate, Pentostam, Syzygium aromaticum.

دراسة تجريبية على فعالية عشبة القرنفل في مكافحة داء اللشمانيا الجلدية مقارنة بعقار البنتوستام

زهرة عبد الله العمري
كلية العلوم || جامعة الملك خالد || المملكة العربية السعودية

الملخص: يعتبر داء اللشمانيا الجلدية إحدى المشاكل الصحية المنتشرة في العالم وهو عبارة عن مرض طفيلي يسببه نوع من الطفيليات السوطية الأولية التي تنتمي إلى جنس اللشمانيا Leishmania وينتقل هذا الداء عن طريق ذبابة الرمل وقد تمّ إجراء العديد من الدراسات للتوصل إلى علاج فعّال وآمن بسبب عدم سيطرة الدواء الحالي على هذا الداء بالإضافة إلى أنه لا يوجد لقاح وقاية لهذا الداء. وقد تم إجراء هذا البحث لدراسة تأثير عشبة القرنفل على تطور بثرة اللشمانيا الجلدية في الفئران المصابة بهذا الداء. ولدراسة تأثير هذه العشبة على تطور البثرة الجلدية التي يسببها طفيل اللشمانيا تم حقن الفئران البيضاء من سلالة BALB/ c بجرعة 107 طفيل/ مل من مزرعة الطفيل سلالة Leishmania major LON-4 في منطقة العجز، وبعد مرور ثلاثة أسابيع تبدأ البثرة بالظهور في المكان المحقون على شكل تورم بسيط (ندبة) يبدأ في النمو إلى بثرة ثم إلى قرحة وغالباً ما يصاحب القرحة التهابات بكتيرية وفطرية. ولتحديد ذلك، تم إعداد

ثلاث مجموعات من الفئران: المجموعة الضابطة السالبة وتتكون من ثمانية فئران والمجموعة الضابطة الموجبة (مجموعة البنتوستام) وتتكون من ثمانية فئران وتحقن بعقار البنتوستام 120ملجرام/ كجم ومجموعة القرنفل تعطى جرعات مختلفة 5، 10، 40، 60ملجم/ كجم عن طريق الفم، وتدهن مباشرة بدهان من عشبة القرنفل على مكان البثرة. وتم إجراء التجربة على الفئران ومن ثم تم القيام بالاختبار النسيجي للبثرات الجلدية والتأكد من نقائها. وفي الجزء الثاني من التجربة تم اختبار نقاء البثرات الجلدية من طفيل اللشمانيا وذلك بأخذ عينات من البثرة الجلدية بطريقة الكشط وعمل الفحص النسيجي في أوقات مختلفة امتدت إلى أربعة أسابيع تقريباً ثم تم صبغ القطاعات الشمعية بصبغة هيماتوكسلين هارس وفحصت بالمجهر الضوئي ثم سجلت النتائج وتم جدولتها وتحليلها إحصائياً. وقد تمّ التوصل من خلال هذه الدراسة إلى أن مجموعة الفئران المصابة والتي عولجت بالقرنفل عن طريق الفم بجرعة 40 ملجم/ كجم وكذلك التي عولجت بالقرنفل عن طريق الدهن على البثرة أعطت نسبة تحسن عالية وصلت إلى 72% يليها المجموعة المعالجة بالقرنفل عن طريق الفم بجرعة 60 ملجم/ كجم حيث بلغت نسبة التحسن فيها 52%، وبالتالي يمكن القول بأن عشبة القرنفل هي الأفضل في علاج داء اللشمانيا الجلدية من بين الكثير من الأعشاب المستخدمة.

الكلمات المفتاحية: داء اللشمانيا الجلدية، طفيل اللشمانيا، الطفيليات السوطية الأولية، عقار البنتوستام، عشبة القرنفل.

Introduction

Leishmaniasis is one of the most common health problems in the world. It is a parasitic disease caused by a parasite known as Leishmania Genus, which is a parasite that helps protect the parasite and facilitates its propagation, leading to human disease without any change. In the shape and structure of the pharyngeal cell. The disease is transmitted by the Sand-fly Phlebotomus, where the dogs are considered to be host families in the Middle East and the Eastern Mediterranean Region. In Asia and Africa, the rodent mice are their host (WHO, 2004) belonging to the genus Leishmania different satisfactory forms ranging from simple skin pustules or the so-called cutaneous Leishmaniasis to skin blisters spread cause severe place of infection deformities and called cutaneous leishmaniasis lesions mucous to a fatal disease if it is not treated intensively affects viscera internal and called (Goldsmid and Melrose, 2005).

The life cycle of the leishmaniasis is complex (Guerin et al., 2002). The human is infected with this parasite when host immunity is weak and thus fails to destroy the parasite (Berman, 2005). The life cycle is characterized by two major developmental stages, the amastigote phase and the front stage is the whip or the stage of the leptomani Promastigote stage (Leptonoas). The first to know that these organisms are primary animals is the physician Brosky in 1898 and was working in the Russian army when he studied similar skin scars in Turkmenistan. In 1903, Leishman linked these organisms to form when he saw them in the visceral leishmaniasis patients with Trypanosoma parasite Trypanosoma from the discovery of the parasite causing Kalazar after isolation from the spleen patient died in India. It is worth noting that the World Health Organization (WHO) has identified leishmaniasis in its various forms as one of the epidemic diseases that it is striving to eradicate (WHO, 2002). Cutaneous leishmaniasis is considered to be one of the most important health problems in most countries (Al-Gindan et al., 1984).

Previous studies indicate that the most common species of leishmania in Kingdom Saudi Arabia (KSA) is leishmaniasis. The main parasite of leishmania in the KSA is the Leishmania major parasite by the female Phlebotomine sand flies (Abuzaid et al., 2017).

Statement of the Problem

In the context of the spread of this disease and the existence of such problems associated with the use of these treatments, many researchers (i.e. Uthman, Satir and Tabbara, 2015; Al-Jaser, 2005) have been searching for less dangerous and more effective treatment alternatives to eliminate the disease. The first officially recorded case of cutaneous leishmaniasis in the Kingdom was in Aramco Hospital (Arab American Petroleum Company) in the eastern region between 1950-1979. In 2007, the Ministry of Health recorded 3286 cases of cutaneous leishmaniasis distributed in various regions of the Kingdom. The Qassim region accounted for the highest percentage, reaching 25.9% of all cases of leishmaniasis, followed by Al-Ahsa with 24.9% and Medina with 18.8%, while the northern border areas and Al-Jouf Al-Qurayyat and Al-Qunfudhah, no cases were recorded. The infection among Saudis (49%) was greater than among non-Saudis (51%) and the infection among males (77.4%) was greater than among females (22.6%). Cases of cutaneous leishmaniasis accounted for the largest percentage in the age group from 15-44 years, reaching (63%), followed by the age group from 5-14 years (18.4%), then the category from 1-4 years (7.7%), and the least exposed groups were infants less than one year old when they reached (1.8%), and the age group from 45 years More than 9.1%. It was observed that the seasonal prevalence of leishmaniasis cases increases during January and February, where the infection rate in these two months combined amounted to 31.1% of the total number of cases, and the least months of infection is June, when the infection rate reached 4.1% (Ministry of Health, 2007) and in 2008 Statistics of the Ministry of Health in the Kingdom of Saudi Arabia recorded a decrease in the incidence of cutaneous leishmaniasis from (13.9) cases per 100, 000 people in 2007 to (9.36) cases per 100, 000 people.

Aim and Objectives

This study aimed to identify the effect of cloves on the development of cutaneous leishmaniasis pimples in mice infected with this disease, to contribute to the extraction of active substances from this herb and the development of methods to treat leishmaniasis using natural compounds, as well as to find different natural alternatives to Pentostam that are safer and more efficient. Also, to identify the effect of these alternatives on the area of cutaneous leishmaniasis pimple in experimental animals. The study also aims to help find more effective ways to treat leishmaniasis and reduce its spread in the Kingdom of Saudi Arabia. Moreover, this study comes to resolve the controversy about the feasibility of clove in treating leishmaniasis.

Theoretical framework

Diagnosis of izmaniasis

There are many methods used to diagnose skin leishmaniasis: microscopy, micro-techniques, pathological diagnosis and laboratory diagnosis.

The method of obtaining tissue for examination is as follows:

1- Needle Aspires: Inject 1 ml of brine solution at 9% of the tip of the blister and place a pipette to obtain a small piece of tissue.
2- Slit Skin Smears: Scrape the edge of the blister using the scalpel after pressing with the thumb and forefinger until a flat area of 1 ml is deep and then pierced and scraped.

The laboratory diagnosis is taken by a biopsy from the tip of the blister for the presence of parasites in a large concentration and not from the middle and then divided into three parts: The first works of the survey and the second to examine the tissue and the third to be used in the work of agriculture is the swab on glass slides and installed by ethyl alcohol 95% and then dyed Gamma dye, hematoxylin and iodine, and then examined with a microscope to confirm the presence of non-whipped phases inside cells. The way the farm works, the samples move to the Novy.Mc Neal-3N environment or the Schneider environment for a period of two to two weeks, where the frontal whip phases appear (Markle and Makhrul, 2004).

Classification of the parasite

- Kingdom: Protista (Haeckel, 1866)
- Sub Kingdom: Protozoa (Gold fuss, 1817)
- Phylum: Sarcomastigophor (Honigberg and Balamuth, 1963)
- Class: Zoomastigophorea (Deising, 1866)
- Order: Kinetoplastida (Honigberg, 1963, emend Vickerman
- Suborder: Trypanosomatina (Kent, 1880)
- Family: Trypanosomatina (Doflein, 1901, emend, Grobben, 1905)
- Genus: Leishmania (Ross, 1903)

Treatment of leishmaniasis

The leishmaniasis of the skin automatically heals in a period of one to three years. However, in the case of leishmaniasis, there are many common medicines. If the ulcers are not severe, they can be treated with heat, exposed to radiation, or radiation or by injecting some compounds, such as Pentostam, into the ulcer. Such as pentavalent antimonials such as sodium stibogloconate, which is commercially produced under the name Pentostam or Glucantime with a dose of 10 to 20 mg/ kg bw for 10 to 30 days (Al-Jasser, 1995; Kirk and Sati, 1947) These were used in the late 1940s (Crof, 1988; Neal, 1987). Although these compounds are effective in eradicating the disease, they have toxic side effects and require a long period of time to achieve the hoped for, in addition to damage that can be caused to patients with heart disease or kidney. Therefore, the response of patients to treatment varies, where a proportion of them respond to treatment during the first treatment period, while others need to repeat the doses more than once (Al-Jasser, 1995) and also the emergence of strains resistant to these drugs in many (Herwaldt and

Berman, 1992). Chemotherapy methods are known to severely weaken the body's immune system and expose the patient to an increased risk of infection. Amphotericin B and Pentamidine for the treatment of Ethiopian leishmania use Miltofosin as an antimicrobial agent and viscous viscera (Croft and Coomb, 2003).

In the presence of such problems associated with the use of these treatments, many researchers have been searching for less serious and more effective treatment alternatives to eliminate the disease and to find sophisticated drugs that are safe and effective against the disease of leishmania, which are based on natural products, which led to the development of hundreds of medicines of natural origin. Herbal medicine is considered a known phenomenon since ancient times. The knowledge of medicine has continued from one breed to another until it has become the so-called popular medicine in the Arab world. Thousands of studies have been conducted in the world on natural compounds to determine the appropriate dose that stops the growth of parasites without harmful effects on the human body and the duration of treatment such as ginger, herbaceous leaves, broccoli, garlic, volatile oils, cloves, cinnamon and many others.

Clove grass

The species of flowers is famous and grows the carnation tree in the warm country, which is small and evergreen and gives a large range of scarlet flowers and the flowering buds are green or reddish before drought and turn into an easy structure of fracture and resemble the shape of the screw. (Azeredo and Soares, 2013). Clove is a mild cinnamon and its smell is acute and is anti-bulging and its oil is therefore used in medicine. It is anti-vomiting and spasticity and its use in the treatment of tooth pain is well known (Lane et al., 1991).

Clove has an active effect against various organisms as well as parasites. Clove has been used for thousands of years, especially in Southeast Asia, and has been considered a panacea for nearly all diseases. In some areas of tropical Asia, Meyer and his colleagues proved that the clove oil has been effective in reducing the Nematoda survival rate to 50% Compared to the control group due to the increasing number of dead eggs (Meyer et al., 2008).

Methodology of the study

The study used white mice of the BALB/ C strain, ranging in age from two months to three months and weighing between 25 and 30 g. The hamster was also used. The animals were obtained from the experimental animal care center of King Faisal Specialist Hospital and College of Pharmacy, King Saud University. Animals were taken care of at the Animal House of the Zoology Department of the University. The animals were caged and provided with every crate, water and food supplied with vitamin D daily. The

animals were subjected to normal environmental conditions. The temperature was fixed at 24 ° C and the lighting was programmed on 12 hours of lightness and 12 hours of darkness. (Figure 1).

Figure (1) Conditions in which mice were cared for (cages of animals with sawdust, water and food)

The Leishmania major parasite, which was classified using lsoenzym analysis, was classified as a leishmania major (zymodwme LON4), which was stored in a liquid nitrogen device at –196 ° C until use The need for the parasite is taken out of the liquid nitrogen, taking into account the wearing of the glove of the device. We then dissolve the leishmania cells in the tube at 37 ° ° C by the water bath, keeping the mouth of the tube out and not dipping it into water and then sterilizing it with alcohol. The first sub culture of the sun in a sterile atmosphere within the suction apparatus c so that the contents of the tube (leishmania) can be emptied into the plastic environment containing 10 ml of RPMI 1640 (Sigma) by sterile pasture. Dropwise, with the alcohol sterilized. The data are recorded on the plastic environment flasks and placed in incubation (Co2 Incubator) And 5% concentration of carbon dioxide with a small opening of the vial of the flask to ensure gas exchange. We then monitor the growth of leishmania for a week by examining the sample under the Inverted Microscope and to ensure that there is no sample contamination. To prepare the Leishmania parasite environments, follow the following:

Preparation of the environments used to cultivate the large leishmania parasite:

RPMI 1640 Environment:

Add 15.89 g of RPMI 1640 to 900 ml of distilled water

(MP, GRMANY) and stir the solution until completely dissolved. Then add 0.85 g of sodium bicarbonate (Na2CO3) Sodinm Bicarbonate, 10 ml of glutamine (Sigma, Germany) L-glutamine and 10 ml of antibiotic penicillin-streptomycin (sigma). Finally, add to the environment 20% of the fresh serum inactivated (FCS) calm (Sigma) and then supplement the volume to 1000 ml with distilled water and filter the environment solution in sterile conditions using a filtration cup and keep at a temperature of 8-8° C until use.

Schneider Insect Medium Environment

Add the powder of the sigma environment to 800 ml distilled water in a standard flask and constantly stir the solution until completely dissolved. Add 0.4 g of sodium bicarbonate and pH of 9.2 for 10 minutes by adding a sodium hydroxide base, Hydrochloric acid gradually to be ph 6.7 and add 0.6 g of calcium chloride anhydrous calcinm chloride dissolved in 50 ml of water). The stirring continues until the solution completely dissolves and then complete the volume with the distilled water to 1000 ml. The pH of the solution is PH = 0.1 - 0.3 Finally serum is added by 20% then the solution is filtered Environment in sterile conditions. The environment is kept at a temperature of 8-2° C until use.

(N-N-N tripe N Media) environment

We take 1.4 grams of agar and put it in a glass flask and add 0.6 g of sodium chloride NaCl sodinm clorid and 90 ml distilled water. The agar is then dissolved in the autoclave at 112° C for 15 minutes at 101 ° C The agar is then cooled at 45° C and added to 13.5 ml sheep Blood Difibrinated (Saudi prepared Meddia laboratory company) and distributed in plastic bottles of 25 cm2 (NUNC. Denmark) and is kept diagonally at room temperature until it freezes. The agar is then tilted and kept at a temperature of 8-2° C. When the agricultural environment is used to grow the parasite, The italic portion of the 1640 RPMI environment.

Extracting the leishmania parasite from the Hamster (isolating the amstigote of the infected animal):

Hamster is injected with a dose of 0.1 ml of the leishmania child in the frontal phase of the promastigote whale at a concentration of 108 infant/ ml cells in the foot bad area. These animals are cared for by providing food, water and straw cages. After 20 days, the blister begins to appear as a small swelling and gradually grows. Before the pimple develops into an ulcer and is infected with a bacterial infection, the animal is killed by carbon dioxide. The foot is removed after the animal is executed with a sharp and sterile scissors. Then heamognaze starts in a sterile atmosphere inside a machine, and remove the nails and hair from the foot and then cut the foot into small pieces and put in the crushing tool heamognaze 40 ml (wheaton USA) and add 10 ml of the RPMI 1640 environment and crush the foot pieces with the crushing tool for 30 minutes continuous until the cells become Transparent color. And then pour into the centrifuge tube 10 ml and placed in the centrifuge at a speed of 500 rpm for five minutes and a temperature of 4° C. Then exit the machine and in sterile conditions the residue is eliminated and keep the fire containing the parasites because they are smaller of other cells so they float while the rest of the cells of hair and bone are deposited to the large size.

The centrifuge-containing tubes are placed at 1500 RPM for 15 minutes. After exiting the device, the residue is discarded and the deposit retained. The parasites have been deposited and added to the 10 mL precipitation of the RPMI 1640 environment. Mix the Vortex and return Centrifuge again at 1500 RPM for 15 minutes to obtain a parasite that is clean and free of tissue impurities. Retain the parasite deposits

and remove the fluid. Add 10 ml of RPMI 1640 and pour in the 3N or 3N to the 10 mL precipitation of the Schneider environment and kept in the flasks Plastic Leah in Elhoudan at a temperature of 26° C and 5% concentration of carbon dioxide gas. The environment will reach the stabilization phase after nine days for the 3N environment and after seven days for the Schneider environment, it will be ready for injection into mice and leishmaniasis or frozen and stored in nitrogenic liquid at a temperature of -196 ° C until the time needed.

Keeping parasites frozen:

After diluting the parasite environment with a concentration of 105 RPMI 1640, transfer to 10 ml centrifuge tubes at 1500 RPM for 5 minutes. The larvae are discarded and retained because the parasites have been deposited and 10 ml of the freezing environment consisting of 80 ml of RPMI 1640, 10 ml serum (FCS), 10 ml Dimethyle Sulfoxide (DMDO) (Sigma) and transported in sterile sterile tubes 1 ml and then transfer the tubes to 86 ° C and leave for 24 hours and then transfer to 196 ° C in liquid Nitrogen.

Follow-up of parasite farms:

The farms are examined using an Inverted Microscope so that farms that are free of bacterial and fungal contamination reduce the RPMI 1640 environment by 3: 1 and the contaminated pollutant is disposed of.

Preparation of the parasite dose for injection of mice:

After nine days in a 3N or 7-day environment in the Schneider environment, parasites in the anterior phase complement the lattice and become ready to infect the mice so that 1 micrometer from the parasite environment is placed on the heamocytometer and then covered with the glass slide cover and the live promastigote cells Using the normal microscope with a 40x magnification lens according to the mathematical equation:

Number of parasites/ number of squares x 104 and then reduce RPMI environment by 1640 concentration. 107

Injection of BALB/ c Mic animals with large leishmania parasites:

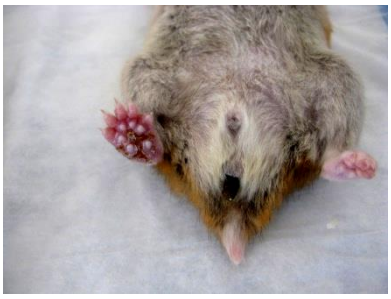
The animals are transported to the area using a sterile shampoo with 70% ethyl alcohol. Each mouse is injected into the treated area with a dose of 0.1 ml from the parasite plant, with 107 parasite/ mL cells from the frontal whip. Three weeks after the injection (incubation period) The blisters begin to appear in the injected spot in the form of a simple swelling (scar) and then gradually develop a nodule closed nodule problem and then grow into an ulcer.

From the first day of the appearance of the blister, the infected mice are divided into study groups in their own cages and all data is written by a form placed on the cages.

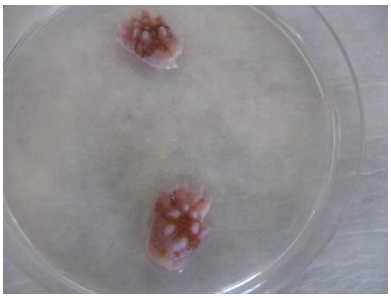
Determination of the appropriate dose of clove and Pentostam:

- Carnation dose: Four doses of clove herb were selected in this study: 5 mg/ kg, 10 mg/ kg, 40 mg/ kg and 60 mg/ kg.
- Pentostam: A 120 mg/ kg dose of the drug is prepared by adding the normal saline solution to the Pentostam.

Experience Tools:



The hamster after his execution and his right foot injected with the parasite swollen

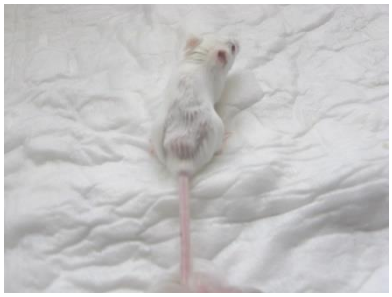


The infected feet are then removed with sterile sterile scissors

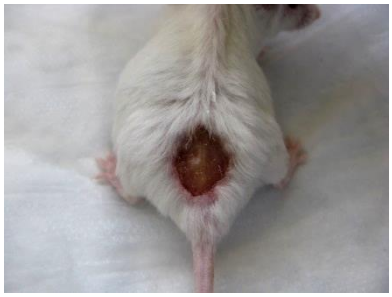
Heamognaze crushing tool used to isolate the whipless phase

Mixing device used to mix the contents of the tube

Inoculation of mice with parasites in the sacral region



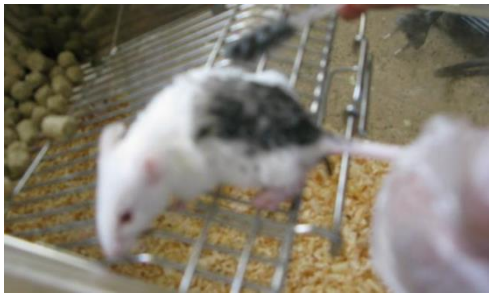
The leishmania pimple is a closed knot



The lemongrass pimple is turned into an ulcer

Method of measuring blister of leishmania area in infected mice using a micrometer

Oral injection for different doses



Method of fatting on Blister

Design Experience

The mice were divided into three main groups as follows:

Group 1: The negative control group consists of eight mice injected with the PBS solution for four weeks a day and the blister are measured three times a week using a special micrometer.

Group 2: Positive control group (Pentostam group) consists of eight mice and injected with Pentostam 120 mg/ kg for fifteen days and measured the blister three times a week using the special ruler (micrometer).

Group III: Carnation group divided into five groups:

1- Oral injection of 5 kg/ kg of oral clove. This group consists of eight mice.
2- A group injected with oral clove herb by 10 mg/ kg. This group consists of eight mice.
3- A group injected with oral clove herb by 40 mg/ kg This group consists of eight mice.
4- A group injected by mouth with clove herb 60 mg/ kg This group consists of eight mice.
5- This group is painted with a cream of carnation on the place of the blister and consists of eight mice.

Each group is given the drug for four weeks a day and the pimple are measured three times a week using the special micrometer and then the appropriate dose is determined.

Purity test of pimples of the skin leishmania parasite:

Through the following steps:

1. Sampling of the pimple of the treated mice from the leishmania parasite by histological examination at different times of approximately 4 weeks.
2. Take a sample of the skin blister in a manner of abrasion.
3. Perform the histological examination to ensure that they are free of leishmania parasite.
4. Processing wax molds.
5. dye wax sectors.
6. Scanning with light microscopy.

Indirect analysis

- **The first hypothesis is the difference between the control group and the Pentostam group.**

Table (1) Tests for independent samples of the differences between the mean of leishmania readings

Groups	SMA	standard deviation	Value t	Degrees of freedom	Level of significance
control	4.7675	0.75833	11.187	144	.0001
Pentostam	3.2919	0.83376			

It is clear from table (2) that there are statistically significant differences between the mean of the leishmania buds for the two groups, since the value of the significance level is less than 0.05. Therefore, we reject the null hypothesis and accept the alternative hypothesis.

In other words, the average of the leishmania pustule in the Pentostam group was less and statistically less than the control group.

- **The second hypothesis is the difference between the control group and the Pentostam group and the carnation groups**

Table (2) Analysis of the differences between the average of the leishmania readings according to the different groups

Source of Contrast	Total squares	Degrees of freedom	Average squares	Value P	Level of significance
Inside groups	86.328	2	43.164	23.633	.0001
Between groups	840.157	460	1.826		

Table (2) shows that there are statistically significant differences between the averages according to the difference of the group because the value of the significance level is less than 0.05, and therefore we reject the null hypothesis and accept the alternative hypothesis. To determine the significance of the differences, the researcher used the post-comparison test: LSD. The following table shows this:

Table (3) LSD test to show differences between group averages.

Group	SMA	Control	Pentostam	Clove
Control	4.7675		*	*
Pentostam	3.2919			
Clove	3.7679		*	

Table (3) shows the following:

1. The average of the leishmania pimple for the pentostam group was less than the rest of the groups and was statistically significant.
2. The average of leishmania pimples for carnivorous groups was less than the control group

- **The third hypothesis: the difference between carnation groups**

Table (4) Analysis of the differences between the mean of the leishmania readings according to the different groups

Source of Contrast	Total squares	Degrees of freedom	Average squares	Value P	Level of significance
Inside groups	184.575	4	46.144	25.521	.0001
Between groups	564.126	312	1.808		

Table (4) shows that there are statistically significant differences between the averages according to the difference of the group because the value of the significance level is less than 0.05, and therefore we reject the null hypothesis and accept the alternative hypothesis. To determine the significance of the differences, the researcher used the post-comparison test: LSD. Table (5) shows that:

Table (5) LSD test to show differences between group averages

Group	SMA	Clove "fat"	Clove "injection 5 mg/ kg"	Clove "Injection 10 mg/ kg"	Clove "injected 40 mg/ kg"	Clove "injection 60 mg/ kg"
Clove "fat"	**2.6606**					
Clove "injection 5 mg/ kg"	**3.8903**	*				*
Clove "Injection 10 mg/ kg"	**4.5652**	*				*
Clove "injected 40 mg/ kg"	**4.5739**	*				*
Clove "injection 60 mg/ kg"	**2.9719**					

Table (5) shows that:

1. The average of the leishmania pimple for the carnation group "5 mg/ kg injection" was greater than the "clove and carnation" injection of 60 mg/ kg "and statistically.
2. The average of the leishmania pimple for the carnation group was "injection 10 mg/ kg" was greater than the clove, "clove, " injection 5 mg/ kg and cloves "injection 60 mg/ kg" and statistically.
3. The average of the leishmania pimple for the carnation group was "injection 40 mg/ kg" was greater than the clove "fat", clove "injection of 5 mg/ kg" and cloves "injection 60 mg/ kg" and statistically.

- **The fourth hypothesis: Differences between leishmania readings in the carnation group "fat" according to the different period.**

Table (6) Tests for independent samples of the differences between the mean of leishmania readings

Periods	SMA	standard deviation	Value t	Degrees of freedom	Level of significance
First	2.7368	1.74216	.291	48	.772
Second	2.5844	1.95532			

Table (6) shows that there are no statistically significant differences between the mean of leishmania for the two periods, since the value of the significance level is greater than 0.05, and therefore we do not reject the zero hypothesis. In the sense that the average of the leishmania pimple in the first period is not statistically different from its average in the second period.

- **The fifth hypothesis: Differences between leishmania readings in the carnation group "injection of 5 mg/ kg" according to the different period**

Table (7) Tests for independent samples of the differences between the mean of leishmania readings

Periods	SMA	standard deviation	Value t	Degrees of freedom	Level of significance
First	3.8695	1.31923	-.151	74	.881
Second	3.9111	1.07300			

Table (7) shows that there are no statistically significant differences between the mean of leishmania for the two periods, since the value of the significance level is greater than 0.05, and therefore we do not reject the zero hypothesis. In the sense that the average of the leishmania pimple in the first period is not statistically different from its average in the second period.

- **The sixth hypothesis: Differences between leishmania readings in the carnation group "injected 40 mg/ kg" according to the different period.**

Table (8) Test for independent samples of the differences between the mean readings of leishmania

Periods	SMA	standard deviation	Value t	Degrees of freedom	Level of significance
First	4.6258	1.02118	.414	60	.681
Second	4.5219	.95492			

Table (8) shows that there are no statistically significant differences between the mean of leishmania for the two periods, since the value of the significance level is greater than 0.05, and therefore we do not reject the zero hypothesis. In the sense that the average of the leishmania pimple in the first period is not statistically different from its average in the second period.

- **The seventh hypothesis: Differences between leishmania readings in the carnation group "injection of 60 mg/ kg" according to the different period**

Table (9) Test for independent samples of the differences between the mean of leishmania readings

Periods	SMA	standard deviation	Value t	Degrees of freedom	Level of significance
First	3.3694	1.24073	2.413	65	.019
Second	2.5862	1.41288			

Table (9) shows that there are statistically significant differences between the mean of the leishmania buds for the two periods, since the value of the significance level is less than 0.05, and therefore we reject the zero hypothesis. In the sense that the average of the leishmania pimple in the first period is larger and statistically significant than the average in the second period.

- **The eighth hypothesis: Differences between leishmania readings in fat groups and injection groups**

Table (10) Test for independent samples of the differences between the mean of leishmania readings

Periods	SMA	standard deviation	Value t	Degrees of freedom	Level of significance
First	3.8589	1.50788	5.672	761	.0001
Second	3.0982	1.66674			

Results

Results of the textile sectors in the control group:

Samples of the pimples of the leishmaniasis-treated mice were taken at different intervals to ensure the purity of the pimples of the leishmania parasite. Where the sector was taken in the blister at the first stage of the emergence and as the sector illustrates the existence of phagocytic cells, figure (2).

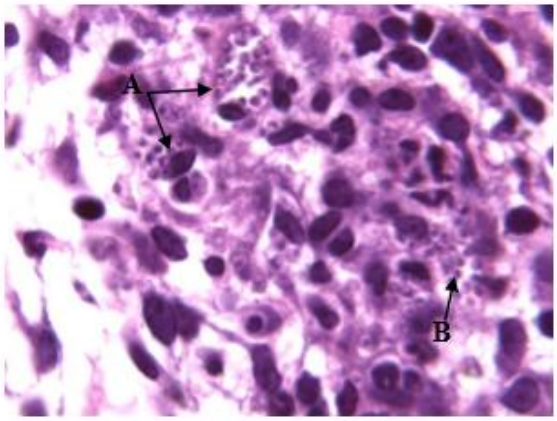


Figure (2) A strip in the blister at the first stage of its appearance, a 100X magnification force

A. Bile cells for parasite B-parasite leishmania

Then 20 days after the appearance of the blister was taken strip in the blister and explains the presence of large numbers of pharyngeal cells containing the parasite, figure (3).

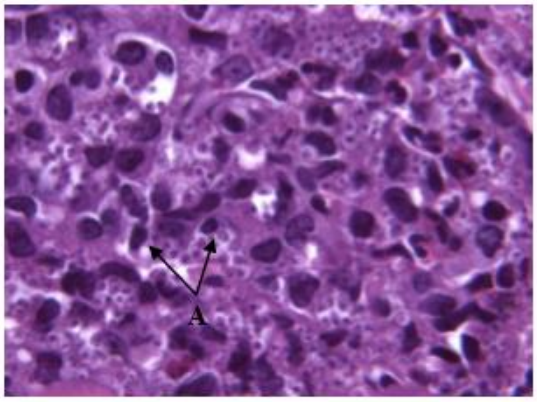


Figure (3) Sector in the blister at a late stage after two months, the force of magnification is 100X

A. cells blister container for parasites

Results of the textile sectors in the positive control group (Pentostam group):

Samples of pimples of pimples of the leishmaniasis parasite and the treatment of Pentostam were taken at different intervals to ensure the purity of the pimples of the leishmania parasite. A segment was taken in the blister after 10 days of treatment with Pentostam, and the sector also showed that there were small numbers of parasites inside the thrombocytopenic cells, figurer (4).

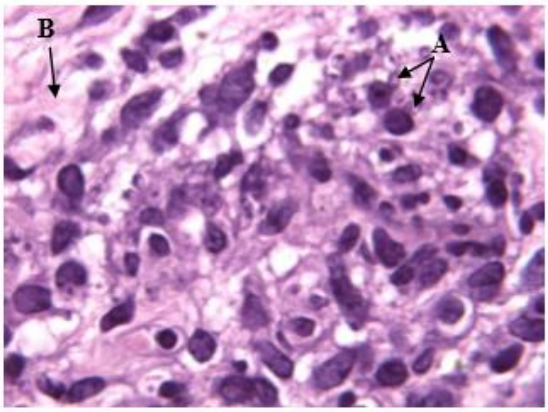


Figure (4) Sector in the blister ten days after treatment with Pentostam, the force is 100X magnification

A. cells of the blister container for parasite B-cell blister are dead

After 30 days of treatment with Pentostam, a section was taken in the blister and the sector was shown to be non-parasite, figure (5).

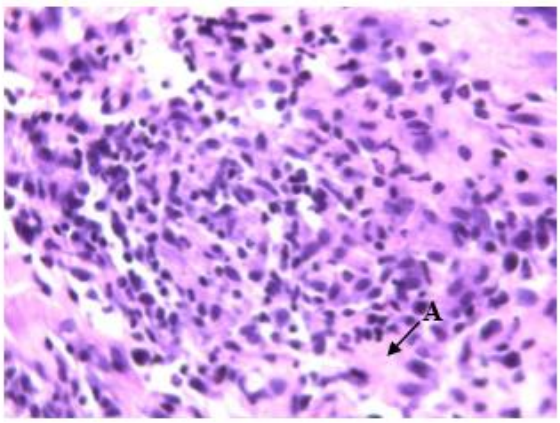


Figure (5) Sector in Blister Thirty days after treatment with Pentostam, 40X magnification power

A. cellular death

Results of the textile sectors in the carnivorous group:

Samples of the pimples of the lashmania parasite were treated and treated with cloves at different intervals to ensure the purity of the pimples of the leishmania parasite.

Group 1: The group treated cloves by fat directly on the blister, where a sector was taken in the blister after 10 days of treatment and the sector shows the presence of large numbers of pharyngeal cells containing the parasite, Figure (6).

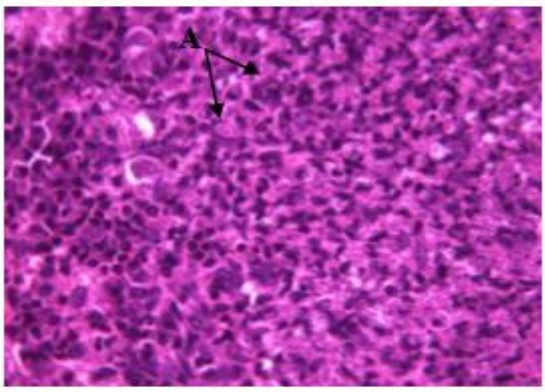


Figure (6) Sector in the blister of the sun after ten days of cinnamon treatment by fat directly on the blister, 40X magnification power

A. cell blister container for parasites

Twenty days after the treatment of cloves by direct fat on the blister another sector was taken in the blister and the sector shows the presence of a small number of pharyngeal cells, Figure (7).

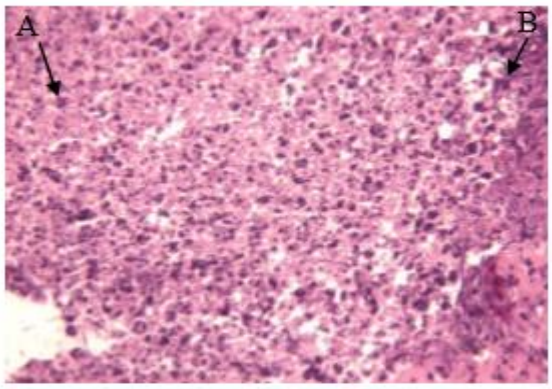


Figure (7) Sector in the blister of the sun after twenty days of cinnamon treatment by fat directly on the blister, 40X magnification power

A cell-death B-cell blister container for parasites

Thirty days after the treatment of cloves by direct fat on the blister was a strip in the blister and illustrates the absence of phagocytic cells, figure (8).

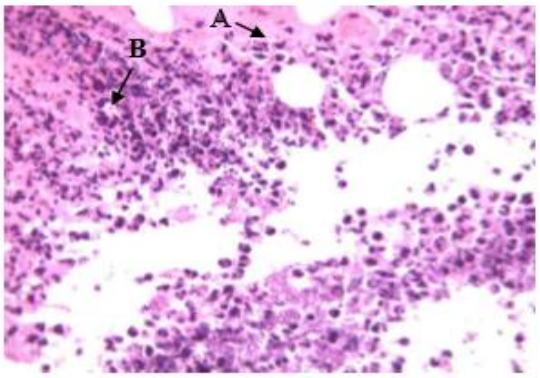


Figure (8) Sector in the blister of the sun after thirty days of cinnamon treatment by fat directly on the blister, 40X magnification power

A dense cell death B-cell blister dead

Group II: The group treated with inhaled cloves at a dose of 5 mg/ kg, where a segment was taken in the blister after 10 days of treatment and the sector showed the presence of large numbers of pharyngeal cells, Figure (9).

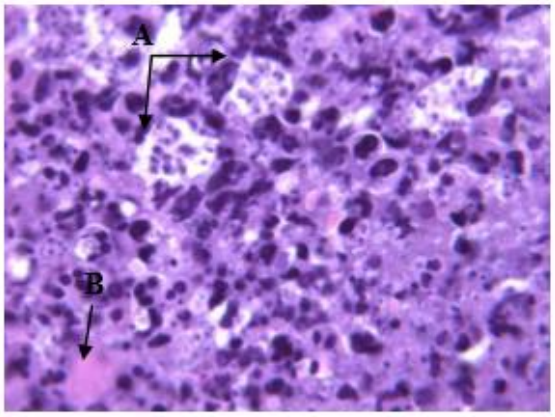


Figure (9) Sector in the blister of the sun after 10 days of treatment with cloves at a dose of 5 mg/ kg, the strength of 40X magnification

A bile-cell container for parasite B-cell death is simple

Then, after 20 days of treatment with cloves with a dose of 5 mg/ kg, a section of the blister was taken and the sector clarified the presence of the parasite cells containing the parasite, figure (10).

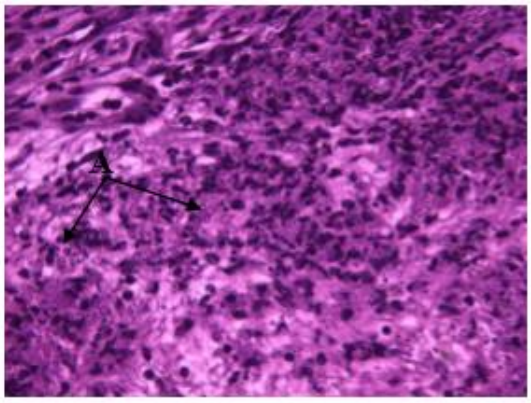


Figure (10) Sector in the blister of the sun after 20 days of treatment with cloves at a dose of 5 mg/ kg, the strength of 40X magnification

A. cells blister container for parasites

After 30 days of treatment with cloves at a dose of 5 mg/ kg, a section was taken in the blister and the sector showed that there were small numbers of pharyngeal cells containing the parasite, figure (11).

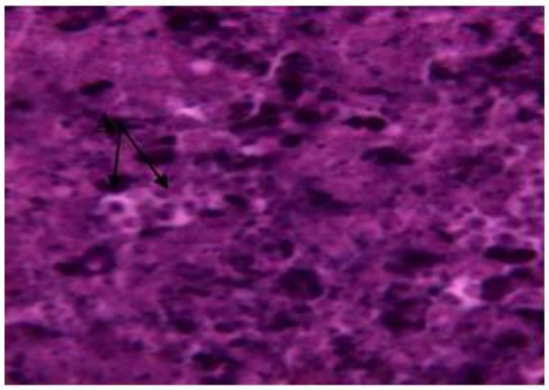
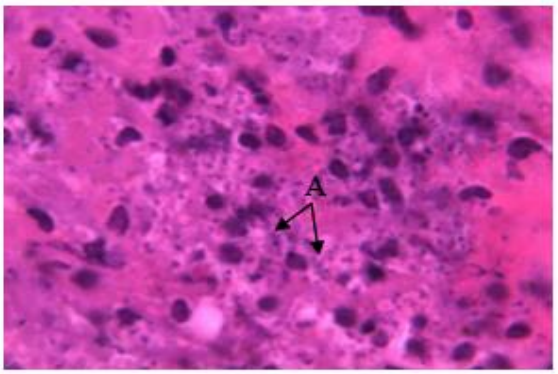


Figure (11) Sector in the blister of the sun after thirty days of treatment with cloves at a dose of 5 mg/ kg, the strength of magnification 100X

A. cell blister

Group III: The group treated with inhaled cloves at a dose of 10 mg/ kg. A section was taken in the blister after 10 days of treatment. The segment shows the presence of the parasite cells containing the parasite, figure (12).



(12) Sector in a blister after 10 days of treatment for a carnivorous mouse with a dose of 10 mg/ kg, 100X magnification

A- cells blister container for parasites

After 20 days of treatment with cloves at a dose of 10 mg/ kg, another sector was taken in the blister, figure (13).

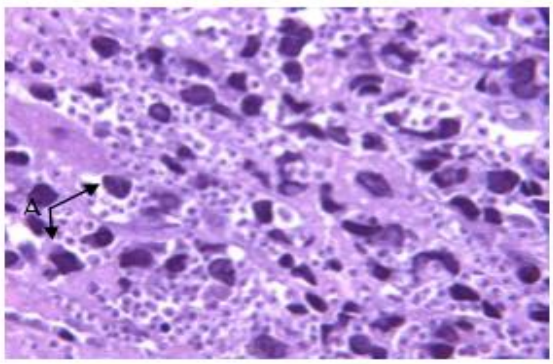


Figure (13) Sector in the blister of the sun after 20 days of treatment with cloves at a dose of 10 mg/ kg, the power of magnification 100X

A. cells blister container for parasites

After 30 days of treatment with cloves at a dose of 10 mg/ kg, a strip was taken in the blister and the segment also showed the presence of the parasite cells containing the parasite, figure (14).

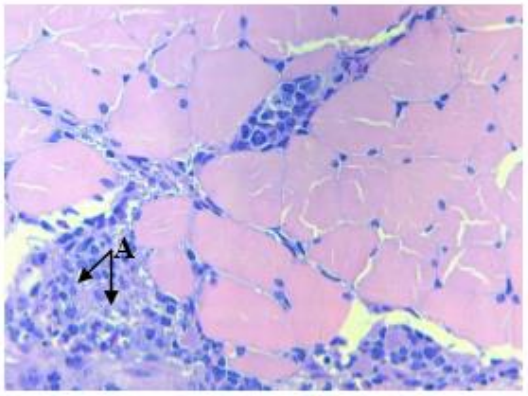


Figure (14) Sector in the blister of the sun after thirty days of treatment with cloves at a dose of 10 mg/ kg, the strength of magnification 100X

A. cells blister container for parasites

Group IV: The group treated with inhaled carnations at a dose of 40 mg/ kg, where a sector was taken in the blister after 10 days of treatment and the sector shows the presence of large numbers of pharyngeal cells, figure (15).

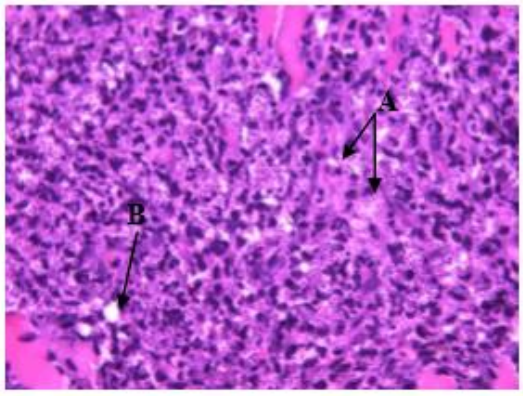


Figure (15) Sector in a blister after 10 days of treatment for a carnivorous mouse with a dose of 40 mg/ kg, 40X magnification

A. cells of the blister container for parasite B-cell blister are dead

After 20 days of treatment with cloves with a dose of 40 mg/ kg, a strip was taken in the blister and the sector also showed the existence of parasitic cells containing parasites, figure (16).

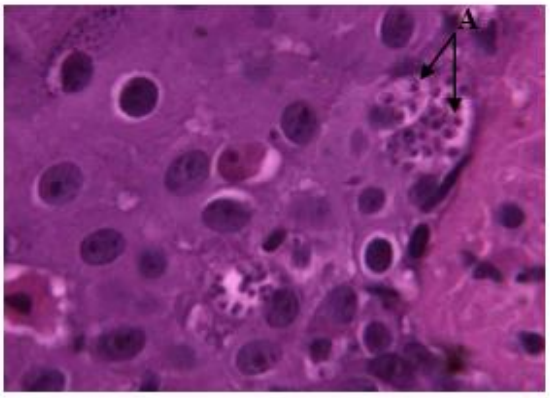


Figure (16) Sector in the blister of the sun after 20 days of treatment with cloves at a dose of 40 mg/ kg, the strength of the magnification 100 X

A. cells blister container for parasites

After 30 days of treatment with cloves with a dose of 40 mg/ kg, a sector was taken in the blister and the sector showed that there were no phagocytes or parasites in the sector, figure (17).

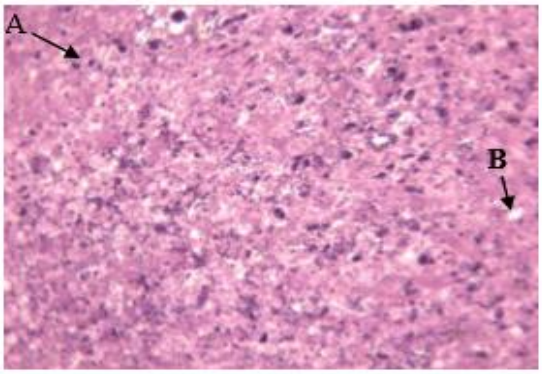


Figure (17) Sector in the blister of the sun after thirty days of treatment with cloves at a dose of 40 mg/ kg, the strength of 40X magnification

A. cell death B-cell blister dead

Group **(5)** The group treated with inhaled cloves at a dose of 60 mg/ kg. A section was taken in the blister after 10 days of treatment. The segment shows the presence of the follicle cells containing the parasite intensively, figure (18).

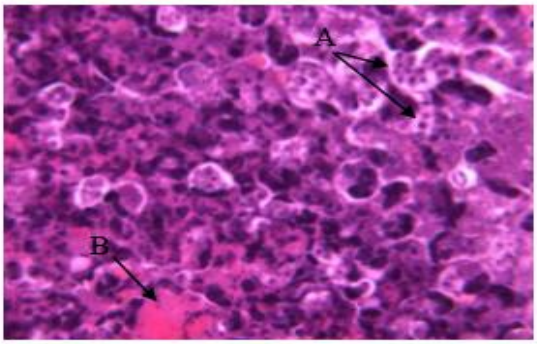


Figure (18) Sector in the blister of the sun after ten days of treatment with cloves at a dose of 60 mg/ kg, the strength of magnification 100X

A. A-cells of a blister container for parasite B-cell death

After 20 days of treatment with cloves at a dose of 60 mg/ kg, another sector was taken into the blister and the sector showed a small number of follicular cells containing the parasite, figure (19).

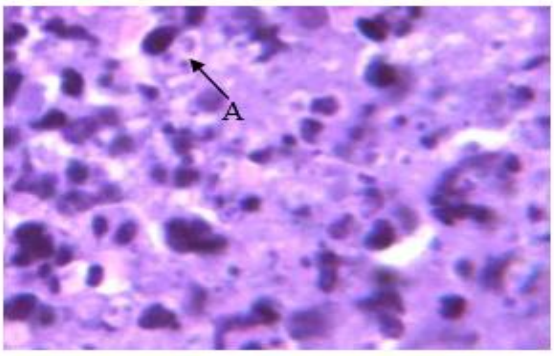


Figure (19) Sector in the blister of the sun after twenty days of treatment with cloves at a dose of 60 mg/ kg, the strength of magnification 100X

A. cells blister container for parasites

After 30 days of treatment with cloves at a dose of 60 mg/ kg, another sector was taken in the blister. The segment also shows that there are few numbers of pharyngeal cells, figure (20).

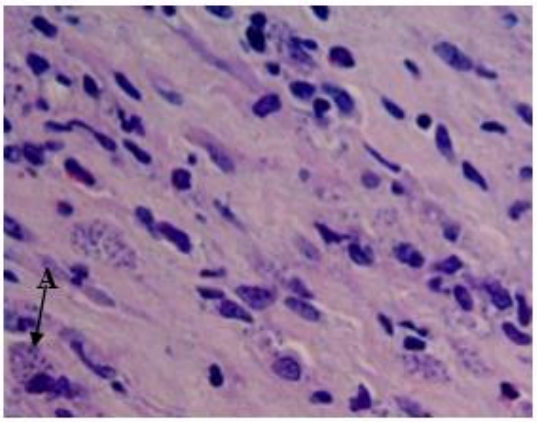


Figure (20) Sector in the blister of the sun after thirty days of treatment with cloves at a dose of 60 mg/ kg, the strength of magnification 100X

A. cells blister container for parasites

Discussion

The incidence of leishmaniasis is an endemic disease in the Kingdom of Saudi Arabia, which the Ministry of Health pays attention to, especially with the increasing cases in recent years. The latest statistics indicate that the rate of skin leishmaniasis reached 10.05 cases per 100, 000 people in 2009 (Ministry of Health, 2009).

In the current study, therapeutic alternatives were selected from natural products. Black seed, cloves and barbaris were used to treat leishmaniasis. It is known that chemotherapy methods severely weaken the body's immune system and expose the patient to an increased risk of infection.

To test the effectiveness of these therapeutic alternatives in the treatment of skin leishmania pimples, animals were used as a model for measuring the efficacy of the drug. The most common animal species were used as a test model (white mice). BALB/ c was selected because it is highly sensitive to all types of leishmania. (Bradley, 1977; Croft and Coombs, 2003)

The results of the present study showed that carnivorous herb had a similar effect to the effect of Pentostam, which contributed to decreasing the volume of pimples. The mean of all groups treated with carnivores was 3.7679 and the group treated with Pentostam was mean (3.2919). This finding was consistent with the results of the sectors taken from the pimple in all groups treated with cloves, where the presence of phagocytic cells in some groups and their absence in other groups in the latter periods of the treatment showed a positive effect of treatment with clove.

The study showed that the mean pimples of the two groups of cloves (fat on the blister directly and injections of 60 mg/ kg) were less than the average of the skin leishmania pimples in the other groups of carnivores and statistically significant, respectively (2.6606) (2.9719). Interestingly, several reports documented that eugenol isolated from Syzygium Aromaticum extracts have shown potent trypanocidal as well as leishmanicidal efficacy against Trypanosoma cruzi, Leishmania donovani, L. amazonensis, L. major and L. tropica (Santoro et al., 2007). Additionally, eugenol showed a potential lethal efficacy against the growth and multiplication of various parasites including Giardia lamblia, Fasciola gigantica, Haemonchus contortus, and Schistosoma mansoni (El-kady et al., 2019). Eugenol exhibited antiviral activity against HSV-1 and herpes simplex -2 (HSV-2) by preventing viral replication and reducing the viral infection (Reichling et al., 2009). Eugenol isolated from Syzygium Aromaticum extracts and their essential oils has shown its free radical scavenging, antioxidant, and antimicrobial properties (Kamatou, Vermaak and Viljoen, 2012).

This is due to the effect of the clove of the cloves, which makes it useful in the relaxation and mitigation of some viral infections and its effect on parasites in general as well as its impact on the various microorganisms, Pathogenic bacteria, anti-microbial and antifungal, anti-oxidant and anti-viral (Chaieb et al., 2007). This finding is in line with the Mayer et al. 2008 study that demonstrated the effectiveness of cloves to reduce the Nematoda survival rate to 50% compared to the control group due to the increasing number of dead eggs (Mayer et al., 2008). As well as with the study showing the positive effect of clove herb in the treatment of Giardia parasite infection and giving better results than the medical drug currently used (Machado et al., 2010).

The anti-inflammatory effects of eugenol were attributed to its effect to prevent neutrophil/ macrophage chemotaxis and prostaglandin synthesis as well as cyclooxygenase II enzyme expressions.

The results of this study also showed that the average of the leishmania pustule in the groups in which the injection method was used - regardless of the quality of the herb and the amount of the dose

used in the treatment - was greater than the groups in which the fat method was used statistically (3.8589) while in fat groups (3.0982).

- **The percentage of improvement in leishmania pustule was studied in the study groups as shown in the following table:**

Table (11) Improvement rate of leishmania pustule in the study groups

Study groups	First reading	Recent Reading	Total average	Percentage improvement
Control group	4.13	5.15	3.37	0
Pentostam Group	3.29	2.81	2.73	14.9
Carnation group "fat"	2.99	0.84	1.511	72
Clove group injected 5 mg/ kg	3.82	3.98	3.36	0
Clove group injected 10 mg/ kg	4.48	5.04	3.22	0
Clove group injected 40 mg/ kg	4.28	1.22	3.22	72
Clove group injected 60 mg/ kg	3.10	1.50	2.27	52

It is clear from the previous table that the group of infected mice treated with carnations by injection of 40 mg/ kg and also treated with cloves by fat on the blister gave a high rate of improvement to 72% followed by the treatment group Carnation by injecting 60 mg/ kg where the rate of improvement (52%). The improvement in the previous group improved by 14.9%. Thus we conclude that carnivorous herb is the best in the treatment of leishmaniasis.

Conclusion:

The study found that clove is the best in treating cutaneous leishmaniasis followed by black seed and then barberry while Pentostam worked out but with less impact.

Recommendations:

In the light of the results, the researcher recommends the following:

Recommendations for Medicine Manufacturers

- Working on extracting active main active compounds in cloves herb since it is more effective than synthetic compounds as well as more effective than herbs covered by previous studies.

Recommendations for Researchers

- Intensifying studies in this field to hopefully reduce the side effects of Pentostam drug.
- Studying the effect of natural alternatives on farms for the preservation of the parasite to know its direct impact on the parasite away from other influencing factors.

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