Republic of Iraq

Ministry of Higher Education

and Scientific Research

University of Babylon

College of Science

Department of Biology

Exploring the Molecular Effects of *Leishmania tropica* Infections on Interleukins Profile in Culture Cell Lines

A Thesis

Submitted to Council of the College of Science,

University of Babylon in Partial Fulfillment of the Requirements for The Degree of Master of Science in Biology

By

Rasha Riyadh jawad Kadim

(B.Sc. Biology, Babylon University, 2009)

Supervised by

Prof. Dr. Maher Ali Jatan Abbas Al-Quraishi

2023 A.D. 1445 A.H.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل – كلية العلوم

قسم علوم الحياة

التحري عن التأثيرات الجزيئية للأصابة باللشمانيا تروبيكا *Leishmania tropica* على هيئة الانترلوكينات في الخطوط الخلوية

رسالة

مقدمة إلى مجلس كلية العلوم،جامعة بابل

كجزء من متطلبات نيل درجة ماجستير في العلوم \علوم حياة

من قبل

رشا رياض جواد كاظم

(بكلوريوس علوم حياة / 2009)

اشراف

ا.د. ماهر علي جتان عباس القريشي

1445 هـ 2024 م

إِنَّمَآ إِلَٰهُكُمُ ٱللَّهُ ٱلَّذِي لَآ إِلَٰهَ إِلَّا هُوَۚ وَسِعَ كُلَّ شَيۡءٍ عِلۡمًا

صدَقَ الله العَظِيم

سورة طه - الاية 98

Supervisor Certification

I certify that this thesis (Exploring the Molecular Effects of *Leishmania tropica* Infections on Interleukins Profile in Culture Cell Lines) was prepared under my supervision at the Department of Biology, College of Science, University of Babylon in partial fulfillment of the requirement for The Degree of Master of Science in Biology by.

"Rasha Riyadh Jawad ".

Supervisor

Prof. Dr. Maher Ali Al-Quraishi

College of Science

University of Babylon Professor

In view of the available recommendation, I forward this thesis for debate by the examining committee .

Assist. Prof. Dr. Adi Jassim Abd Al-Razzak

Head of the Department of Biology

College of Science

University of Babylon

Dedication

To the one who has mercy and contentment in a Paradise as wide as the heavens and the earth, prepared for the righteous ------**my father**,

and to the Paradise under her feet and whose supplications are the secret of my happiness ------- **my mother**

To my life partner and companion, you were and still are, God willing, my support in my time of distress ------ **my husband**

To the radiance of hope, the secret of challenge, and my soul mate ----- **my children**

My apologies to you, my dears, if I have been somewhat negligent towards you. To everyone who provided me with help or advice to complete my academic journey. To all of them, I dedicate the fruit of my humble effort.

Rasha -2023

Acknowledgments

First, I would like to express my gratitude to Allah (God) for providing me the blessings to complete this work. My gratitude to the department of Biology, College of Science, and University of Babylon for providing me with this opportunity to complete the M.Sc. study. I would like to express my thanks and gratitude to my supervisor professor **Prof. Dr. Maher Ali Al-Quraishi** for his guidance, encouragement and great advice throughout the work of this research. whose expertise was invaluable in formulating the research questions

and I also extend my thanks to my **Prof. Dr. Alaa Tariq Al-Hassnawi**, who provided me with his help and valuable advice during the writing of this thesis. Your insightful feedback has greatly contributed to sharpening my thinking and taking my work to a higher level.

Special thanks to **Prof. Dr. Rana Al-Ghalib and Prof. Dr. Qaisar Naama** for helping me with the cellular aspect of my research work while I was in the Tissue Culture Laboratory - College of Medicine / University of Babylon

Special thanks to A. **Ali Nayef,** College of Science, University of Mosul, and my friend **Zahraa Mohammed** for all the help you provided me to complete this dissertation

Finally, my thanks go to all the people who have supported me to complete the research work directly or indirectly.

Summary

During the period from October 2022 to June 2023, the parasite and its growth are examined, and some experimental items are conducted in the Advanced Parasitology Laboratory of the College of Science and the Tissue Culture Laboratory at the College of Medicine / University of Babylon.

Leishmaniasis is a disease caused by protozoan parasites. These parasites are transmitted to humans through the bites of infected female phlebotomine sandflies. The parasite can stimulate the immune system to produce inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-12 (IL-12), and interferon-gamma (IFN-γ). These cytokines play a role in activating immune cells and promoting local inflammation. Additionally, *Leishmania* can also stimulate the production of anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β), which regulate the immune response and help reduce inflammation. The cytokine response during *Leishmania* infection can vary depending on the *Leishmania* species and the host's immune response.

In this study, sample are taken from patient with suspected skin infections of *Leishmaniasi*s who visited the dermatology outpatient clinic in the Sinjar region, Mosul Governorate. The parasite is grown in NNN culture medium and RBMI 1640 medium. After that, *Leishmaniasis* were identified, by sequencing and genotyping. This study, amplified one specific PCR fragment using a partially covering the IS1, 5.8S rRNA, and ITS rRNA in *L. tropica*. The amplified PCR fragments are directly exposed to Sanger sequencing experiments to assess the pattern of genetic polymorphism in the *L.tropica* samples. A specific comprehensive tree is built to assess the accurate genotyping of the observed variants and their phylogenetic

distribution .the presence of one nucleic acid variant (94-95ACdel) in the investigated sample. It is inferred from the tree that our investigated sample is positioned in the same phylogenetic position within the *Leishmania tropica* sequences with a noticeable tilt from the nearest strains of FN677341.1 and OL413428.1 that are isolated from Palestinian and Malaysian sources, respectively.

The parasite is then exposed to concentrations of sodium stibogluconate (Pentostam) 2000, 1500, 1000, 500, 250, and 62.5 µM to determine its toxicity. Both the U937 subunit line and primary human monocyte-derived macrophages (MDMs) are infected with the parasite with various concentrations of the standard treatment for *Leishmania* and then cell viability and toxicity are considered. the levels of interleukins (TNF-α, IL-10, IL-B1, and TGF-B1) as well as the level of (MDA, ROS, GPX, TAC) are measured in U937-derived macrophages and monocyte-derived macrophages (MDMs).

The study also included an analysis of the effect of sodium stibogluconate on the secretion of cytokines from immune cells. The results showed that sodium stibogluconate is directly proportional to the secretion of important cytokines such as TNF and inversely proportional to IL-10, IL-B1, and TGF-B1. Overall, the study suggests that sodium stibogluconate has a toxic effect on cutaneous leishmaniasis and suppresses the immune system response.

The results of this study showed that a decrease in the concentration of GPX and TAC was observed in normal cells as well as cancer cells, and this decrease is due to the production of more reactive oxygen species by phagocytes. The observed inverse relationship between TAC levels, ROS, MDA, and GPx activity in *Leishmania*-infected macrophages suggests a disruption of the normal antioxidant defense mechanisms. The parasite's

ability to modulate the host immune response and interfere with the antioxidant system contributes to oxidative stress, lipid peroxidation, and a decrease in the overall antioxidant capacity. These imbalances can have implications for the pathogenesis of cutaneous leishmaniasis and may affect the outcome of the disease. *Leishmania* infection may cause changes in the immune response and activation of anti-tumor immune cells.

The use of normal macrophage and malignant cells helps in our understanding of different immune responses and provides researchers with options for combating *Leishmania*. Normal cells may have a higher inherent resistance or lower susceptibility to the effects of the antiparasitic treatment compared to cancer cells Incorporating these findings into the development of new treatments for *Leishmania* infection has the potential to enhance therapeutic outcomes, reduce drug resistance, and improve patient care in combating this parasitic disease

List of contents

List of Tables

List of Figuers

List of Abbreviations

Abbreviate	Title
µmol	Micromole
bp	Base pair
CL	Cutaneous Leishmaniasis
DGM	density gradient media
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DTNB	**(**dithiops (**2-nitrobenzoic acid))**
ELISA	Enzyme Linked Immunosorbent Assay
et al	And other
FBS	Fetal Bovine Serum
GPX	glutathione peroxidase
HIV	Human immune deficiency virus
HRP	Horseradish Peroxidase
IFN-γ	Interferon- Gamma
IL	Interleukin
IL-10	Interleukin-10
iTOL	Interactive Tree of Life
K DNA	Kinetoplast DNA
L.V.	Leishmania Viannia

MCL	Mucocutaneous Leishmaniasis
MDA	Malondialdehyde
Mg	Milligram
mM	Millimolar
MNC	mononuclear cells
MPA	phorbol 12-myristate 13-acetate
MTT	"3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCBI	National Center for Biotechnology Information
NK	Natural Killer cell
Nm	Nano Meter
NNN	Novy-McNeal-Nicolle
NO	Nitric Oxide
OD	Optical Density
PBMCs	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PGE	prostaglandin E
PKDL	Post Kala-azar Dermal Leishmaniasis
qPCR	quantitative Polymerase Chain Reaction
RBMI 1640	Roswell Park Memorial Institute
ROS	reactive oxygen species
TAC	Total Antioxidants Capacity

TGF-B1	Transforming Growth Factor – β1
Th1	T helper 1 cell
Th2	T helper 2 Cell
TNFα	Tumor Necrosis Factor
VL	Visceral Leishmaniasis
WHO	World Health Organization
µl	Micro Liter
µM	Micromolar

Chapter One

Introduction

1 . Introduction

Leishmaniasis is a vector-borne disease transmitted by the bite of the female sand fly caused by different species of *Leishmania*. It manifested in three major clinical forms: cutaneous, mucocutaneous, and visceral Leishmaniasis (Reithinger & Dujardin, 2007) Leishmaniasis is a group of neglected tropical diseases caused by infection with the *Leishmania* parasite genus (Armitage *et al*., 2018)

Leishmania is an obligatory intracellular parasite that exclusively infects the macrophage cells of their mammalian hosts (Zeng *et al*., 2016) All *Leishmania* parasites have two main life stages and require two hosts to complete their life cycle, the first of which is the invertebrate host (sand flies), known as the promastigote stage.

The second is the amastigote stage of the vertebrate host (human), which invades the vertebrate host's macrophages (Abdulla *et al*., 2018) is seen in humid seasons such as spring, autumn, and winter, where increased wetness may provide a suitable environment for microbial growth and reproduction. However, the amount of moisture varies by season and geographical location (Afshar *et al*., 2022).

Prevalent in tropical and subtropical regions around (12) million individuals are infected in 98 countries by this tropical and subtropical disease, with (350) million more at risk of infection globally (Ali, 2021) . *Leishmania* can be eliminated by effective immunity after infection, or the parasite can survive by using several evasion methods (De Pascali *et al*., 2022) .

A common factor that may encourage the return of clinically apparent leishmaniasis in people with compromised cell-mediated immune responses

is that the majority of *Leishmania* infections are asymptomatic (De Pascali *et al*., 2022).

Further more, there are few and a variety of definitions in clinical and laboratory studies addressing asymptomatic people. The difficulty of detecting people who momentarily and covertly contain the parasite is one commonly cited drawback of these investigations. the identification of anti-*Leishmania* antibodies, a less precise indicator of infection or ongoing disease, and an intradermal skin test, which reveals the cellular immune response connected to prior exposure to *Leishmania*, are the two most often employed tools (de Gouvêa Viana *et al*., 2008).

Leishmania is mostly found in macrophage-like cells. As a result, the mechanism involved in their macrophage-to-macrophage transfer in cutaneous or visceral lesions is an important area of research. However, the steps of the intracellular lifecycle in mammalian hosts require the obligatory egress of *Leishmania* amastigote forms from host cells for the parasite to spread to new host cells and other tissues (Real *et al*., 2014) .

As a result, focusing on immunological components is an effective way to fight the illness. Additionally, *leishmania* infection-specific host innate immune markers may aid in early illness outcome prediction. These comprise components of the innate immune system, such as the natural killer (NK) cells, and mononuclear and polymorphonuclear phagocytes, which serve as the first line of defense (Hawn *et al*., 2002) In general, the *Leishmania* parasite resists their uptake by phagocytic dendritic cells (DCs) Macrophag cells (Dayakar *et al*., 2019) by inhibiting reactive oxygen species (ROS) production that delays phagolysosome formation (Gueirard *et al*., 2008).

these infections induced both cellular and humoral immune responses and the balance of expression varies with the type of disease.

In cutaneous leishmaniasis, the immune response can be divided into two phases: the early Th1 response and the later regulatory response. Initially, during the Th1 response, there is an upregulation of pro-inflammatory cytokines like TNF-α, interferon-gamma (IFN-γ), and interleukin-12 (IL-12). These cytokines activate macrophages, which are important immune cells in controlling *Leishmania* infection. The activated macrophages produce reactive oxygen species and other molecules that kill the parasites (Al-Bajalan *et al.*, 2021).

However, as the infection progresses, there is a shift towards a regulatory response characterized by the production of IL-10. The production of IL-10 helps to downregulate the inflammatory response, preventing excessive tissue damage caused by prolonged inflammation. IL-10 acts as a negative regulator of the immune response, inhibiting the production of pro-inflammatory cytokines like TNF-α and IFN-γ (Saha & Souravi, 2021).

Cytokines and chemokines play a role in immunity to a host of infectious diseases, including *Leishmania* infections. According to Guha et al. (2013), some of these secreted protein immune modulators are involved in the activation and differentiation of immune cells important in parasite clearance, such as IL-12, TNF-α, and IFN-γ. On the other hand, as mentioned in studies by Mattner *et al*. (1996) and Swihart *et al.* (1995), others could either dampen the immune response against *Leishmania* or activate and differentiate immune cells that will ultimately favor the persistence of the parasite, such as IL-4, IL-10, IL-13, and TGF-β.

Proinflammatory cytokines are largely produced to boost the body's defenses against *Leishmania* infection. TNF-, IFN-, IL-1, IL-2, IL-8, IL-12, IL-15, IL-18, and IL-17 are the main proinflammatory cytokines, whereas anti-inflammatory cytokines are immunoregulatory molecules that work to

counteract the effects of proinflammatory cytokines to prevent inflammation from being brought on by excessive production of proinflammatory cytokines. The main anti-inflammatory cytokines are TGF-, IL-5, IL-6, IL-4, IL-10, and IL-13 (Andargie & Diro Ejara, 2016).

In vitro infection research is critical for evaluatin specific elements of *Leishmania* biology, as well as for more meaningful in vitro screenin of interestin chemical entities. Macrophage-like cell lines of various origins are susceptible to *Leishmania* infection. Cell lines are highly recognized for their ability to provide repeatable infections and reliable data due to their stability and standardization capability (Santarém et al.,2019).

The induction of oxidative stress during parasitic infections triggers the activation of the host's antioxidant response to counteract the damaging effects of the oxidative burst. Macrophages play a crucial role in disease progression or control because the ultimate outcome depends on the interactions between the infecting *Leishmania* species and the type and strength of the host immune response (Reverte *et al*., 2022).

Effective medications are few, expensive, and challenging to use, and they all have negative side effects. Glucantime and Pentostam, two pentavalent antimonials, are frequently used as medicinal drugs, yet both seem to have comparable toxicity and efficacy. In some cases, the condition is resistant to antibiotics, necessitating the use of pentamidine and amphotericin B as substitute medications. Both have significant toxicity issues, with the pentamidine causing diabetes and kidney damag (Naman, 2018) .

There is no ideal vaccination or acceptable medicine to entirely eradicate leishmaniasis. Until yet, no vaccine or medication has been developed to give long-term protection and effective immunity against leishmaniasis as a result,

important research is needed in the areas of immunity, medicines, and vaccines to achieve specific results (Ghorbani and Farhoudi, 2017).

1.1 Aim of Study

Investigation of some interleukins, oxidants, and anti-oxidants. In cell lines infected with the *Leishmania* parasite. by the following objectives: -

1- Confirm the identity of *Leishmania* ssp using conventional PCR.
2- Assess the exted of *Leishmania* genetic variation and evaluate the role of these variation in altering the phylogenetic position of the parasite compared with the other relative species.
3- Peripheral blood monocytes be isolated from a blood sample and differentiated into macrophages using PMA (Phorbol 12-Myristate 13-Acetate).
4- U937 cancer cell line Differentiation of mononuclear cells into macrophages using (PMA).
5- ELISA technique was use to look at interleukins such IL-10, IL-B1, TGF-B1, and TNF-α in a cell line infected with the *Leishmania* parasite.
6- Determine the impact of the parasite infection on the amount of oxidative damage. Malondialdehyde (MDA), reactive oxygen species (ROS) and levels of antioxidants Tests for total antioxidant capacity (TAC) and glutathione peroxidase (GPx).

1.2. Ethical approval

The Biology Department of the College of Sciences of the University of Babylon gave its approval to the experimental protocol. The study protocol the subject information and the consent form were reviewed and approved by a local ethics committee according to the document number z221201 (6/12/2022).

Chapter two

Literatures Review

2 Literatures Review

2.1 History of *Leishmania*

The doctors Leishman and Donovan initially identified the visceral leishmaniasis pathogen in 1903; they subsequently named it Leishmania donovani. It is first discovered in spleen by stained smears of infected patients who had signs of a condition comparable to malaria (Sherif El Shanat, 2015)

During the Paleogene or Paleocene era, after the extinction of dinosaurs and the emergence of the first placental mammals, it is postulated that the genus *Leishmania* originated. It is possible that early mammals or reptiles served as the parasite's sole hosts. At that time, the ancestor of *Leishmania* split into Sauroleishmania, which affected reptiles like lizards, and the modern *Leishmania*, which affects mammals. These creatures are currently the actual and permanent hosts of *Leishmania* (Tuon *et al*., 2008)

The earliest known reports of leishmaniasis date to 2,500 B.C., and current DNA research usin ancient archeological material has revealed multiple early descriptions of the disease (Akhoundi *et al*., 2016).

The development of *Leishmania*-like organisms in earlier periods has been supported by the discovery of two types of remains. The first evidence comes from a fossil found in Burmese Cretaceous amber dating back 100 million years ago. Within the proboscis and blood-filled gut of a female *Palaeomyia* burmitis, a *Leishmania*-like organism was identified. This organism was named P. proterus and classified under the newly established historical genus Paleoleishmania.

Alon with promastigotes and amastigotes suggested that the parasite was acquired by the sand fly from the blood of a vertebrate while feeding. The

presence of amastigotes suggests that *P. proterus* has a digenetic life cycle. Subsequent analysis revealed that the blood cells found in the first *Leishmania*-like fossil were actually reptile blood cells. The second *Leishmania*-like fossil, named Paleoleishmania neotropicum, was discovered in a 20-30-million-year-old fossilized sand fly called Lutzomyia adiketis, preserved in Dominican amber. Within the gut and proboscis of this sand fly, promastigotes, paramastigotes, and amastigotes were detected. However, no vertebrate blood cells were found. Despite the absence of monogenetic flagellates in sand flies, the presence of amastigotes suggests that P. neotropicum has a digenetic life cycle involving a vertebrate host, as described by Hassan et al. in 2021.

Cutaneouse leishmaniasis (CL) is a developin community health concern in various countriese, includin Iraq (Al-hucheimi *et al*., 2009) There are very few accounts left that describe leishmaniasis' presence in the early history of humanity. The 7th-century Assyrian Kin Ashurbanipal's library has descriptions of lesions that resemble Oriental sores on tablets (Steverding, 2017)

The primary chroniclers of CL durin the Middle Ages are Arabic scientists. Ab Bakr Muhammad ibn Zakariyya al-Rz, a Persian polymath who lived from 854 to 935, wrote on cutaneous sores in the Baghdad region in 930 (Edrissian *et al*., 2016)

The incidence of (CL) may be increasin in locations hit by a refugee crisis, such as Sanliurfa; as a result, local rare species of *Leishmania* be encountered in these areas. This could make diagnosis and treatment more difficult in the future (Yentur Doni *et al.*, 2020)

CL caused by the *L. tropica* complexe is found in many parts of Asia, Africa, Mediterranean Europe, and the former Soviet Union's southern region (Ibrahim Al-Jubori *et al.*, 2019)

CL is also known as Oriental sore, Aleppo boils, Jeriho boils, Baghdad boile, Balkh soree, Penjdeh sore, Briska button (clou de Briska), Bouton de Crete, and Bouton D 'Orient in the Old World. The disease is known as Uta, Espundia, Chiclero'e ulcer, Pain bois, and forest yaws 2 in the New World (Steverding, 2017)

the middle of the 19th century. The causal agent of CL was found in 1885 by David Cunningham and later by the Russian surgeon P. F. Borovsky (1863-1932), who published it in an area military magazine in 1898. James Homer Wright, an American pathologist (1869–1928), is credited with discoverin it. William Leishman (1865-1926) and Charles Donovan (1863-1951), two Scottish doctors, published a paper in 1903. Professor of Physiology at Madras University in India separately identified the causal agent of human visceral leishmaniasis *(L. donovani*) in the spleens of Kala-azar patients. The Sandflies are discovered in 1921, and the real route of disease transmission is discovered in 1941 (Azizi *et al*., 2016)

2.2 Leishmanaisis

In the Americas, Asia, and Africa, leishmaniasis is regarded as a severe public health tissue. There are three main types: cutaneous leishmaniasis (CL), mucocutanous leishmaniasis (MCL), viscerale leishmaniasis (VL). More than 20 different species of Leishmaniaecause these diseases, which are spread through insect bites. *Leishmania* is parasitic obligate intracellular protozoans that infect mononuclear phagocytes, caused by infected female Phlebotomine sand fly (Alemayehu & Alemayehu, 2017)

2.2.1 Cutaneouse Leishmaniasis (CL)

The most prevalent type of illness is skinned leishmaniasis (Gurel *et al*., 2020) World wide, it is estimated that 2 million individuals are infected with the parasite, with up to 1 millione new cases reported each year and more than 1 billion people risk of infection (Ferreira *et al.*, 2023) Cutaneous leishmaniasis resolves spontaneously within between one month and six years (Mokni, 2019)

The tropics and neotropics are endemic for cutaneous leishmaniasis. Because of the wide variety of clinical symptoms, which vary from tiny cutaneous nodules to severe mucosal tissue damage, it is sometimes referred to as a group of disorders. CL is caused by numerous *Leishmania* species and is spread to humans and animals by sandflies (Reithinger *et al*., 2007)

In the old world, the causative agents of CL are *L. aethiopica, L. major*, and *L. tropica*, but in the new world, the causative agents are *L. mexicana*, *L. amazonensis*, *L. guyanensis*, *L. panamensis,* and *L. braziliensis*. There are two forms of CL:localized cutaneous leishmaniasis (LCL) and diffuse cutaneous leishmaniasis DCL (Bayram, 2017) DCL is more frequent in immunocompromised patients (Sabzevari *et al*., 2020).

Patients with *Leishmania* braziliensis infection develop chronic lesions that frequently do not respond to antiparasite medication treatment. (Amorim *et al*., 2019)

2.2.2 Mucocutaneous Leishmaniasis (MCL)

Because of its low frequency worldwide, MCL has beeneless frequently suspected and as a result, lower frequency of reports. MCL is caused by the lymphatic/hematogenous diffusion of parasites from the skineand affects the mucous membranes of the mouth, nose, lips, throat, and larynx. MCL often

appears days to years after the previous CL, however, it can also appear alongside other skin lesions.(Sabzevari *et al.*, 2020)

Mucosal involvemente may be detected in up to 20% of patients in endemic locations. 22 The most common cause of MCL is *L. braziliensis*, however other species involved include *L. amazonensis*, *L. guyanensis*, and *L panamensis* (Abadías-Granado *et al.*, 2021)

Dependin on the patients' immunological condition, CL and MCL have different clinical symptoms. Comparatively to immunocompetent patients, immunosuppressed individuals frequently have many skin lesions with torpid development, a higher recurrence rate, and a more challengin course of therapy. (Banuls *et al.*, 2011)

2.2.3 Visceral leishmaniasis

Visceral leishmaniasis (VL) is a potentially lethal vector-borne illness that ranks second and seventh in terms of fatality amon tropical diseases (Scarpini *et al*., 2022) Around 13,000 cases of VL is reported by the World Health Organization (WHO) in 2020 (Ruiz-Postigo *et al*.,2021)

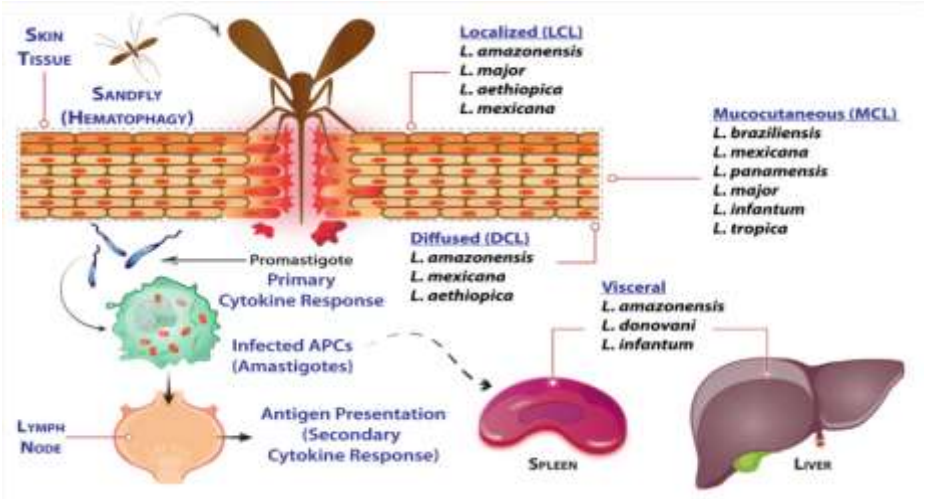
VL is also known by the local name’s kala-azar and black fever, the latter of which is related to the incidence of melanocyte activation and skin darkenin associated with infection, also known as infantile VL. The parasites attack the liver, spleen, bone marrow, and lymphatic organs, causin splenomegaly, Dumdum fever, and Assam fever (Lidani *et al.*, 2017)

Fever, hepatosplenomegaly, hypergammaglobulinemia, Weight loss, cachexia, anemia, and leucopenia are some of the clinical signs of VL infection Malnutrition symptoms associated with VL infections include hypoalbuminemia. Moreover, diarrhea may develop, and liver functions may

be damaged, resultin in a drop in pro-thrombin and leadin to the late stage of illness, which is mucosal bleedin and sepsis (Abdulla *et al.*, 2018).

It may also occur after recoverin from a tropical skin illness known as Leishmaniasis post-Kala-Azareg dermal leishmaniasis (PKDL) The PKDL is in the latter stages of parasite infectioneg (Mishra *et al.*, 2013)

In India, around 5-20% of VL patients acquire PKDL followin effective treatment, while somee PKDL cases are documented without any VL episode or treatment history (Singh *et al.*, 2011)



Figuer (2.1) highlights the *Leishmania* species specific tissue tropism for developmenteg of different forms of Leishmaniasise disease(Singh *et al.*, 2011)

2.3 Classification of leishmaniasis

Leishmania parasite categorization is a difficult task. Nevertheless, different classifications are utilized for the genus *Leishmania* since the proposed classifications, which are Monothetic Linnean Classifications, were made between 1916 and 1987 AD. and the Viannia , which is unique to the New World (Doherty, 2002)**.**

Leishmania species are divided into "old world" and "new world" categories dependin on their geographic distribution. *L. major, L. tropica*,

and *L.aethiopica* are regarded to be "old world," while *L.mexicana*, *L. amazonensis*, and *L. guyanensis* strains are known as "new world" strains (Alvar *et al.*, 2012).

Initially, the classification of *leishmania* is based on the clinical signs and symptoms it produced (Table 2). *L. tropica* was thought to be the parasite responsible for cutaneous leishmaniasis over the globe, and the cause of visceral leishmaniasis is *L. donovani*. The initial step towards avoidin this Nicolle (1908) took the stance that the clinical and epidemiological characteristics of Mediterranean visceral leishmaniasis are distinct from those of that of India, referrin to it as *L. infantum*. It has been established that *L. infantum*, which is common in the Mediterranean region, also produces atypical cutaneous leishmaniasis in addition to visceral leishmaniasis. Domestic dogs and wild canids are this organism's natural hosts.

The parasite that caused a case of disseminated leishmaniasis from Brazil was given the name *L. braziliensis* three years later, in 1911, by Vianna. In light of this, Yakimoffeg and Schokhore (1914) hypothesized that the parasites causin urban and rural cutaneous leishmaniasis in Asia were distinct types of *L. tropica*, designatin the urban form as var. minor and the rural form as var. major.(Espinosa *et al.*, 2018).

Over 20 *Leishmania* parasite strains are known to produce various forms of leishmaniasis and constitute an endemic threat to people everywhere (Rabaan *et al*., 2023)**.**

One such instance is *L. major*, which is originally thought to be a subspecies of *L. tropica* major based on morphology and about the similarly now-abandoned subspecies *L. tropica* minor, which corresponded to smaller forms and is now the species *L. tropica*. *L. infantum*, which is thought to be

more common in newborns, is named and described based on epidemiological data, whereas species like *L. guyanensis*, *L. mexicana*, *L. braziliensis*, etc. are named and defined based on their geographic distribution or the area in which they are first reported (Hassan *et al*., 2021)

Table (2.1) Classification of *Leishmania* parasites According to the World Health Organization (WHO, 1990)

Kingdom	**protista**
Subkingdom	**Protozoa**
Phylum	**Sarcomastigophora**
Subphylum	**Mastigophora**
Class	**Zoomastigophora**
Order	**Kinetoplastorida**
Suborder	**Trypanosomatina**
Family	**Trypanosomatidae**
Genus	***Leishmania***

Leishmania's initial designations of species are based on temporary principles that combined ecological, morphological, phenetic, and clinical

ideas. Many species definitions and names so take inspiration from this tradition (Sunter & Gull, 2017)

2.4 Morphological Forms of The Genus *Leishmania*:

The *Leishmania* parasites cellular morphology is very accurately defined by factors such as cell shape, flagellum length, location of the kinetoplast and nucleus, and ultrastructurale characteristics. has lon been used to describe observable cell types (Sunter & Gull , 2017)

However, in some instances, these morphologicale descriptions of cell shapes have been used to define particular cell types at different stages of the life cycle in the literature. few molecular markers are now available to help define life cycle forms more precisely, therefore attention and caution are required when classify in cell types simply based on cell shape (Doyle, 2022)

Leishmania has two distinct cell morphologies, typified by the promastigote morphology in the sand fly and the amastigote shape in the mammalian host the core cellular architecture, however, is retained between the two *Leishmania* cell forms and is determined by cross-linked sub-pellicular corset microtubules. Because this array is maintained throughout the cell cycle, cell division is dependent on the insertion and elongation of microtubules into the existin array. The nucleus and a collection of single-copy organelles, such as the mitochondrion and the Golgi apparatus, are housed within the cell. The kinetoplast, a mass of concatenated mitochondrial DNA that is directly related to the basal body from which the flagellum extends, is located anterior to the nucleus. The flagellum's base is (Sunter & Gull, 2017)

Some parasites undergo two different environments during their life cycle and must quickly adapt to each. They exist as extracellular promastigotes in

a sugar-rich and slightly alkaline environment in the fly's digestive system, which is characterized by a moderate temperature of around 26°C. On the other hand, intracellular amastigotes are found inside cells in higher temperatures in the skin and internal organs. Amastigotes encounter a sugar-poor, fatty acid- and amino acid-rich acidic environment within the phagolysosome, which represents their habitat.(Tsigankov *et al*., 2014)

a. Amastigote phase

Microscopically detectin amastigotes is the gold standard for leishmaniasis diagnosis (Roy *et al*., 2022)

The amastigote is a small, round oval body that measures around 2-5m and is identified only in the macrophages of affected vertebrate hosts. The double membrane of the amastigotes is supported by a layer of subpellicular fibrils. From the area of the flagellar base to the posterior apical end, they travel in a spiral motion. Durin the amastigotes' metamorphosis, one of the membranes is removed; the fibrils are kept nevertheless.

Although amastigotes lack a flagellum, one can occasionally be spotted emergin from the kinetosome. This is located outside of the flagellar pocket and has a 9x2 fibril shape. Amastigote mitochondria are uncommon. The only mitochondria in the organism are found in the kinetoplast (Ul Bari & Rahman, 2008)

b. Promastigote phase

The flagella form occurs in cells prepared from the invertebrate host in a circular shape or it may take a different shape dependin on the stage of the parasite life cycle. a round or oval nucleus with dimensions of (2–6) microns in length and (1-3) microns in breadth. The kinetoplast is the focus of this phase, and the cytoplasm houses state bodies and vacuoles as well as a

patch-like Blepharoblast from which a fine axial thread emerges. The cell membrane includes polysaccharides and is not encased in an envelope (Kadhim & Al-Quraishi, 2020)

The kinetoplast can be found in all protozoa of the kinetoplastid ae group (eg. *Leishmania, Trypanosoma, Crithidia*). The kinetoplast-DNA (kDNA) is a rod-shaped mitochondrial structure composed of a DNA network of around 10,000 minicircles and approximately 50 maxicircles, from which a tiny axoneme emerges.

kinetoplas is next to the Para basal body. Until recently, the function of the kinetoplast is unknown. Maxicircles are discovered to encode mitochondrial ribosomal RNAs. The minicircles are involved in the editin of these mRNA (Ali, 2019)

2.5 The life cycle of *leishmania*

A family known as that of the leishmaniases caused by parasitic protozoa from more than 20 different species of *Leishmania*. (Figure 2.2) The female phlebotomine sandfly, a tiny (only 2-3 mm long) insect vector that bites humans and spreads these parasites (WHO; 2021) the afflicted Phlebotomine sandflies *(Phlebotomus* spp., *Lutzomyia* spp.) and antigen-presentin cells (APCs), such as macrophages, are the hosts for the *Leishmania* spp. They are found in the sandflies as proliferating, elongated "promastigotes" with flagella. They are phagocytosed by neutrophil, dendritic, and macrophage cells once they have entered a mammal. They undergo a reversible stage change into the ovoid, flagellated "amastigotes," which proliferate within the host cells at moderate to minimum growth rates, in response to the acidic environment inside phagosomes and the temperature of mammalian tissue *(*Grünebast *et al.*, 2021)

Leishmania spp. have a polymorphic life cycle involvin Two types of hosts: - insects and vertebrate hosts (primarily mammals) The infected phlebotomine female sand fly finds a suitable host and injects the promastigote parasites into the skin durin a blood meal. The promastigotes are elongated, flagellated and infective parasites that grow in the midgut of the female sandfly When these promastigotes parasites reach the wound, they are phagocytized or ingested by macrophage cells. In macrophage cells, these promastigotes transform into amastigotes, which is the tissue stage of the parasite. Amastigotes parasites multiply by the process of simple division. After multiplyin and formin a larger group, they infect other phagocytic cells (Ticha *et al.*, 2022)**.**

The contact of metacyclic promastigotes with cutaneous macrophages starts the developmental cycle. Metacyclic promastigotes are ingested and internalized in a phagosome, whereupon normal lysosomal fusion takes place and the parasite occupies a secondary lysosome or phagolysosome (Singh, 2006).

Durin 12 to 24 hours of this process, the metacyclic promastigote changes into an amastigote and continues to develop and split inside the phagolysosomal compartment. These parasites often infect humans at a certain life cycle stage, livin in human blood or tissues. Kinetoplastida is known to have lon and thin morphological forms at various stages of their life cycles, as well as aflagellate forms linked with their infectivity in humans. Kinetoplastid intracellular parasites' cell cycles take many morphological forms, and cell division in these parasites is linked to disease manifestation (Bhattacharjee & Biswas, 2023).Infections can be started by both the promastigote and amastigote types of *Leishmania*. When infections begin with promastigotes, these parasites change into amastigotes within parasitophorous vacuoles (PVs) over a 24-72-hour period. Infections are

then perpetuated by amastigote forms in infected cells and hosts. Amastigotes can live in cells for several days, if not weeks. The host cell reaction differs dependin on whether the promastigote or amastigote form is used (Kima, 2007).

Such variations in host response are to be expected because various parasite forms express stage-specific chemicals that can act as virulence factors. The lipophosphoglycan (LPG) molecule, expressed on the promastigote form's surface but is expressed on the surface of the promastigote form but is minimally in the amastigote form, is an example of a stage-specific expressed on the promastigote form's surface-specific virulence factor (Ilgoutz & McConville, 2001)

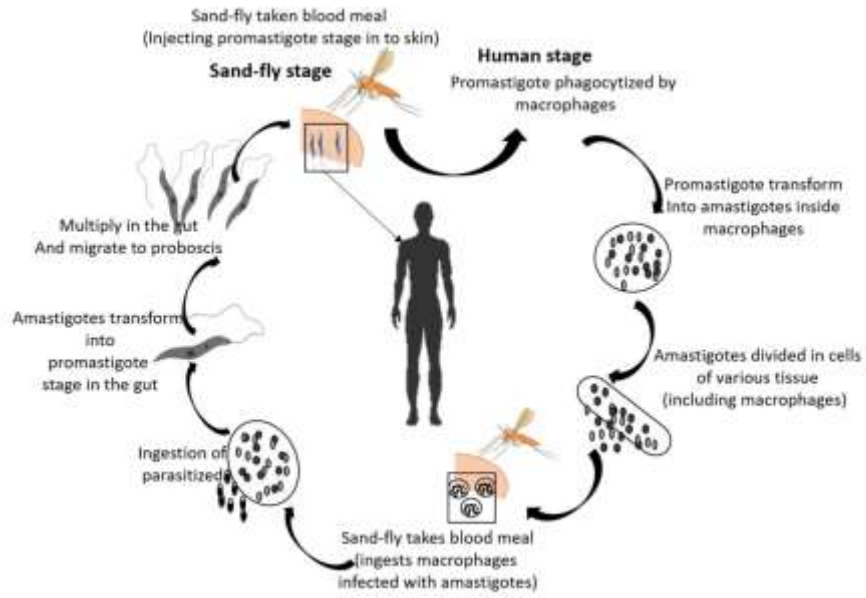


Figure (2.2) shows how *Leishmania* is transmitted through the bite of a female sandfly. (1) Sandflies infuse the transferrable stage, promastigotes, through blood feeds. (2) Promastigotes are phagocytized by macrophages when they reach the wound and become amastigotes. (3) Amastigotes divide into infected cells and cause havoc in a variety of tissues. (4) Clinical *Leishmania* signs begin when the amastigote is consumed by the sandfly. (5) The transmission cycle is completed when an amastigote in the sandfly gut changes to a promastigote. (Rabaan *et al*., 2023)

Within the sand flies gut, amastigotes convert into infective metacyclic promastigotes, which are regurgitated and injected again into the mammalian skin to continue the life cycle (Yasmin *et al*., 2022) .

2.6 Epidemiology

2.6.1 Epidemiology of Leishmaniosis in the world

Leishmaniasis is a neglected tropical illness that is a major source of morbidity and mortality in disadvantaged nations. It is a worldwide illness with clinical and epidemiological diversity, with many distinct species of *Leishmania* impactin humans (Mann *et al*., 2021)

Through migration, tourism, and military activity, leishmaniasis has spread to formerly leishmaniasis-free areas and new epidemics are developin in endemic regions. The majority of leishmaniasis cases occur in isolated rural areas with subpar housin and little to no access to cutting-edge medical facilities. Leishmaniasis is a disease of the poor. Every diagnosis of leishmaniasis in endemic locations places a tremendous financial load on already scarce financial resources at both the individual and community levels. 90% of newly diagnosed cases of visceral leishmaniasis are observed in India, Bangladesh, Nepal, Ethiopia, Sudan, and Brazil. Accordin to estimates, there are between 200,000 and 400,000 cases of visceral leishmaniasis each year around the world. Visceral leishmaniasis (VL) has two major infectious agents*: Leishmania (L) L. donovani and L. infantum* are dangerous to one's health (World, 2010).

In various regions of the world, parasites from the *L. donovani* complex are the cause of (VL), commonly known as "kala azar". Asia, North Africa, Latin America, and Southern Europe are all home to the *L.donovani* complex, which mostly affects unprotected and vulnerable populations. In its

most severe form, VL, untreated, nearly usually results in death. It is characterized by an undulatin fever, weight loss, anemia, lymphadenopathies, and/or splenomegaly. The other VL-causin agent, *L. infantum*, is present in Southern Europe, North Africa, West Asia, and Central Asia. Another clinical manifestation of kala azar is post-kala azar dermal leishmaniasis (PKDL), which is present in all places where *L. donovani* is prevalent. With incidence rates of 50 and 10 percent, respectively, it makes specific mention of East Africa and the Indian subcontinent (Inceboz, 2019)

Cutaneous leishmaniasis: Brazil and Peru account for roughly 90% of all cases in Afghanistan, Pakistan, Sudan, Syria, Saudi Arabia, Algeria, Iran, and Iraq. The yearly number of cases of visceral leishmaniasis in the world. is estimated to be between 700,000 and 1.2 million *L. major, L. infantum*, and *L. tropic*a are Old World species*; L. amazonensis, L. chagasi, L. mexicana, L. viannia* (V*), L. braziliensis*, and L. *guyanensis* are New World species. Cutaneous antroponotic *leishmaniasis* (Inceboz, 2019)

The number of cases in Eastern Africa climbed from 2015 to 2016, whereas the Indian subcontinent decreased durin the same two-year period, while VL cases in Brazil stayed consistent in 2015 and 2016. The Eastern Mediterranean and European regions are also thought to be endemic. Even though the fact that reported cases are lower in comparison to South-East Asia and Africa. In contrast to other exotic vector-borne illnesses, such as Dengue and Chikungunya, which produced health catastrophes in Europe in the previous decade and so piqued the scientific community's interest, VL has been over looked (Berriatua *et al* .,2021).

It can affect persons of all ages, however in endemic places, the frequency is greater amon children because to adults' established immunity. Children

contribute for 60%-70% of the entire illness burden in CL-endemic areas. The greater prevalence might be attributable to children bein exposed to the parasite at a youn age when they lack CL-specific immunity (Rather *et al*., 2021)

In 2019, more than 87% of skin cases occurred in Afghanistan, Algeria, Brazil, Colombia, Iran, Iraq, Libya, Pakistan, Syrian Arab Republic, and Tunisia. More than 90% of mucocutaneous leishmaniasis cases occurred in Bolivia, Brazil, Ethiopia, and Peru, while Brazil, Ethiopia, Eritrea, India, Iraq, Kenya, Nepal, Somalia, South Sudan, and Sudan concentrated more than 90% of VL cases (Raimundo ,2022) as shown in Figure (3).

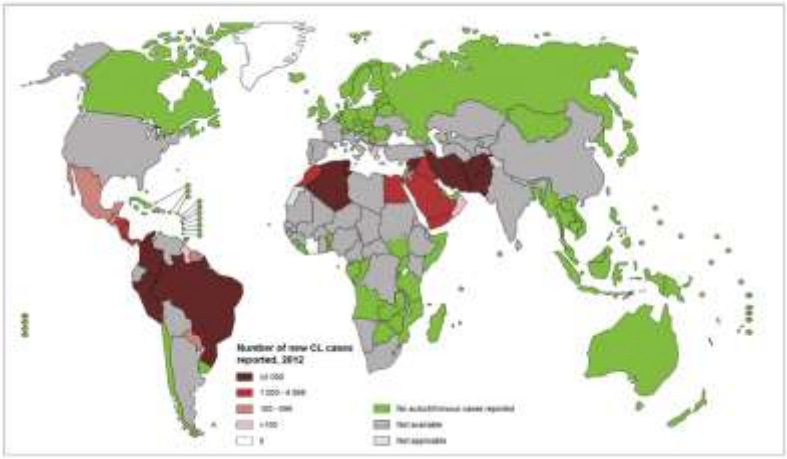


Figure (2.3): Worldwide distribution of CutaneousLeishmaniasis (WHO, 2016)

2.6.2 Epidemiology of Leishmaniasis in Iraq

Both the cutaneous (Baghdad boil) and visceral (Kala-azar) manifestations of leishmaniasis are prevalent in Iraq. With a population of almost 32 million, 23% of whom live below the national poverty line, Iraq has seen a great deal of conflict and sufferin durin the previous 25 years. In the early years of conflict and population relocation, a maximum number of leishmaniasis cases are documented; in 1992, the incidence peaked at 45.5 cases per 100,000 people (Salam *et al.*, 2014) .

There are many different climatic, geographic, and racial characteristics in Iraq, some of which may be linked to the prevalence of visceral leishmaniasis. While northern Iraq has continental climatic regimes, the climate is mostly subtropical. Understandin these climatic and regional variations is crucial because climate change is anticipated to have an impact on the seasonality and spatial distribution of leishmaniasis in Iraq (Al-Warid *et al.*, 2019). previous studies in Iraq based on some immunological effects such as interleukins and interferon by showed immune value, especially in patients serum infected with cutaneous leishmaniasis and many other skin diseases (Ali, 2019)

This may be crucial in temperate regions where risin average temperatures may enable the expansion of the existin Phlebotomus species' reproductive seasons or the emergence of tropical and/or subtropical vector species. Those modifications would enable the disease to remain in regions where historically low temperatures have prohibited the vector from overwintering.(Al-Hayali & alkattan, 2021)

Unfortunately, few studies have used PCR to identify *Leishmania* strains in human cutaneous lesions. Yet, in a US military post in southern Iraq, a phylogenetic research used molecular and phylogenetic analysis to assess the frequency of various *Leishmania* species in sandflies (Al-Bajalan *et al.*, 2021). from 2008 to 2015, there were 17001 confirmed cases of leishmaniasis in Iraq, with incidence rates rangin from 2.9 to 10.5 per 100,000 individuals. The war on terror and military operations that are connected with emigration may be thought to be the reason for the disease's prevalence in towns and cities, accordin to the results of an outbreak of CL cases in Iraq that are explained by emigration in various parts of the nation. Moreover, poor health and cleanliness in the nation contribute to the spread of the disease (Kadhim & Al-Quraishi, 2020)

between September 2019 and July 2020 in Iraq's southern regions In Basrah, Maysan, and Thi-Qar, the incidence of VL is 6.61%, 18.32%, and 75.06% over the previous five years, respectively. Thi-Qar province had the largest sickness rate (295)(Hameed & ; Jihad A. Ahmed, 2019) At Baghdad General Hospitals and a few private clinics, 444 cases of cutaneous leishmaniasis were documented and examined between 2019 and 2020 (Al-naimy & Al-waaly, 2021)

the role of climate change in the emergence and reemergence of human infectious diseases, particularly vector-borne diseases, is well recognized; temperature and humidity are the two most important climatic factors (Hameed & Jihad , 2019)

Table (2.2). Species and distribution of *Leishmania* according to Bailey, 2017.

Species	**Distribution**	**Main leishmania**
L. tropica	**Mediterranean area, South West and Central Asia**	**Cutaneous leishmanisis**
L. major	**South- West and Central Asia, North and West Africa**	**Cutaneous leishmanisis**
L.donovani ***L.d.donovani*** ***L.d. infant***	**Southern Asia Mediterranean area and Central Asia**	**Visceral leishmaniasis**
L.d. sinensis	**East Asia**	
L.d. Archibald	**East Asia**	
L. aethiopica	**East Africa**	**Cutaneous**

		leishmaniasis
L. Mexicana		**Cutaneous leishmanisis**
L. m. Mexicana	**Central America**	
L. m. amazonensis	**South America**	
L. m. venezuelensis	**South America**	
L. m. pifanoi	**Barazil**	
L.braziliensis		**Cutaneous and Mucocutaneous leishmaniasis**
L.v. baraziliensis	**South America**	
L. v. guyanensis	**South America**	
L. v. panamensis	**Central America**	
L. v. peruviana	**South America**	
L. tropica	**Mediterranean area, South West and Central Asia**	**Cutaneous leishmanisis**

2.7 Mode of Transmission of *Leishmania*

Leishmania parasites are spread by the bites of infected female phlebotomine sandflies, which rely on blood to lay their eggs. *Leishmania* parasites may be found in 70 different animal species, includin humans (Pal *et al*., 2021)

1. **Transmission by vectors:** The most prevalent method of transmission in the globe might happen when infected sand flies are crushed into a skin wound or mucous membrane (Cunha *et al*., 2019) Direct amastigote injection or personal contact are the two main methods of parasite transmission from human to human or animal to human (Cunha *et al*., 2019)

2. **Transfusion-transmitted:** Leishmaniasis cases have been extensively recorded from a number of nations, includin India (Singh, 2006) Through exchangin needles . state that more than 85% of the 200 cases of HIV-associated leishmaniasis of HIV-associated leishmaniasis found in Spain were amon intravenous dru users (Alvar *et al*., 2008)

3. **Laboratory-acquired transmission:** when infections caused by *L. tropica*, *L. braziliensis*, and *L. donovani* are reported (Dey & Singh, 2006) Several of those were from needle-stick injuries, which resulted in ulcers at the inoculation site. , and the story of the first year in a few days (Singh, 2006) .

2.8 Pathogenesis of cutaneous leishmaniasis

Cutaneous Leishmaniasis is a disease caused by Leishmania parasites that are transmitted to humans through the bites of infected sandflies. The symptoms and severity of the disease can vary depending on the individual's immune system and the type of Leishmania involved. Typically, the disease begins with the appearance of a small ulcer or lesion at the site of the sandfly bite, which may initially be painless. Over time, the parasites can multiply

within the wounds and spread to other areas of the skin, resulting in multiple skin lesions. The lesions are characterized by itching, redness, and can be painful or painless (Guha *et al*., 2013).

2.9 Immunity Against *Leishmania*

The parasite has a complex life cycle with two developmental stages: infectious, non-flagellated amastigotes that live and reproduce inside host macrophages, and promastigotes with flagella. The sandfly penetrates the host skin with its mouthparts durin the blood meal, rupturin the extracellular matrix and blood capillaries to cause a lesion in the dermis. Innate immune cells such as Dendritic Cells (DCs), macrophages, and neutrophils are drawn to the wound as it develops (Kupani *et al*., 2023).

Leishmania parasites must change the host's physiological makeup in order to inhibit the immune system or encourage pro-parasitic host responses in order to survive (Mirzaei *et al*., 2021) . Immunity to Leishmania can be divided into autoimmunity and acquired immunity.

1. Autoimmunity:

This response includes cellular and molecular factors that are present in the body and work to neutralize and attack the Leishmania parasites present in the tissues.

The cellular factors in innate immunity include natural killer cells (NK cells), natural killer T cells (NKT cells), and gamma-delta (γδ) T cells. These cells are capable of detecting and directly destroying cells infected with Leishmania. Natural killer cells work by neutralizing and breaking down the infected cells using a variety of chemical compounds. Gamma-delta (γδ) T cells recognize the parasites and activate the immune response.

In addition to cellular factors, the molecular factors in innate immunity include specialized proteins that are secreted by immune cells as a natural response to infection. These distinctive proteins, such as interferons and interleukins, work to activate and enhance the immune response and stimulate other cells to attack the infected parasites (Kupani *et al*., 2023).

2. Acquired immunity:

Acquired immunity, also known as adaptive immunity, refers to the immune response that develops after the body is exposed to the actual pathogen causing the infection. It is a process that the body acquires over time. Acquired immunity plays a crucial role in the defense against Leishmania infection (Dubie & Mohammed, 2020).

2.9.1 Neutrophil *Leishmania* interaction

Durin the first several hours after the *Leishmania* invasion, neutrophils are the first immune cells to arrive at the site. Their early recruitment is crucial, especially durin the first containment phase (Charmoy *et al.*, 2007).

Neutrophils boost the immune response by influencin several of processes, includin the engulfment of *Leishmania* promastigotes, the production of antimicrobial compounds such as neutrophil extracellular traps (NETs), and the manufacture of lytic enzyme (Kienle & Lämmermann, 2016). Differential cytokine production sush as (TNF-α), (IL-1β) and reactive Oxygen species (ROS) (Pitale *et al*., 2019 ; Oualha *et al*., 2019).

The coordinated immunological responses of neutrophils to *Leishmania* infection are under the direction of Toll-like receptors (TLRs). TLRs have been shown to mediate neutrophil activation and death as well as early and optimal neutrophil recruitment to the infection site (Iwasaki & Medzhitov, 2004) .

as well as the development of neutrophil extracellular traps (NETs). Furthermore, they actively contribute in the modulation of T cell responses by secretin various chemokines and cytokines (IL-6), (IL-12) (Kupani *et al*., 2023).

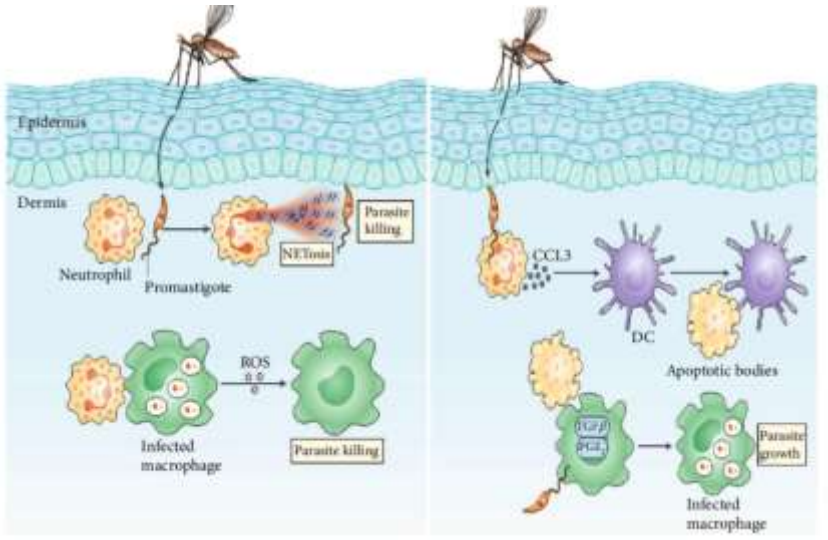


Figure (2. 4) The role of neutrophils in leishmania infection control and persistence of infection (Dubie & Mohammed, 2020)

2.9.2 Macrophage *Leishmania* interaction

Intracellular amastigotes modify various antimicrobial defense pathways and interfere with several of essential macrophage functions to sustain and multiply inside the cell. *Leishmania* parasites are engulfed by macrophages and are eliminated by the production of Interferon-gamma (IFN- γ), reactive oxygen species (ROS) and nitric oxide (NO) derivatives inside the phagolysosome (Mitjà *et al*., 2017).

The next line of defense for the host is the macrophage, which secretes nitric oxide and pro-inflammatory cytokines (IL-1, IL-6, IL-12, and TNF- α) when the *Leishmania* parasite has effectively invaded neutrophils (Jafarzadeh *et al.*,2020) The consumption of promastigotes by macrophages involves several numbers of receptors, includin toll-like receptors (TLR),

complement receptors (CR), kinases, and transcription factors (Liu & Uzonna, 2012).

The interaction of macrophages and *Leishmania* is critical in the development of the infection. In experimental leishmaniasis models, macrophages are critical cells not only for parasite survival, replication, and development, but also for parasite eradication (Horta *et al*., 2012)

Various factors, such as sandfly saliva, play an essential role in determinin disease establishment durin the early stages of infection. The inhibition of the first proinflammatory immune response by *Leishmania* parasites and sandfly saliva has been linked to parasite survival (Liu & Uzonna, 2012)

The potential of saliva to specifically block pathogen identification, nitric oxide (NO), and hydrogen peroxide generation, thereby decreasin macrophages' ability to destroy parasites, has been linked to increased infectivity (Andrés *et al*., 2022)

Additionally, it has been demonstrated that *Leishmania* vector saliva decreases the synthesis of protective type 1 cytokines such as IL-12 and IFN- γ, while increasin the production of IL-10, IL-4, IL-6, and prostaglandin E (PGE) 2 (Carregaro *et al*., 2013).

Different species of *Leishmania* depend on on a variety of macrophage receptors durin the early recognition processes, includin complement receptors (CRs), mannose receptors (MR), fibronectin receptors, and Fc receptors (FcRs), which may subsequently influence the course of infection (Phumee *et al*., 2022).

The surface molecules of the *Leishmania* parasite perform significant functions in host-parasite interaction, such as parasite adhesion and absorption by macrophages. Examples of these are examples of acid

phosphate and lipophosphoglycan (LPG) such as glycoprotein GP63 are examples of these. the LP is only found in promastigotes. it shields the promastigotes from host complement lysis and aids in the parasite's establishment in the macrophage (Shaddad, 2012).

Macrophages are specialized for pathogen elimination and primin of the host adaptive immune response. However, *Leishmania* parasite surface molecules such as slipophosphoglycan, proteophosphoglycan, glycosylinositol sphospholipids and glycoprotein p63 sub-version in the host cell ssignalin pathways and modulatin expression of various cytokines, sproduction of microbicidal molecules and antigen presentation. This allows the parasite to elude the innate immune response, replicate within the infected macrophage's phagolysome, and cause disease development (Andargie & Diro Ejara, 2016).

Arginine-derived metabolites, which have a major impact on parasite survival in macrophages, are amon the most critical actors. Polyamines are important metabolites in trypanosomatid protozoa and help to synthesize thiol trypanothione. A metabolic mechanism produces polyamines. Induction of the arg1 enzyme enhances *Leishmania* growth and dispersion in vivo, accordin to animal studies (Rostami & Khamesipour, 2021).

The elongated promastigote inside the macrophage develops into a spherical body with a shorter flagellum. The amastigote grows at a slower rate than the promastigote, probably to reduce the metabolic burden and multiply and live for a longer time hence increasin infectivity. by keepin the flagellar pocket neck tight, the amastigote defends itself from the surroundin acidic media containin proteases (Sunter & Gull, 2017).

The amastigotes multiply inside the phagosome and subsequently escape via cell rupture to infect other phagocytic cells (Mougneau *et al*., 2011).

Lymphocytes have a role in adaptive immunological responses to *Leishmania* infection, notably by producin cytokines that activate or inhibit macrophage antiparasitic action. T cells are widely established to have an important role in immunity to various types of *Leishmania*. In general, IFN-producing Th1 cells are required for the resolution of *L. major* infection, where they promote nitric oxide synthesis in macrophages. Susceptibility, on the other hand, is linked to the production of cytokines by Th2 cells such as IL-4 and IL-13 (Guha *et al*., 2013).

Natural killer T (NKT) cells, a specialized subset of T lymphocytes involved in innate immunity to pathogens, also play important roles in the immune response durin the early stages of *Leishmania* infection (Amprey *et al*., 2004; Ishikawa *et al*., 2000).

Moreover, it is found that *Leishmania* exosomes increase the inflammatory cytokine IL-23/IL-17 in the lymph nodes of mice, a ravatin the skin pathology associated with cutaneous leishmaniasis. This further established *Leishmani*a exosomes as important vector-inoculated virulence factors (Olivier & Zamboni, 2020).

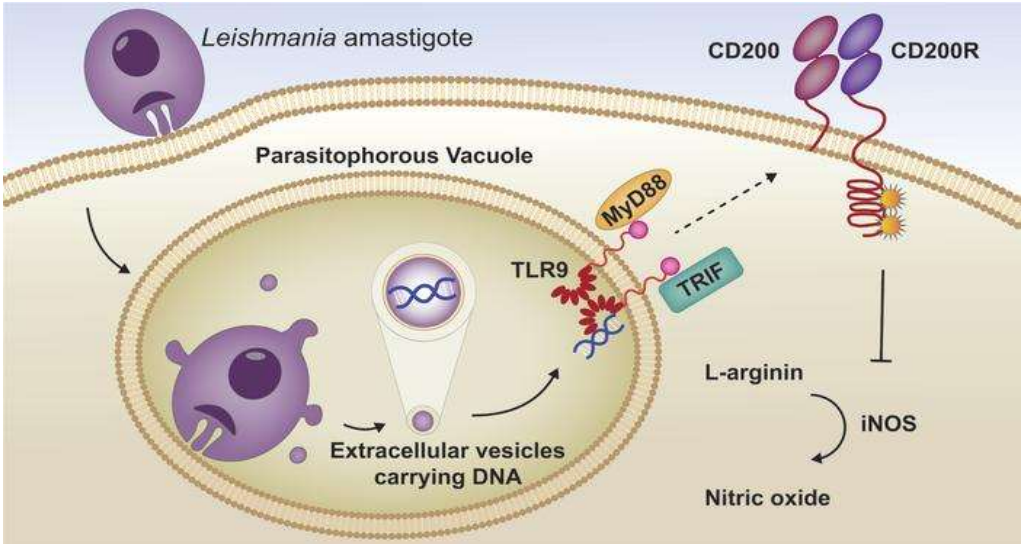


Figure (2.5) Macrophage *Leishmania* interaction (Sauter *et al*., 2019).

2.9.3 Dendritic cell *Leishmania* interaction

Specifically, skin macrophages and dendritic cells (DC) are where the parasite is found the parasite discreetly invades, there is a stress response whereas DC gets activated, releases IL-12, and primes T cells that are specific to an antigen (Cunningham *et al*., 2023).

Macrophages and DC (dendritic cells) are closely related myeloid cells and represent the most potent phagocytic cells in the body. Macrophages are described as large cells with a conspicuous nucleus. whereas DC show their distinctive protrusions and have a relatively smaller nucleus (von Stebut & Tenzer, 2018) The way DCs interact with *Leishmania* parasites differs based on the species, parasite morphology, and host type (Feijó *et al*., 2016).

Dendritic cells (DCs) are a kind of professional antigen-presentin cell (APC) that exists in immature form in all peripheral tissues and is capable of antigen absorption and processing. As so, they serve as immune system sentinels. After comin into touch with microorganisms or chemicals linked with infection or inflammation, DCs mature and move to the T cell regions of lymphoid organs. They transmit antigens to immature T lymphocytes and modify their responses there (Margaroni *et al*., 2022).

Interaction between dendritic cells and *Leishmania* Pattern recognition receptors (PRRs) such as toll-like receptors on DC (TLRs) and C-type lectin identify danger signals known as pathogen-associated molecular patterns (PAMPs), activatin DCs and causin enhanced cytokine production and activation of T lymphocytes for pathogen internalization (Dudek , 2013).

The maturation of immature dendritic cell morphologies is necessary for the initiation of a protective immune response against *Leishmania* parasites. This shift is characterized by increased expression of major histocompatibility complex (MHC) and the activation of CD40, CD80, and

CD86, as well as the production of the pro-inflammatory cytokine IL-12 (Iwasaki & Medzhitov, 2004).

these DCs can produce the anti-inflammatory cytokine IL-10 drivin the differentiation and act- vacation of regulatory T cells that are responsible for visceral disease establishment (Margaroni *et al.*, 2022).

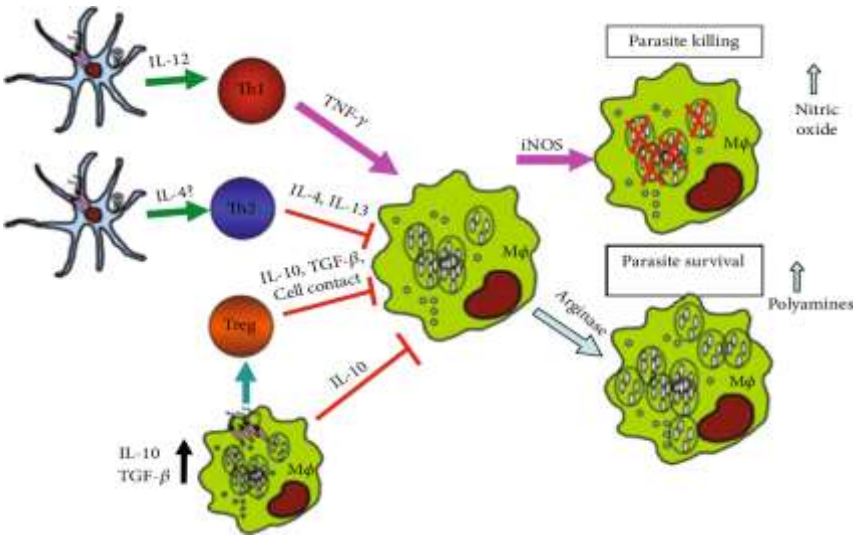


Figure (2.6) Dendritic cells and macrophages regulate the outcome of *Leishmania* infection.

Dendritic cells and macrophages regulate the outcome of *Leishmania* infection. Followin infection, both macrophages and dendritic cells phagocytose *Leishmania* leadin to different functional outcomes. Infected dendritic cells produce IL-12, which is critical for the development of IFN-γ-producin CD4+ Th1 cells. IFN-γ acts on infected macrophages leadin to their activation (classical activation), upregulation of iNOS, and production of nitric oxide and other free radicals that are important for intracellular parasite killing. In contrast, the production of IL-4 by other cell types (includin keratinocytes and Vβ4 T cells) supports CD4+ Th2 development. Th2 cells produce IL-4 and IL-13, which leads to upregulation of arginase activity, alternative macrophage activation and the production polyamines that favor intracellular parasite proliferation. In addition, naturally occurrin

regulatory T cells and infected macrophages also produce some immunoregulatory cytokines including IL-10 and TGF-β, which further deactivate infected cells leadin to impaired parasite killing) (Dubie & Mohammed, 2020) As in the figure (2.6) .

2.10 Enzymatic oxidants and antioxidants

2.10.1. Reactive oxygen species (ROS) and nitric oxide (NO)

ROS are crucial for maintainin human life and play important roles in a number of signalin and pathogenic processes (Kwon *et al*., 2021)

The complicated relationship between Leishmania's immune subversion arsenal and the mononuclear phagocytes' microbicidal capabilities is essential for the development of infection and ongoin parasite persistence. Lysosomal enzymes, reactive oxygen species (ROS), and reactive nitrogen species are examples of such systems (RNS). The best defenses against *Leishmania* species are oxidative burst and nitric oxide (NO) release (Pinho *et al*., 2022) .

T cells are the primary source of IFN- α production; initial macrophage activation by IFN- α is required for parasite death via oxidative-burst processes. In addition to IFN- α, additional inflammatory cytokines such as IL-1, tumor necrosis factor (TNF-α), interferon alpha (IFN-α), and interferon beta (IFN-β) are implicated in macrophage activation and the promotion of iNOS expression and NO generation (Rostami & Khamesipour, 2021).

Activated macrophages may successfully kill parasites by producin Nitric Oxide (NO) after activatin inducible nitric oxide synthase (iNOS) and other leishmanicidal chemicals, such as Reactive Oxygen Species (ROS). NO and ROS are two important actors in macrophage defense (Karampetsou *et al*., 2019) .

Durin infection, *Leishmania* spp. settles in the macrophages where it uses a variety of strategies to elude the host immune system, includin a decrease in iNOS (inducible Nitric Oxide Synthase) activity and an increase in Th2 response by inducin the release of anti-inflammatory cytokines IL4, IL10 (Jadhav, 2022)

When pattern recognition receptor ligands and phagocytosis occur, neutrophils and macrophages release reactive oxygen species (ROS) (Horta *et al*., 2012).

The Pattern recognition receptors PRRs detect can either be of pathogenic origin (pathogen-associated molecular patterns PAMPs), or they can be caused by danger patterns (damage-associated molecular patterns, or DAMPs), which generally signal tissue damage while remainin undetectable to Examples of DAMPs include ATP (Carta *et al*., 2009).

Infected macrophages contribute to the parasite-killin process by producin NO and ROS derivatives through respiratory blasts. In contrast, there is evidence that NO or ROS have a protective role in human leishmaniasis, which might serve as the foundation for future research on the function of NO/iNOS in humans and its potential as a biomarker (Olivier *et al*., 2005).

Mononuclear cells produce two important anti-*Leishmania* components: ROS, which is created durin phagocytosis by a respiratory burst, and NO, which is produced by iNOS in response to IFN-γ (Rostami & Khamesipour, 2021).

2.10.2. Malondialdehyde (MDA):

Malondialdehyde MDA is a byproduct of lipid peroxidation, which occurs when ROS react with unsaturated fatty acids in cell membranes. Increased levels of MDA indicate oxidative damage and lipid peroxidation. In the

context of *Leishmania* infection, the parasite-induced oxidative stress can lead to lipid peroxidation, resulting in elevated MDA levels (Asmaa *et al*., 2017) .

2.10.3. Glutathione Peroxidase (GPx):

GPx is an antioxidant enzyme that plays a crucial role in neutralizing hydrogen peroxide (H2O2) and lipid peroxides. It uses reduced glutathione (GSH) as a co-substrate to convert H2O2 into water and lipid peroxides into less harmful compounds. However, the *Leishmania* parasite has developed strategies to impair the host's antioxidant defense system, including the downregulation of GPx activity. This reduction in GPx levels compromises the ability of infected macrophages to efficiently neutralize ROS and contributes to oxidative stress and damage (Pinho *et al*., 2022).

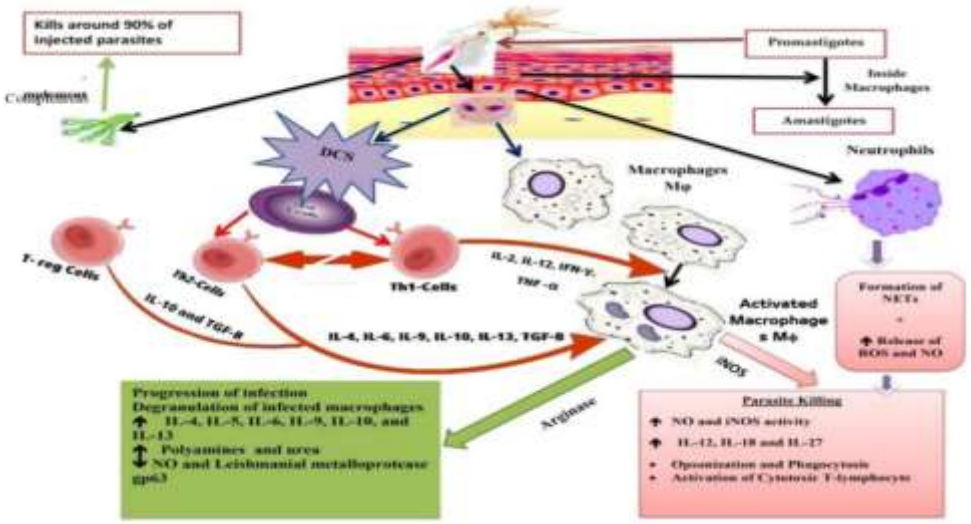


Figure (2. 7) The complex mechanisms of immune response against *Leishmania* infections that include various immune cells and effector molecules (Elmahallawy *et al*., 2021).

2.11 Influencin of Immune System on The Infected Macrophage:

Durin an immunological host, macrophages can be stimulated by inflammatory cytokines to create toxic compounds that result in intracellular death of *Leishmania*, or their microbicidal activity can be suppressed or eliminated by suppressive cytokines, resultin in clinical symptoms. The immune system cells and cytokines influence Leishmania's survival within the host macrophage (Giudice *et al.*, 2007).

2.11.1 The Immune System Cells

In human leishmaniasis, immunity is predominantly mediated by T lymphocytes T1, T2 and NK cell, which play a major role in generatin specific and memory Tcell responses to intracellular parasitic infections and these have been extensively characterized in *Leishmania* infection. Th1 and Th2 cells can be distinguished by the cytokines they secrete: Th1 cells secrete activators of cell-mediated immunity such as IFN-γ, while Th2 cells secrete cytokines such as IL-10, which represent the main macrophage-deactivatin cytokine (Ikeogu *et al.*, 2020).

once established After an infection has been established, different myeloid and lymphoid cells may be drawn to the infection site and nearby lymph nodes. It is widely known that the CD4+ TH1-type response (IFN, TNF) will favor control and healin of the lesion within weeks after infection, dependin on the kind of T helper cell involved and released cytokines (Olivier & Zamboni, 2020).

Recently, Extracellular vesicles (EVs) isolated from *L. amazonensis* were shown to modulate immune responses in B-1 cells by inducin the production of IL-6 and TNF-α and by inhibitin IL-10 (Barbosa *et al*., 2018). However,

how those mechanisms contribute to the severity of the disease in New World species of *Leishmania* is still unknown. Recognizin the importance of glycoconjugates and EVs in the pathogenesis of Leishmaniasis (Nogueira *et al*., 2020) As in the figure(2.7) .

2.11.2 Cytokines Production

Leishmaniasis is influenced by cytokines that are both pro- and anti-inflammatory. The results of an experimental infection showed that proinflammatory cytokines, such as TNF-α and IL-12, are essential antortant for the start of immune defense but that anti-inflammatory cytokines help parasites survive.(Chanyalew *et al.*, 2021)

The production of proinflammatory and antiinflammatory immune response has long been linked to the regulation of *Leishmania* infection and disease progression. A prolonged Th1 response that is characterized by increased IL-12, IL-2, IFN-, and TNF production while downregulating IL-4 and IL-10 production encourages Macrophage activation which appears to be essential for host control of the *Leishmania* parasite burden and clinical cure. The survival of the parasite is aided by Th2-related cytokines (IL-4, IL-5, IL-10, and IL-13), which prevent Macrophag activation

Both IL-10 and TNF-α are cytokines involved in the immune response to *Leishmania* infection, but they have different roles and effects. IL-10 is generally considered an anti-inflammatory cytokine and is known to suppress immune responses, including those involved in controlling infections. On the other hand, TNF-α is a pro-inflammatory cytokine that plays a crucial role in initiating and sustaining inflammation.

Cytokines are powerfulgchemicals that can influence cell activity. Cytokines are messengers, immune cells that respond to cytokine stimulation are receivers, and immune cells that are controlled by the

infectious disease *leishmania* are followers. The parasite takes advantage of cell plasticity to switch them from parasite eliminatin to parasite survival favorin form via a process known as reciprocity, which is mediated by cytokines and results in a pro-inflammatory to anti-inflammatory switch, causin immune cell populations to switch phenotype (Saha & Silvestre, 2021).

2.11.2.1 Pro-inflammatory cytokines

The primary purpose of pro-inflammatory cytokines is to enhance inflammatory responses, which in turn sets off the immunological response to *Leishmani*a infection. This cytokine is essential for a protective reaction, but it can also result in excessive inflammation and unintended tissue injury. Because of this, anti-inflammatory cytokines work to restrict the presence of pro-inflammatory cytokines and their consequences (Andargie & Diro Ejara, 2016)

- **Interleukin-12 (IL-12):**

IL-12 is a heterodimer made up of two subunits (35 and 40 kDa) that are connected by a disulfide bond and are mostly generated by activated M8s and DCs. It is a proinflammatory cytokine that bridges the gap between innate and adaptive immune responses (Dayakar *et al.,* 2019) .

IL-12, also known as a natural killer (NK) cell stimulatin factor, cytotoxic lymphocyte maturation factor, and a fundamental immunoregulation of the start and maintenance of the Th1 response, is essential for inducin IFN- production by T and NK cells (Trinchieri, 2003).

DCs release IL-12 and encourage Th1 differentiation, which is characterized by IFN- production by Th1 cells and results in the eradication of parasites. L. major amastigotes are internalized more than promastigotes

by epidermal Langerhans cells (LCs), which are found close to the inoculation site. It is connected to the elevation of MHC-class I and class II antigens that the parasite uptake activates protective Th1 response (Yasmin *et al*., 2022)

Moreover, IL-12 promotes T-cell proliferation and the generation of lymphokines. The presence of IL-12 lowers CD4+ T-cells' capacity to generate IL-4 while increasin their ability to create IFN-γ. Hence , IL-12 and NK cells appear to play a crucial role in the establishment of the Th1 response (Dayakar *et al.*, 2019).

have demonstrated that individuals with active illness had much greater levels of IL-12 than asymptomatic and healthy participants In a 2012 research by Costa and colleagues, it was established that IFN- and IL-12 plays a significant part in the immune response and helps the parasites die. Its absence might be a sign of an immune system malfunction (Snyder *et al.*, 2021).

supports the synthesis of IFN-γ and the Th1 response. limits the growth of Th2 and IL-4 production produces NO and increases the expression of NOS2 stimulates the synthesis of lymphokines and cell proliferation(Andargie & Diro Ejara, 2016)

- **IL-6 :**

A pro-inflammatory and anti-inflammatory cytokine, IL-6 is a pleiotropic cytokine. Many different cell types, includin macrophages, DCs, and T cells, generate IL-6. The cytokine also functions as a B-cell proliferation factor (Maspi *et al*., 2016).

discovered that lethality was related with high levels of IL-6, supportin the prior study. TNF-α production can be directly inhibited by IL-6, which

can also decrease Th1 responses or stimulate Th2 responses.(Wynick *et al*., 2014).

As a result, the high correlation between IL-6 and illness severity and mortality may be explained in two ways: first, by preventin the production of TNF-α durin the early stages of infection, and second, by its suppression of Th1 responses (Dos Santos *et al*., 2016)

- **Interferon-γ (IFN-γ) and Tumor Necrosis Factor Alfa (TNF-α):**

Key cytokines in the defense mechanisms against intracellular infections include IFN and TNF, and single nucleotide polymorphisms control the amounts of their gene expression (SNP) (Soon *et al*., 2012).

According to Hussein and Ali (2022), the activation of TNF-α and IFN-γ has been demonstrated to destroy the *Leishmania* parasite by inducing macrophages to produce nitric oxide and reactive oxygen species. However, it has been discovered that patients exhibit varying abilities to produce these cytokines and subsequently control the disease. TNF-α has been implicated in the pathogenesis of leishmaniasis, and significantly elevated levels of this cytokine have been observed in cases of active leishmaniasis. Conversely, excessive TNF-α levels can cause tissue damage, resulting in skin sores in cutaneous leishmaniasis (CL) (Maspi *et al*., 2016). The tumor necrosis factor (TNF-α) was first identified as an in vivo necrosis inductor in sarcomas. TNF-α is a proinflammatory cytokine with several biological effects (Michlewska, 2011).

TNF-α production is closely regulated since it can mediate the detrimental consequences of septic shock such as arterial hypotension, disseminated vascular coagulation, and fatal hypoglycemia. TNF-α seems to have a pivotal role in mycobacterial infection management, operatin on a

wide range of cells. Activated macrophages, T lymphocytes, and dendritic cells are the primary producers (Cavalcanti *et al*., 2012).

This cytokine interacts with IFN-γ to stimulate the synthesis of reactive nitrogen intermediates (RNIs) hence facilitatin macrophage single-phase activity (Jafarzadeh *et al*., 2020).

TNF- α also increases immune cell migration to the infection site, which contributes to the creation of granulomas, which are capable of regulatin disease progression (Mohan *et al*., 2001) . which induces the macrophages to eliminate *Leishmania* IFN- γ, a kind of macrophage activatin factor (MAF) that was initially identified as a lymphokine (LK) implicated in leishmaniasis, is a type of leishmaniasis (Mirzaei *et al*., 2021).

IFN- γ serves as a master regulator of immune responses and has lon been recognized as a key pro-inflammatory cytokine in the pathophysiology of inflammatory disorders. Studies have also shown that early IFN-γ production is reliant on IL-12 production and that in vivo IL-12 neutralization at early and late stages of *L.donovani* infection may result in significant downregulation of IFN-γ, TNF-α, and iNOS (Engwerda *et al*., 1998; Rostami & Khamesipour, 2021).

- **IL-1B**

The pro-inflammatory cytokine L-1β, which immune cells produce to promote inflammation, is discovered to be strongly present in the lesions brought on by *Leishmania* infections. The increased production of IL-10 seen after treatment with IL-1β may speed up the disease's course (Patil *et al*., 2018)

Th1 response is impacted by IL-10, which also suppresses the production of the pro-inflammatory cytokine IL-12. This can compromise anti-leishmanial resistance and increase disease (Patil *et al*., 2018)

a powerful pro-inflammatory cytokine that is primarily produced by innate immune system cells like monocytes and macrophages and released

by a range of cell types (D. Santos *et al*., 2018) .L-1β synthesis is positively linked with both the size of the necrosis in lesions and the length of the lesions. Intermediate monocytes (CD14++CD16+) are the predominant source of IL-1β (D. Santos *et al*., 2018).

Inflammatory molecules (TNF-α , IL-1B , and IL-6) are most likely responsible for the increased cellular recruitment seen at infection sites (Latifynia *et al*., 2023).

2.11.2.2 anti-inflammatory cytokines

- **Interleukin -10 (IL-10):**

Unlike IFN-γ, IL-10 increases parasite viability by preventin macrophage activation and decreasin these cells' capability to destroy *Leishmania* (Belkaid *et al.*, 2001) The findings show that IL-10 is necessary for parasite survival and show the amazin therapeutic benefits of anti-IL-10 receptor Ab in eradicatin chronic infection and the possibility of disease recurrence.

Initially, Langerhans cells (LCs) are thought to be crucial for the management of cutaneous leishmaniasis. Further research, however, showed that the production of protective immunity against *Leishmania* parasites is not dependent on these cells (Lemos *et al.*, 2004)

In fact, it has been shown that these cells can perform a regulatory function. The loss of LCs promotes the generation of IFN-γ and reduces the parasite load while dampening IL-10 production and Treg (T regulatory cells) levels (Yasmin *et al*., 2022).

For the transportation of *Leishmania* antigens from the infection site to the drainin lymph node and for the development of a particular T cell response, cutaneous DCs (CD11c+ CD8 Langherin) and monocyte-derived DCs are shown to be more important than LCs It's interestin to note that IL-4, a type 2 cytokine, is necessary for DCs to optimally induce Th1 responses since it

prevents the synthesis of IL-10.(Yasmin *et al*., 2022) IL-10 is a critical component of the suppressive immune response

- **IL-4**

prevents IFN-γ production, activates macrophages alternatively, and promotes parasite survival reduces ROS and NO in macrophages, which prevents oxidative bursts.(Moll *et al*., 2002) .

Infection with *L. major* shows significant IL-4R expression. fosters a disease-promotin humoral response by modifyin antigen uptake and endosomal processing. induces CD8+ T cells to secrete IFN- durin *L.donovani* infection.

IL-4 is crucial for the transformation of Th0 cells into Th2 cells. Mast cells, basophils, activated eosinophils, and Th2 cells are the major producers of IL-4 (Zamorano *et al*., 2003). Inhibitin the leishmanicidal action of macrophages and prolongin parasite life, IL-4 production increases arginase and polyamine biosynthetic activity. Accordin to its down-regulation, IL-4 prevents the production of Th1 cytokines. IL-12 synthesis (Lazarski *et al*., 2013)

IL-4 reduces the chemokine synthesis that attracts Th1-type cells to the infection site (Lazarski *et al*., 2013)

Pro-inflammatory cytokines such as IFN-γ stimulate M1 macrophages, resultin in NOS2 activation, NO release, and parasite death, whereas Th2 cytokines such as IL-4 and IL-13 stimulate M2 macrophages, resultin in parasite survival and inflammation inhibition by counteractin the effects of NOS2 activation and nitric oxide. Indeed, a balance between conventionally activated (M1) and alternatively activated (M2) macrophages regulates inflammatory responses and contributes to immune system homeostasis and wound healin (Gordon & Martinez, 2010)

IL-4 actions are not limited to lymphoid cells. Consequently, IL-4 can control proliferation, differentiation, and death in a variety of haematopoietic

and non-hematopoietic cell types such as myeloid, mast, dendritic, endothelial, muscle, and neural cells (Lutz *et al.*, 2002).

High IL-4 expression has been linked to virulence due to its anti-inflammatory properties as well as its potential to exacerbate tissue damage when combined with TNF- α (Cavalcanti *et al*., 2012)

- **Transformin Growth Factor - β (TGF-β):**

After a cytokine profile survey in ML and CL, the function of IL-10 and transformin growth factor- (TGF-β) for reinforcement of wound healin and control of inflammatory responses is identified (Mirzaei *et al*., 2021)

TGF-beta (Transformin growth factor beta / TGF-β) is a type of cytokine that controls proliferation, cellular differentiation, and other functions in most cells. TGF-beta, is a factor synthesized in a wide variety of tissues a recognized inhibitor of Th1 and macrophage activities (Bogdan, 2020).

TGF gets its name to the findin that it can occasionally be a factor inducin malignant transformation in vitro. These molecules work through a shared receptor. Its effects on the immune system are inhibitory immunosuppressive, as evidenced by a decrease in the proliferation of B, T, and NK cells as well as a reduction in their function, the secretion of some cytokines, and antagonizin the effects of pro inflammatory TNF-like cytokines while it strongly attracts macrophages. Its functions span a wide range of activities, includin the regulation of cell growth and development, dependin on the type of cell on which it acts (Ali, 2021)

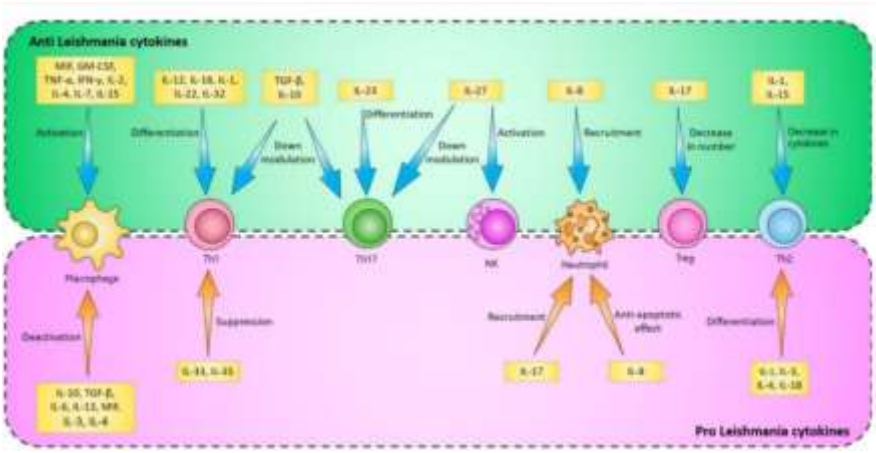


Figure (2.8) The cytokines involvin in leishmaniasis were categorized accordin to pro and anti-Leishmania effects and their interaction with immune cells which illustrate the possible interventions concernin each cytokine and their role in resolvin and a ravatin the outcome of leishmaniasis (Mirzaei *et al*., 2021).

2.12 Diagnoses of leishmaniasis

The traditional diagnostic procedures are useless and difficult to differentiate between various organisms at the genus level (Flaih *et al*., 2021).

The biest obstacle to developing a successful strategy plan to manage and eradicate the illness is the absence of a gold standard test for diagnosing *leishmania*. a combination of clinical signs (supported by epidemiological data) and laboratory testing, such as direct parasitological (microscopy, histopathology, and parasite culture), serological, and molecular tests, are used to determine the disease's diagnosis (Abdulla, 2018).

2.12.1 Diagnoses of cutaneous leishmaniasis (CL)

diagnosed usin a variety of approaches, accordin to (Abdulla, 2018) including:

1. Direct identification usin a microscope when a smear is taken from the center at the boundary of amastigotes-containin tumors treated with Giemsa stain.
2. Antileishmanial antibodies are utilized in serological testing, albeit their usage is limited due to their inaccuracy.
3. PCR identification of parasite DNA and culture.

2.13 Cell lines as leishmanial experimented models

In vitro infection research is critical for evaluatin specific elements of *Leishmania* biology, as well as for more meaningful in vitro screenin of interestin chemical entities. Macrophage-like cell lines of various origins are susceptible to *Leishmania* infection. Cell lines are highly recognized for their ability to provide repeatable infections and reliable data due to their stability and standardization capability. Moreover, for more than 40 years, these cells have been a cornerstone of leishmaniasis research (Santarém *et al*.,2019).

The total number of cell lines may be calculated usin the American Type Culture Collection (ATCC) Cell Biology Collection, which has over 3,600 cell lines from more than 150 different species (Sadhu, 2023)

2.13.1 Several types of cell lines, including:

1. **THP-1 cell line,** isolated from the peripheral blood of a male patient with acute monocytic leukemia who was one year old (Shah *et al.*, 2022).

2. **U937 cell line,** isolated from human lymphoblast lun the origin and stage of maturation of U937 and THP-1 cells are the primary differences. THP-1 cells are from a blood leukemia origin and are less developed than U937 cells since they are of tissue origin (Takahashi *et al*., 2014).
3. **J774 cell line**, is a cell line that was found in the ascites of a female adult patient who had reticulum cell sarcoma in 1968 (Ullah *et al.*, 2017).
4. **RAW264.7 mouse macrophage cell line,** The RAW264.7 cell lineage has been well-characterized in terms of macrophage-mediated immunological, metabolic, and phagocytic functions (21), and it is rapidly bein utilized and acknowledged as a cellular model of osteoclastogenic study (Kong *et al.*, 2019) .

2.14 Treatments

Cutaneous Leishmaniasis is thought to be a self-healin condition. All the same, prompt treatment is still necessary to minimize unsightly scars and parasite spread. There is currently no one best therapy for CL (Kadhim & Al-Quraishi, 2020)

The aim of CL medication is to improve the overall appearance of the lesion and to decrease the length of the disease. Several factors influence CL treatment, includin clinical presentation, illness duration, the number, size, and location of lesions, and the occurrence of secondary infection *(*Arenas *et al*., 2017).

The standard therapy for all kinds of leishmaniasis is sodium stibogluconate (SSG) (Silva *et al*., 2021) . Liposomal amphotericin B is very potent agent for Cutaneous Leishmaniasis (Ramos *et al*., 2022). Additional antileishmanials include paromomyin, miltefosine and others.

1. Sodium stibogluconate (SSG)

Sodium stibogluconate is a pentavalent antimonial that has lon been used to treat all kinds of leishmaniasis, includin cutaneous illness. Antimonials must be administered parenterally, either intravenously or intramuscularly, in order to be absorbed. For New World cutaneous illness, the typical dosage is 20 m antimony/kg/day for 20 days (Shaddad, 2012)

In amastigote form, antimonials are known to inhibit glycolytic enzymes and fatty acid oxidation. Serious adverse effects were noted, includin pancreatitis, which caused nausea and stomach discomfort in several individuals Its method of action is based on inhibitin glycolysis and fatty acid oxidation in protozoan cells (Severino *et al*., 2022) .

2. Amphotericin B

Amphotericin B is a polyene antifungal antibiotic discovered in 1956 in a Streptomyces bacterium. Amphotericin B binds to cell wall sterols, but preferentially to ergosterol, the main cell membrane sterol of fungus and *Leishmania*, but not to human cell membranes. It specifically inhibits the parasite's membrane production and produces holes in the membrane, resultin in parasite death (Singh & Sivakumar, 2004) **.**

The adverse effects of this medication include high fever, thrombophlebitis, myocarditis, renal failure, hypokalaemia, and death (Oliveira *et al*., 2011). However, the cost of manufacture makes its usage challengin in developin nations (Shaddad, 2012).

3. Paromomycin

An aminoglycoside known as paromomycin is effective against certain protozoa, cestodes, and many Gram-positive and Gram-negative bacteria. It was approved in India in 2007 as an effective, well-tolerated, and reasonably priced therapy for visceral leishmaniasis (VL) at a dosage of 11 mg/k (base)

for 21 days, even though it is no longer in use as an antibiotic *(Davidson et al*., 2009) .but better at combatin cutaneous leishmaniasis (Shaddadg, 2020)

4. Pentamidine

Leishmania infections that are resistant to Sb V have been treated with pentamidine, a synthetic aromatic diamine. Its effects on polyamine synthesis and mitochondrial function impairment are connected to its anti-leishmanial activities (Neamah *et al*.,2022) . The adverse effects of this medication include myalgia, nausea, headaches, permanent insulin-dependent diabetes, hypoglycemia, and even death (Christen *et al*., 2018)**.**

5. Miltefosine

There have been a few clinical trials that have looked into miltefosine's use in treatin cutaneous leishmaniasis. As compared to conventional parenteral medications in the context of widespread usage in the interior regions of endemic nations, this medication's oral administration represents a significant benefit. Moreover, miltefosine has action in severe or resistant instances (Machado & Penna, 2012) .

The safe and affordable therapy for cutaneous leishmaniasis is miltefosine. It works in CL situations that are resistant to antimony compound (Tahir *et al.*, 2019).

Alkyl phosolipid miltefosine was created as an anti-tumor agent. It activates T cells, macrophages, and increases interferon-, which promotes the hematopoietic and immunological systems (Cruz *et al.*, 2009).

2.15 Control of Leishmaniasis

Leishmaniasis is a serious public health hazard, particularly for people affected by poverty, conflict, poor nutrition, and weakened health systems. The East African area is one of the most affected regions in the world, and a lack of data on the prevalence, distribution, social/risk factors, and transmission of leishmaniasis remains a barrier to disease treatment in the

majority of affected nations. As our globe changes, both politically and environmentally, it is critical that we understand the present epidemiology of leishmaniasis in endemic areas, enhance surveillance and disease treatment, and undertake vector control where appropriate (Jones & Welburn, 2021)

A vector control program's goal is to limit or stop disease spread. Controllin sandfly vectors, particularly in domestic and peridomestic transmission settings, is an effective technique for lowerin human leishmaniasis. Chemicals, environmental management, and personal protection are amon the potential control strategies (Steffen *et al*., 2007)

Leishmaniases are a common and medically significant category of parasitic illnesses, with some posin a substantial health risk in communities throughout the Mediterranean basin. Scientists from Israel, Turkey, Portugal, and the Netherlands began a combined, collaborative investigation of the Mediterranean leishmaniases in 1993. The goal of this study is to create a multi-component method to successfully control all kinds of leishmaniasis, with a focus on the more severe, visceral leishmaniasis (Ozbel *et al*., 2000)

Sandflies breed in the gaps in walls that have rich humus and moisture. The idea of environmental management is to make the environment unfavorable for breedin Indoor residual sprayin is a straightforward and cost-effective technique for eliminatin endophilic vectors, and DDT continues to be the pesticide of choice for leishmaniasis management (Chen *et al*., 2019). Nonetheless, pesticide resistance is expected to grow in the population, particularly in places where insecticide has been used for years (Kishore *et al*., 2006)

Many programs have been put in place in the countries of the world to control leishmaniasis, especially in regions where Chemicals, environmental

management, and personal protection are all options for control (World Health Organization, 2010) includes these programs :-

1. Sprayin houses with insecticides to eliminate the vector to reduce the risk of infection with leishmaniasis, especially the carrier of human leishmaniasis parasites. Anthroponotic Leishmaniasis Cutaneous (Sharma & Singh, 2008)
2. Control of animals that store *Leishmania* parasites. Cutaneous Zoonotic Leishmaniasis, by makin changes in the environment, as is the case in Jordan and Tunisia, where operations are carried out to fill animal burrows and remove bushes from the are as surroundin the population by 2-3 km (World Health Organization, 2010)
3. Control success is dependent on understandin the epidemiology of leishmaniasis and some of the practices used, such as sleepin outside without mosquito nets to guard against sand fly bites or sprayin buildings with unsuitable chemicals to kill the vector insect (Stockdale & Newton, 2013)
4. Medical testing, early diagnosis, especially in areas where leishmaniasis is widespread, and laboratory research to identify and classify different parasites are all important for disease plannin (Gow *et al*., 2022)

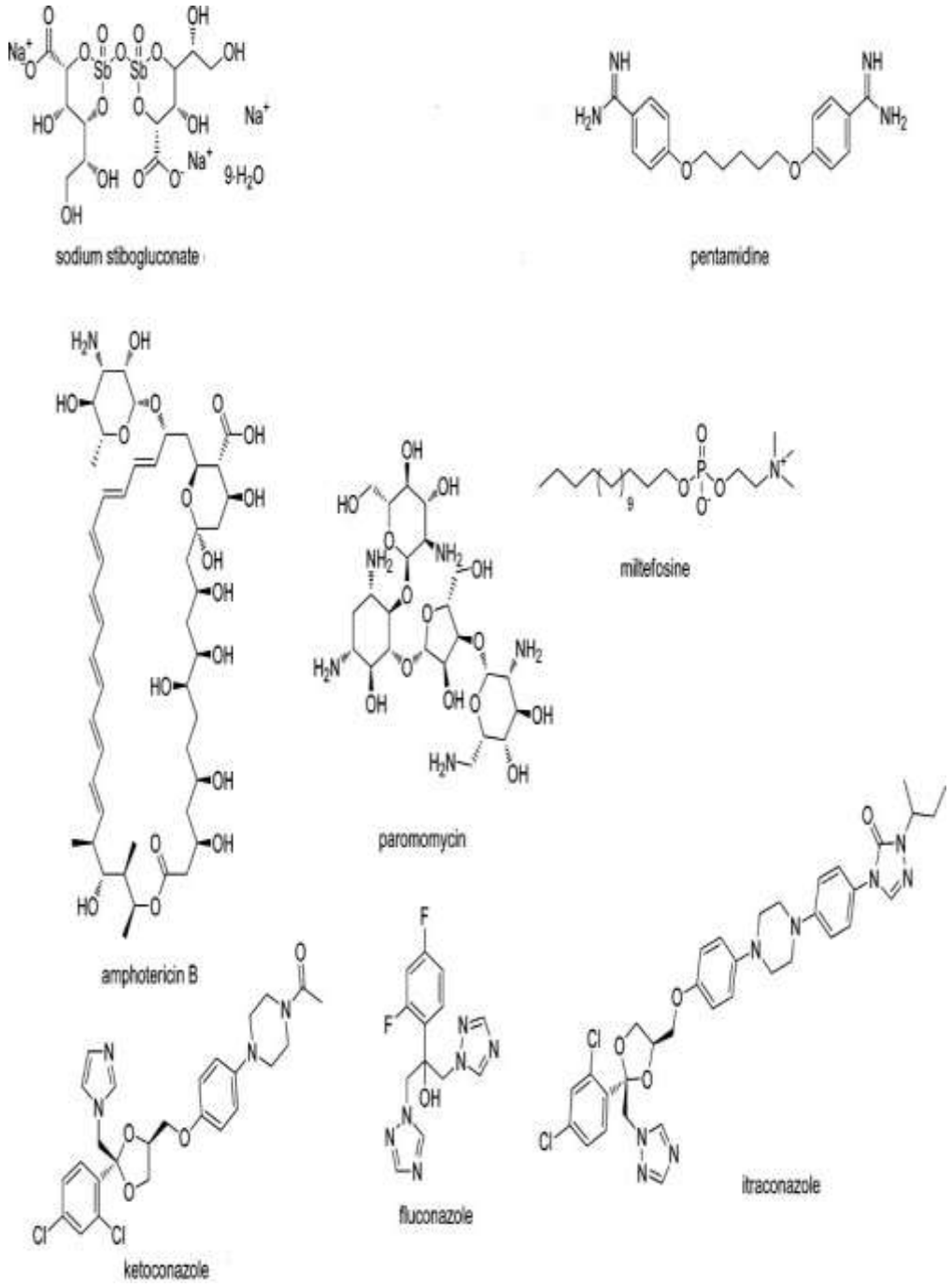


Figure (2. 9) Drugs that have been repurposed for the treatment of leishmaniasis (Reguera *et al*., 2019).

Chapter Three

Materials and Methods

3 Materials and Methods

3.1 Materials

3.1.1 Equipment and Instruments

Table (3.1) The equipment and instruments used in this study with their companies and countries of origin .

NO.	Equipment and Instrument	Company\ origin
1.	Autoclave	Stermite/Japan
2.	Aerosol Resistant Micropipette tips (1000,100,10µI)	Promeg/USA
3.	Digital Camera	Canon/Japan
4.	Disposable syringe (3 ml)	Sterile EO / China
5.	Disposable tips	Netheler-Hinz/ Germany
6.	Eppendorf tubes	Bioneer/ Korea
7.	Flasks	Pyrex/ UK
8.	Gel electrophoresis	unite Cleaver scientific/ Japa
9.	Glass Slide	Citoclase / China
10.	Gloves	Laleh/ Iran
11.	High-Speed Cold centrifuge	Eppendorf /Germany
12.	Light Microscope	Olympus/Japan

13.	Medical scalpel	OEM/China
14.	ELISA reader	Avusturya
15.	Rack	SUN (H.K.J)
16.	Refrigerator	Concord /Lebanon
17.	Sensitive Balance	Sartorius/Germany
18.	Thermocycler PCR -96	BioRad /USA
19.	Vortex	CYAN/ Belgium
20.	Water Bath	Memmert/Germany
21.	Cell culture dishes	Falcon/USA
22.	Cell culture plates (42, 48,96) wells	Falcon/USA
23.	Cell culture dishes, tubes, flasks	Falcon/USA
24.	Millipore filter 0.22	Falcon/USA
25.	Sterile pasture pipette	Falcon/USA
26.	Co2 incubator	SANYO/Japan
27.	Microcentrifuge	Hettich/Germany
28.	Plate Rotor Centrifuge	HERMLE\ Germany
29.	Hood with U.V. light	CRYSTE \ Korea
30.	Digital PH-meter, Neubauer improved	Griffin/Germany

3.1.2 Chemicals

Table (3.2) The chemicals with their companies and countries of origin used in this study .

NO.	Chemical	Company and Origin
1.	Agarose gel	Promega1 (USA)
2.	DNA Extraction Kit	Favorgen / Europe
3.	DNA ladder (100bp)	Biolabs/ UK
4.	Ethanol1 (96%)	Himedia (India
5.	Giemsa stain	Conda /Spain
6.	Loading dye	Biolabs/ UK
7.	Nuclease free water	Promega /USA
8.	Primers	Macrogen/ Korea
9.	Proteinase k	Biolabs/ England
10.	Red save	BIO BASIC INC/ USA
11.	TBE buffer	BIO BASIC INC/ USA
12.	Master mix	Intron/USA
13.	Oil immersion	China /CC

14.	DMSO Dimethyl sulfoxide	SDFCL s d fine-Chem Limited
15.	FBS (fet bovine serum)	Biochrom GmbH
16.	MTT (3-(4,5-Dimethylthiazole-2-yl)- 2,5-diphenyl-2H-tetrazolium bromide) dye powder	Germany
17.	PBS (phosphate buffer saline)	Sigma-Aldrich
18.	Penicillin / Streptomycin	Euroclone® / Europe
19.	RPMI1640 medium	Sigma-Aldrich
20.	Fetal Bovine Serum	Gibco ™
21.	PMA (phorbol myristate acetate)	Sigma-Aldrich
22.	Cytokine kit TNF-α	BIOSORURCE Europe S.A.
23.	Cytokine kit TGF-B1	BIOSORURCE Europe S.A.
24.	Cytokine kit IL-B1	BIOSORURCE Europe S.A.
25.	Cytokine kit IL-10	BIOSORURCE Europe S.A

3.2 Study Design

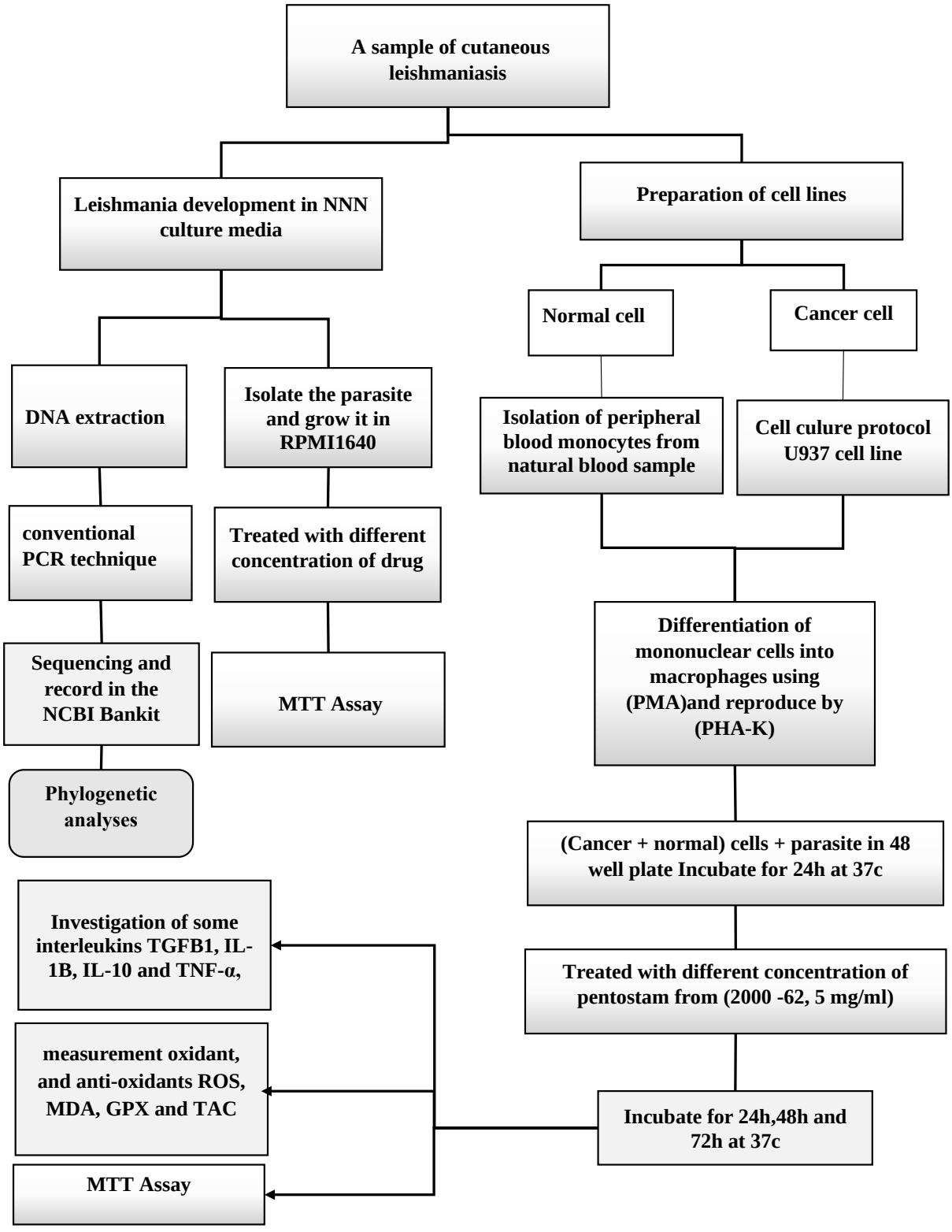


Figure (3.1) Study Design

3.3 Methods

3.3.1 Leishmania parasite

The sample was taken from an infected person from the Sinjar area in west Mosul, and it was diagnosed by the laboratory. During the period from October 2022 to June 2023, the parasite was examined and developed, and some experimental items were conducted in the Advanced Parasitology Laboratory of the College of Science and the Tissue Culture Laboratory at the College of Medicine / University of Babylon.

3.3.2 Culture Media

3.3.2.1 Biphasic medium (Nova-MacNeal-Nicolle) (NNN):

Leishmania parasites were maintained and grown in vitro using the developed biphasic medium. Typically, it has two stages according to (Chang and Hendricks, 1985) as shown in the figure (11) in the Appendix.

1. Solid Phase

The solid blood agar phase consists of the following ingredients for 1liter final volume:

Ingredients	**concentration**
Agar	**20 gm**
Sodium chloride	**8 gm**
Pepton	**5 gm**
Meat extract	**4 gm**
Human blood	**200 mL**

Distilled water	**1000 mL**

Antibiotics	
Ingredients	**concentration**
Gentamycin sulphate	**0.08 gm/mL**
Streptomycin sulphate	**0.4 gm/mL**

1- The above ingredients were dissolved (except for antibiotics) in distilled water and the pH was adjusted to 7.2 and then sterilized by Autoclave at 121 ° C for 20 minutes at a pressure of 1.5 Bar.

2- the solution is then left to cool to 50 ° C and heat-inactivated human blood and antibiotics were aseptically added.

3- The prepared culture medium is poured with a volume of 5 ml into sterile 25 ml bottles and allowed to solidify in a slanted position. The medium was then incubated in the incubator for 24 hours at a temperature of 37 ° C to ensure sterilization and kept in the refrigerator until used.

2. Liquid phase

The Lock solution and its ingredients were used per liter according to (Chang and Hendricks, 1985):

Ingredients	**concentration**
Sodium chloride	**8 gm**
Potassium chloride	**0.2 gm**
CaCI22H2O	**0.2 gm**

Sodium bicarbonate	**0.2 gm**
D-glucose	**2 gm**
Distilled water	**1000 ml**

Antibiotics	
Ingredients	**concentration**
Gentamycin sulphate	**0.08 gm/ml**
Streptomycin sulphate	**0.4 gm/ml**

1- The above ingredients were dissolved (except antibiotics) in distilled water and the pH was adjusted to 7.2 and sterilized by Autoclave at 121 ° C for 20 minutes at a pressure of 1.5 Bar

2- The solution is left to cool and antibiotics are added to prevent bacterial and yeast contaminations.

3- Five ml of liquid medium was added to the solid medium.

4- The medium was inoculated with 0.1 ml of logarithmic phase promastigotes and incubated at 26 ° C for six days.

3.3.2.2 RPM I 1640 (Roswell Park Medium Institute)

Roswell Park Memorial Institute (RPMI) media are a series of media developed by Moore Kenny *et al.* (1972) for the culture of human normal and neoplastic cells, in vitro. RPMI 1640 is the most commonly used medium in the series. A modification of McCoy's 5A medium, the medium was specifically designed to support the growth of human lymphoblastoid

cells in suspension culture. Presently, the medium is extensively used for a wide range of anchorage dependent cell lines. The medium needs to be supplemented with 5-20% Heat Inactivated Fetal Bovine Serum HI FBS. The medium is also known to support the growth of cells in the absence of serum (Hassan & Dhumad, 2021).

3.3.3 Direct Giemsa Stain

1. Twenty microliters of the sample (Pus) are placed on a glass slide and left it to dry for (2-3) minutes.
2. Proven the prepared slide swab by using methanol alcohol one-minute duration
3. The prepared swab is examined and standing by the dye of Giemsa stain as (Figure 3.4) compound microscope and on the strength of 40X magnification lens oily slide where examined to detect the presence of the parasite (Hakim *et al*., 2023)

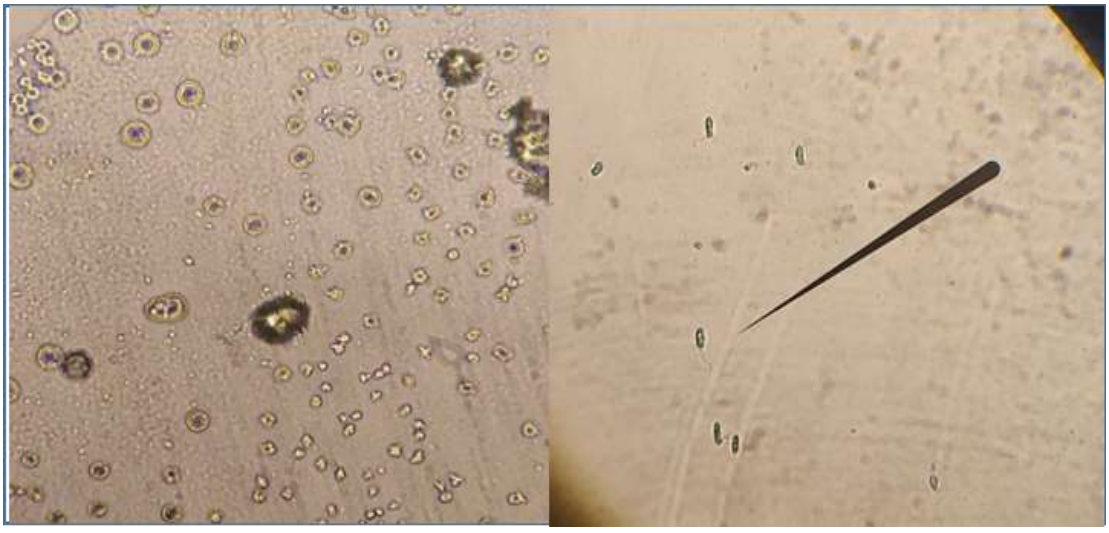


Figure (3.2): Figure showing promastigotes on the right and Amastigotes on the left by 40X

3.4 Molecular Methods

3.4.1 Genomic DNA Extraction

The DNA of the developing *Leishmania* parasite was extracted in an NNN medium by Favorgen Plasmid DNA Extraction Mini Kit as shown in the figure (13) in the Appendix) and done according to company instructions as follows:

1. Sample Preparation

Transfer up to 200 ul of serum from culture to a 1.5 ml microcentrifuge tube. If the sample volume is less than 200 VI, add the appropriate volume of PBS. Add 30 ul Proteinase K (10 mg/ml) to the sample and briefly mix. Then incubate for 24 h at 60 ^{0}C.

2. Cell Lysis

Add 200 ul FABG Buffer to the sample and mix by vortexing. Incubate In a 7 C cc water bath for 15 min to lyse the sample. During incubation, invert the sample every 3 min. Preheat the required Elution Buffer in a 70 oc water bath for DNA Elution. (If RNA-free genomic DNA is required, add 5 kilograms of 1 0 mg/ml RNase A to the sample and mix by vortexing. Incubate for 5 min at room temperature.

3. DNA Binding

Add 200 uI of ethanol (96-100%) to the sample vortex for 10 sec. Pipette the sample mix well if there is any precipitate formed. Place FABG Column 10 a Collection lube. Transfer the sample mixture carefully to the FABG Column. Centrifuge Cit speed 18,000 for I min. Discard the Collection and place the FABG Column la a new Collection Tube.

4. Column Washing

Add 400 ul of W1 Buffer to the FABG Column and centrifuge for 30 sec at speed 1 4,000 rpm or 18,000 x g. Discard the flow-through and place the FABG Column back into the Collection tube Add 600 ul of wash Buffer to the FABG Column and centrifuge for 30 sec at a speed of 14000 rpm or 18.000 x g. Discard the flow-through and place the FABG Column back in the Collection Tube. Centrifuge for an additional 3 min at speed 14.000 rpm or 18.000 x to dry the column.

5. Elution

Place the dry FABG Column in a new 1.5 ml microcentrifuge tube. Add 100 ul of Preheated Elution Buffer or TE to the membrane center of the FABG Column. Incubate the FABG Column at 37 ^{o}c for 10 min in an incubator. Centrifuge for I minute at full speed 14.000 rpm or 18.000 x to elute the DNA. Store the DNA fragment at 4^{0}C or -20C.

3.4.2 PCR Examination

The conventional Polymerase chain reaction PCR technique was performed to detect Cutaneous Leishmaniasis based on the kinetoplast DNA (kDNA) for their detection of *L.major* and *L. tropica* This method is carried out according to Noyes et al. (1998)

Polymerase chain reaction (PCR) is performed using *Leishmania* - specific primers with forward IR1 and reverse IR2 (LITSRCTATCATTTTCCGATG),eL5.8Se(TGATACCACTTATCGCACTT) : A total reaction volume of 20 µL was prepared of PCR reaction buffer, 1.5 mM of MgCl2, 60 µM of each dNTP, 1 µM of IR1 and 1 µM of IR2 primers in cycling conditions: 95 °C for 12 min followed by 35 cycles consisting of

94 °C for 20 s, 56 °C for 30 s and 72 °C for 1 min. Followed by a final extension step of 72 °C for 10 min (Aransay *et al*., 2000) .

3.4.3 Nucleic acids sequencing of PCR amplicons

The resolved PCR amplicons are commercially sequenced from both directions, forward and reverse directions, following the instruction manual of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI (Applied Biosystem) sequence files were further analyzed, ensuring that the annotation and variations were not because of PCR or sequencing artifacts. By comparing the observed nucleic acid sequences of local samples with the retrieved nucleic acid sequences, the virtual positions, and other details of the retrieved PCR fragments were identified.

3.4.4. Interpretation of sequencing data

The sequencing results of the PCR products of the targeted samples are edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The observed nucleic acids were numbered in PCR amplicons as well as in their corresponding positions within the referring genome. Each detected variant within the *Leishmania tropica* sequences was annotated by Snapgene Viewer ver. 4.0.4 (https://www.snapgene.com).

3.4.5. Deposition of sequences to GenBank

Both of the investigated and analyzed sequences were submitted to the NCBI Bankit portal and all the instructions described by the portal were followed as described by the server (Benson *et al.,* 2017). The submitted

sequence was provided as nucleic acid sequences in the NCBI to get a unique GenBank accession number for the investigated sequences.

3.4.6. Comprehensive phylogenetic tree construction

A specific comprehensive tree was constructed in this study according to the neighbor-joining protocol described by Sarhan *et al.* (2019). The observed variants were compared with their neighbor homologous reference sequences using the NCBI-BLASTn server (Zhang *et al*. 2000). Then, a full inclusive tree, including the observed variant, was built by the neighbor-joining method and visualized as a circular cladogram using the iTOL suit (Pearson, 2013). The sequences of each classified phylogenetic group in the comprehensive tree were colored appropriately**.**

3.5. Isolation of Mononuclear Cells (MNC) From Buffy Coats Samples

Peripheral Blood Mononuclear Cells (PBMCs) are contained within the buffy coat alongside platelets, leukocytes, and granulocytes. To isolate PBMCs from the buffy coat, density gradient centrifugation can be used.

PBMCs may be removed from the buffy coat using manual or automated methods, with or without density-gradient materials such as albumin, Ficoll, and Percoll. These hydrophilic colloids (polymers formed by copolymerization of sucrose and epichlorohydrin or polyvinylpyrrolidone-coated colloidal silica) remove essentially all RBC and myeloid elements, leaving only mononuclear cells (Efthymiou *et al*, 2022)

1- The day before your scan, your buffy coats were collected. The buffy coat should be isolated from whole blood samples, or it can be procured from a

biorepository. Our protocol for buffy coat isolation from whole blood can be read here.

2- The buffy coat should be diluted with PBS in a 1:1 ratio in a 50 mL conical tube. Gently mix the contents by inversion.
3- Prepare a 50 mL conical tube and dispense 15 mL of room temperature density gradient media (DGM) into it. Pour 35 mL of the blood/PBS mixture on top of the DGM, ensuring that they remain separate without mixing. This will create two distinct phases.
4- Centrifuge the tubes at 900 ×g for 22 minutes at room temperature.
5- Take a 15 mL tube and add 10 mL of PBS. Carefully transfer the PBMC layer from the centrifuged tubes to the 15 mL tube, being careful not to disturb the interphase or aspirate the DGM. Gently invert the tubes to mix.
6- Centrifuge the 15 mL tubes containing the PBMCs-PBS mixture at 250 ×g for 5 minutes. Discard the supernatant.
7- Resuspend the pellet in PBS and centrifuge it at 250×g for 5 minutes at room temperature. Discard the supernatant.
8- If the supernatant is still cloudy, repeat washing steps 6-7 (optional).
9- Resuspend the washed beads in PBS at the desired concentration for downstream use. If you require assistance with cell counting, a protocol is outlined in the next section of this article.

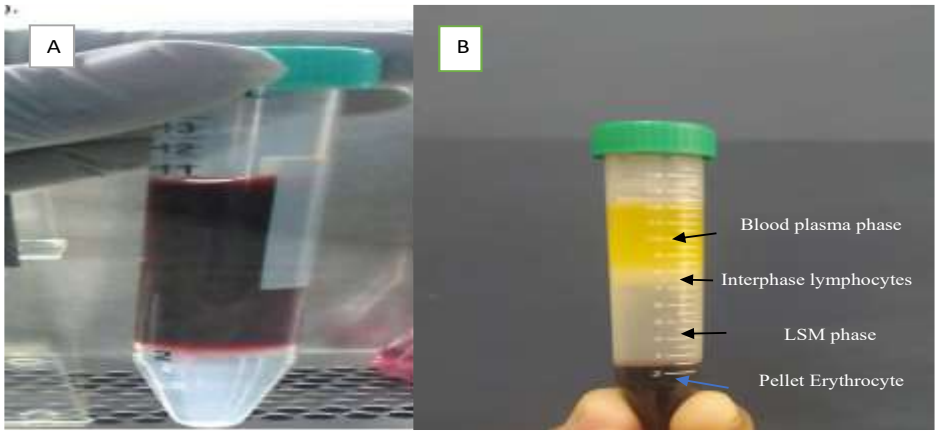


Figure (3.3)A- Blood Separation Tube with lymphocyte separation medi. B - after centrifugation Diluted plasma, buffy coat Interphase lymphocytes and Pellet Erythrocyte

3.5.1. Culturing the Isolated Peripheral Blood Monocytes

The previously prepared media (RPMI+1%pencillin-strep. + 10%FBS) was added to the pellets of PBMC after isolation and washing to be cultured in a T25 flask and incubated for 24 h. at 37C and 5%CO2, in this step the monocytes will adhere to the surface and the lymphocytes remain in suspension

3.5.2.U937 cancer cell line

The Institut Pasteur provided the U937 macrophage cell line. (Tehran, Iran). U937 is a human pro-monocyte myeloma cell line, obtained from the histiocytic lymphoma of a 37-year-old man. (Sundström and Nilsson, 1976). This cell line exhibits many characteristics of monocytes and is easy to use the cell line U937 is was grown in RPMI1640 medium with 10% HIFBS and 1% penicillin/streptomycin at 37°C in a humidified incubator with 5% CO2. An inverted microscope is used to inspect the flask of cells, which are thoroughly scrutinized for any signs of microbial contamination. Also, see whether any more cells are adhering to the flask's bottom (adherent cells) (Prasad *et al.*, 2022).

3.5.3.Cell Line and Culture Conditions

A human pro-monocytic myeloid leukemia cell line U937 is maintained in suspension culture in RPMI-1640 supplemented with L-glutamine (0.3 g/L), 10% (v/v) heat-inactivated fetal bovine serum (FBS), and antibiotics [1% (v/v) penicillin–streptomycin] purchased from Biosera (Nuaille, France). The cells were grown at 37°C in 5% CO_2 in a humidified atmosphere and subcultured every 3rd or 4th day. Dimethyl sulfoxide was used as an organosulfur compound, a polar aprotic solvent for PMA (Sigma Aldrich GmbH, Germany), and its final concentration in cell suspension is always maintained below 1%. U937 cells are induced to differentiate by exposing the cells (5×10^5 cells/mL) to 100 ng/ μL of PMA for 24h, 48h, and 72h the final concentration of 500 ng/ μL and 1000 ng/ μL PMA was based on previous studies (Baek *et al.*,2019)

3.5.4. Monocyte to macrophage differentiation

Preparation of stock solution of Phorbol myristate acetate (PMA). Add of DMSO and vortex until completely dissolved. then Prepare serial dilutions. 1000 ng/ μL, 500 ng/ μL ,100 ng/ μL. Before using monocytes, they are distinguished by adding 500 ng/ μL from phorbol 12-myristate-13-acetate (PMA) to monocyte cells to convert them to macrophage after incubator at 37c for 24h. As shown below, U937 monocytic cells are transformed from a non-adherent, rounded, lymphocyte to an adherent flattened "macrophage-like" cell with PMA addition. Transformed cells develop elongated arms or pseudopods which increase surface area for antigen uptake. These physical characteristics can be used to differentiate microscopically between monocyte and macrophage morphologiesas shown in the figure (3.4), (3.5) Then the cells are multiplied by adding PHA-K (Phytohemagglutinin-K) as shown in the figures (5),(6) in the Appendix.

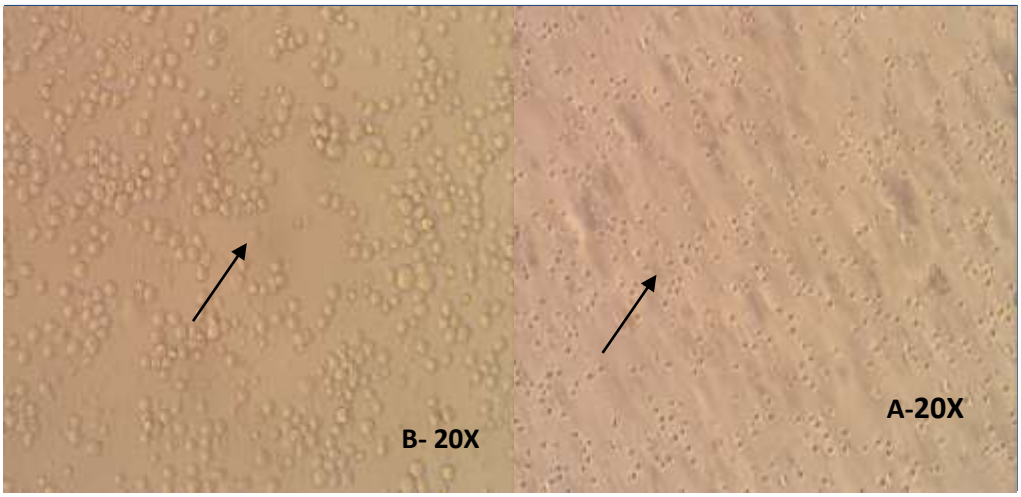


Figure (3.4): A- Normal monocyte cells under an inverted microscope at 20X power B - Normal monocytes differentiate into macrophages

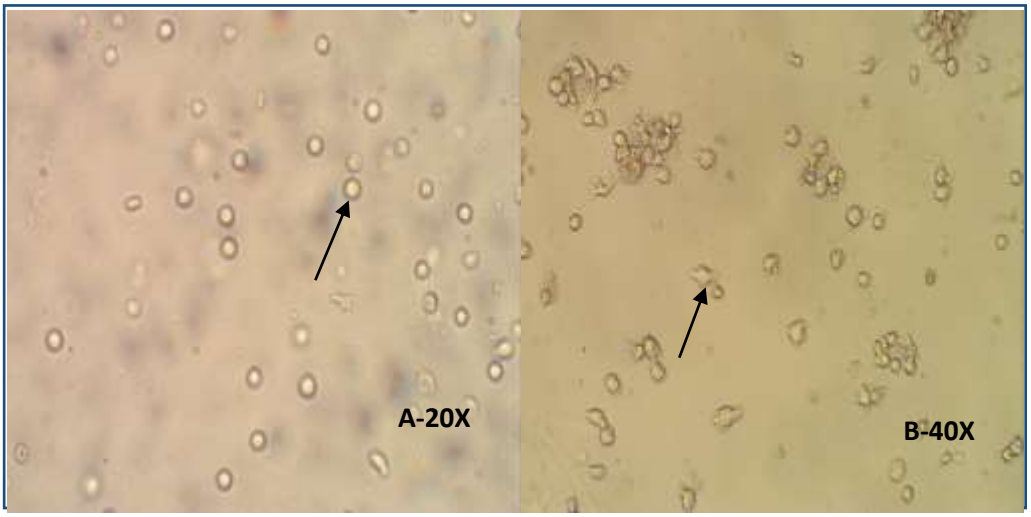


Figure (3.5): A- Microscopic pictures were taken of the untreated U937 cell B - after the 24-hour. Monocyte-to-macrophage differentiation at 40X power

3.5.5. Cytotoxicity assay (Colorimetric MTT assay) for parasites Treated with different concentrations of Pentostam

L. tropica promastigotes (2 x 105 parasites/well) and test compounds (and pentostam drugs) are prepared and dispensed in a flat-bottom 48-well microtiter plate with different concentrations starting from (2000,1000,500,250,125,62,5 mg/ml) and then incubated at 37˚C. These cultures were repeated in triplicate. The microtiter plate was incubated at 37˚C for one day. After 24, incubation periods of the wells, 10 µl of MTT solution is added per well to achieve a final concentration of 0.5 mg/ml. The microtiter plate is incubated for 4h at 37˚C. The media was removed and 100µl of DMSO solution was added to solubilize the formazan crystals. The microtiter plate is stirred gently and then left for 15 minutes. The optical absorbance of these plates was measured by the ELISA reader at 570 nm. The viability percentage was calculated

For all concentrations of pentostam, the results were plotted and compared to the control group. Cytotoxicity was measured using data obtained from the microtiter-plate reader and computed as the mean ± standard deviation (SD). The percentage of viability was estimated using the following equation

$$\%\ viability = \frac{mean\ absorbance\ of\ test\ sample\ at\ 620\ nm}{mean\ absorbance\ of\ control\ \ sample\ at\ 620\ nm} \times 100$$

Also, IC50 was estimated for each cytotoxicity test, the concentration that inhibited 50% of the cell growth, which was calculated by SPSS soft-wear 2010 and excel application was considered as IC50 value (Hagiya *et al*., 2012).

3.5.6. Ex-vivo infection of normal macrophage cell with *L. tropica* Amastigotes and Treated with different concentrations of Pentostam

infection normal macrophage cell with *L.tropica* promastigotes (1:10 parasites/cell) and after 24h pentostam drugs were prepared and dispensed in a flat-bottom 48-well microtiter plate with different concentrations starting from (2000-1500-1000- 500- 250- 125- 62,5 mg/ml) and then incubated at 37˚C. These cultures are repeated in triplicate. The microtiter plate was incubated at 37˚C. After 24h, 48h, and 72 h incubation periods of the wells, 10 µl of MTT solution was added per well to achieve a final concentration of 0.5 mg/mL. The microtiter plate was incubated for 4h at 37˚C. The media was removed and 100µl of DMSO solution was added to solubilize the formazan crystals. The microtiter plate was stirred gently and then left for 15 minutes. The optical absorbance of these plates was measured by the ELISA reader at 570 nm. The viability percentage was calculated

3.5.7. Ex-vivo infection of U937 macrophages with *L. tropica* amastigotes treated with different concentrations of Pentostam

infection U937 macrophage cell with *L.tropica* promastigotes (1:10 parasites/cell) and after 24h pentostam drugs were prepared and dispensed in a flat-bottom 48-well microtiter plate with different concentrations startingfrom (2000-1500-1000- 500- 250- 125- 62,5 mg/ml) and then incubated at 37˚C. These cultures are repeated in triplicate. The microtiter plate was incubated at 37˚C. After 24h, 48h, and 72h incubation periods of the wells, 10 µl of MTT solution was added per well to achieve a final concentration of 0.5 mg/ml. The microtiter plate is incubated for 4h at 37˚C. The media was removed and 100µl of DMSO solution was added to

solubilize the formazan crystals. The microtiter plate was stirred gently and then left for 15 minutes. The optical absorbance of these plates is measured by the ELISA reader at 570 nm. The viability percentage was calculated (Neamah *et al*.,2023)

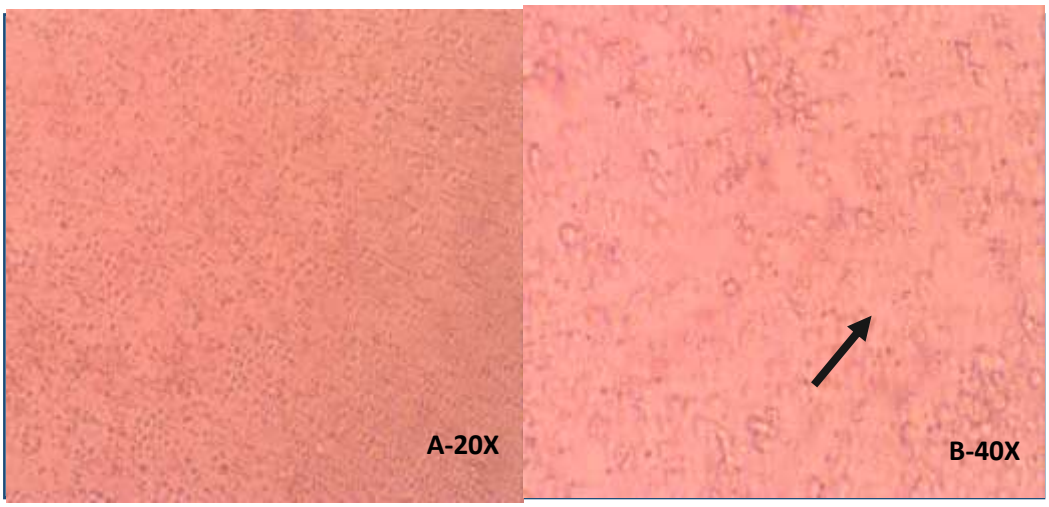


Figure (3.6): A- infection of U937 macrophages with *L. tropica* Amastigote (Parasite inside macrophage cell) at power 10X. B- infection of U937 macrophages with *L. tropica* Amastigote at power 40X

3.6. Measurement of reactive oxygen species (ROS) Erel (2005).

In this method, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to the ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the assay being calibrated with hydrogen peroxide, and the results are expressed as Lmol H_2O_2 equiv/l). The total amount of oxidant molecules presents in the sample. The assay is carried out as follows: 225 µl solution of Reagent 1 (xylenol orange) 150 µM, NaCl 140 µM and glycerol 1.35 M in 25 µM H_2SO_4 solution, pH =1.75) was mixed with 11 Ll Reagent

2 (ferrous ion 5 mM and o-dianisidine 10 mM in 25 mM H_2SO_4 solution) and 35 Ll sample extract was added to it. The mixture was then read at 560 nm. The assay is calibrated with hydrogen peroxide, and the results are expressed as µmol H_2O_2 equiv/l)

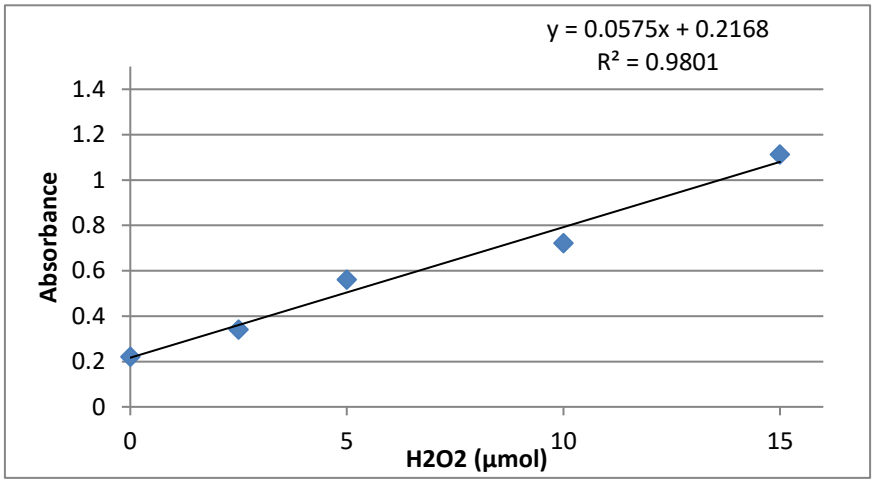


Figure (3.7): Standard curve of H_2O_2

3.7. Determination of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was determined according to the method of (Hafemann *et al* ; 1974).

Principle: The activity of GPx is determined by measuring the decrease in GSH content after incubating the sample in the presence of H2O2 and NaN3.

$H_2O_2 + 2\ GSH \longrightarrow 2H_2O + 2\ GSSG$

Procedure

The following are added to 0.1 ml of sample 0.1 ml of 5 mM GSH, 0.1 ml of 1.25 mM H_2O_2, and 0.1 ml of 25 mM NaN_3, and phosphate buffer (0.05

mM, pH 7) in a total volume of 2.5 ml at 37oC for 10 min. The reaction was stopped by adding 2 ml of 1.65 % HPO32- and the reaction mixture was centrifuged at 1500 rpm for 10 min. 2 ml of the supernatant was mixed with 2 ml 0.4 M Na_2HPO_4 and 1ml of 1mM (dithiops (2-nitrobenzoic acid)) DTNB. The absorbance of the yellow-colored complex was measured at 412 nm after incubation for 10 min at 37oC against distilled water. A sample without the tissue homogenate processed in the same way was kept as a nonenzymatic reaction

3.8. Estimation of Serum Malondialdehyde (MDA):

Malondialdehyde is estimated by the Thiobarbituric acid (TBA) assay method of Buege & Aust, 1978 on a spectrophotometer. This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. The basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of thiobarbituric acid to form a red MDA-TBA complex which can be measured at 535 nm.

Stock TCA – TBA – HCl Reagent:

It is prepared by dissolving 15% W/V trichloroacetic acid 0.375% W/V thiobarbituric acid and 0.25N HCl to make 100 ml (2.1 ml of concentrated HCl in 100 ml). This solution was mildly heated to assist in the dissolution of TBA. Dissolved 15 gm TCA and 0.375 mg thiobarbituric acid in 0.25 N HCl and volume was made up to 100 ml with 0.25 N HCl.

Procedure:

To 0.4 ml of serum, 0.6 ml TCA-TBA-HCl reagents are added. It was mixed well and kept in a boiling water bath for 10 minutes. After cooling 1.0 ml freshly prepared 1N NaOH solution was added. This absorbance of pink

color is measured at 535 nm against blank which contained distilled water in place of serum. In blank 0.4 ml distilled water and 0.6 ml TCA-TBA-HCl reagent was mixed and boiled. Blank was always taken.

Calculation: -

Malondialdehyde (μmol/l) =Absorbance of sample / E_0*L *D

Where:

E_0 = Extinction coefficient1.56* 10^5 M^{-1} cm^{-1}

L = Light path cm.

D = Dilution factor =6.7*10^6

3.9. Total Antioxidants Capacity Assay: The Cuprac Method

Principle: (Apak et al., 2007)

Total antioxidants + Cu+2 ⟶ Cu^+

Cu^+ + 2,9 - dimethyl- 1,10-phenanthroline ⟶ complex (λ max at 450 nm)

Reagents:

1. Copper (II) chloride solution at a concentration of 10-2M was prepared from $CuCl_2 \cdot 2 H2O$ weighing 0.4262 g, dissolving in H2O, and diluting to 250 ml with water.
2. Ammonium acetate (NH4Ac) buffer pH = 7.0 was prepared by dissolving 19.27 gram of NH4Ac in water and completing the volume to 250 ml.

3. Neocuproine (Nc){2,9-dimethyl-1,10-phenanthroline} solution at a concentration of 7.5 * 10^{-3}M was prepared by dissolving 0.039 gram Nc in 96% ethanol , the volume was completed to 25 ml with ethanol.
4. The standard solutions of sample antioxidants are prepared at 1.0 * 10^{-3}M Torolox.

Procedure

Reagents	Test	STD	Blank
Copper (II) chloride solution	1ml	1ml	1ml
Sample	50 µl	------	-------
Working standard solution	-------	50 µl	-------
D.W	-------	-------	50 µl
Neocuproine (Nc) solution	1ml	1ml	1ml
Ammonium acetate (NH_4Ac) buffer	1ml	1ml	1ml
Test tubes was mixed by vortex and incubated for 30 minutes at 37°C, after that the absorbance was read on a spectrophotometer at 450 nm.			

Calculation: $\text{Total antioxidants levels} = \frac{\text{A.test}}{\text{A.STD}} * Conc.of \quad STD \ (\text{mmol/l})$

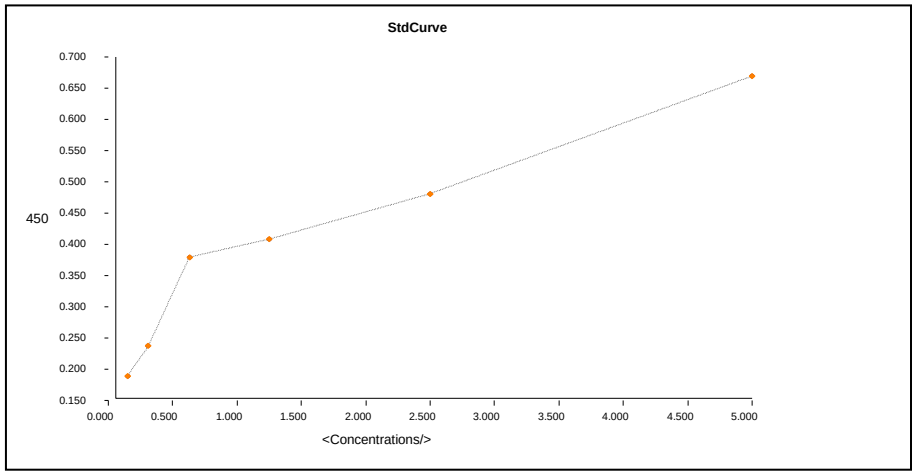


Figure (3.8): Standard Curve of Total Antioxidant Capacity Concentration

3.10. ELISA method

3.10.1. Cytokine Assay

To determine the cytokine concentrations in serum samples, standard curves for each cytokine were created by running the assays and optimizing them. Sensitivity ranges were measured in pg/ml for IL-10, IL-B1 pg/ml , TNF- α pg/ml , TGF-B1 pg/ml , As shown in figure (3.9), (3.10), (3.11) ,(3.12) .

The coating solution used in ELISA often contains phosphate buffer3. sodium carbonate (Na2CO3) and bicarbonate (NaHCO3) to the coating solution to increase the pH and make it more alkaline (pH=9). This basic environment provides ideal conditions for the binding of proteins to the polystyrene surface of the ELISA plate.

The coating solution promotes the formation of covalent bonds between proteins in the sample and the surface of the ELISA plate. This ensures that the proteins remain immobilized during subsequent steps of the ELISA, such as washing and detection, optical density O.D value was Determined for

each well at once with a micro-plate reader set to 450 nm (Chiswick *et al*., 2012).

3.10.1.1. Interlukin-10 (IL-10)

3.10.1.2. cytokine Assay Preparation of Items and Solutions for ELISA Technique

1. Brought all reagents to room temperature (18~25℃) before use. Followed the Microplate reader manual for set-up and preheat it for 15 min before optical density OD measurement.

2. Wash Buffer: A volume of 30 mL of Concentrated Wash Buffer diluted into 750 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved. 50

3. Standard working solution: the standard was Centrifuged at 10,000×g for 1 min, 1.0 mL of Reference Standard and Sample Diluent were added, it let stand for 10 min and it turned upside down several times. After it dissolved fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 2000 pg/mL. Then serial dilutions as needed. The recommended dilution gradient is as follows: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/mL.

4. Dilution method: Eppendorf tubes (7) taken, a volume of 500uL of Reference Standard & Sample Diluent added to each tube. Pipette 500 of the 2000 pg/mL working solution to the first tube and mix it up to produce a 1000 pg/mL working solution. Pipette 500 uL of the solution from the former tube into the latter one according to these steps

5. Biotinylated Detection Ab working solution: the required amount Calculated before the experiment (100 μL/well). In preparation, slightly

more than calculated should prepared. The stock tube was Centrifuged before use, and the 100× concentrated Biotinylated detection Ab diluted to a 1×working solution by Biotinylated Detection Ab Diluent

6. **Concentrated HRP Conjugate working solution**: the required amount Calculated before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. The 100×Concentrated HRP Conjugate Diluted to 1×working solution by Concentrated HRP Conjugate Diluent

Assay procedure

1. Standard working solution added to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 uL for each well). Added the serum samples to the other wells (100 uL for each well). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃.
2. Removeed the liquid from each well, do not wash. Immediately added 100 μL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37°C
3. Aspirate or decant the solution from each well, and add 350 uL of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeated this wash step 3 times. Add 100 μL of HRP Conjugate working solution to each well. Covered with the Plate sealer. Incubated for 30 min at 37 °C.
4. Aspirate or decant the solution from each well, and repeat the wash process five times as conducted in step 3.
5. A volume of 90 μL of Substrate Reagent Added to each well. Covered with a new plate sealer. Incubated for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes.

6. Stop Solution 50 µL of Added to each well.
7. the optical density O.D value was Determined for each well at once with a micro-plate reader set to 450 nm immediately and the results were calculated as seen in the figure

3.10.1.2. TGF-B1, TNF-α and IL-B1 Cytokine Assay

The assay max human TGF-B1, TNF-α and IL-B1 ELISA kit was conducted according to the manufacturing company (Elabscience / China) using the same procedure described in (IL-10) as seen in figures (3-9) ,(3-10) and (3.11).

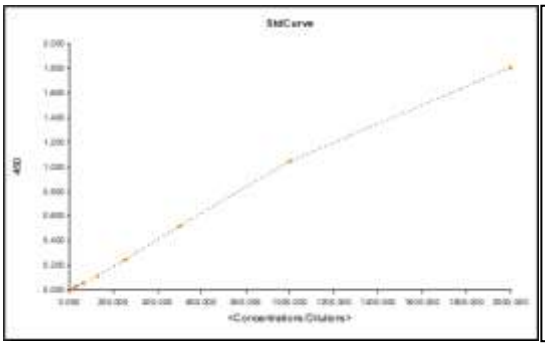


Figure (3-9): Standard Curve of IL-10 Concentration

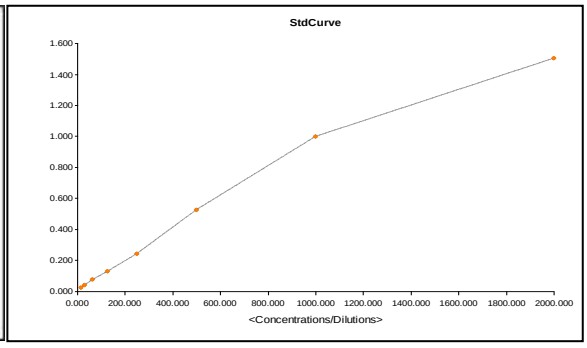


Figure (3-10): Standard Curve of TNF-α Concentration

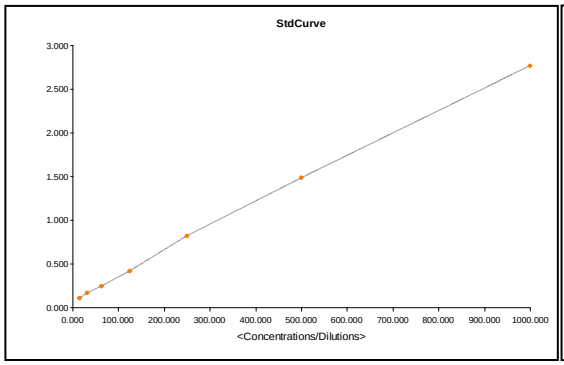


Figure (3-11): Standard Curve of TGF-B1 Concentration

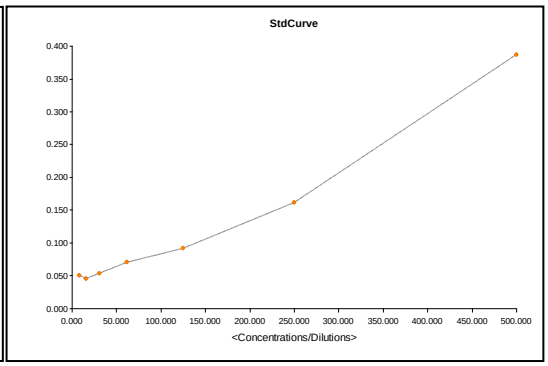


Figure (3-12): Standard Curve of IL- B1 Concentration

3.11. Calculate the results

According to the concentration of IL-10, TNF- α, TGF-B1, and IL-B1 in the samples by drawing the calibration test, Figure (3.13), which performs the same test as in the sample according to the instructions and steps of the kit. The absorbance value is projected on the standard curve and the corresponding concentration was calculated

3.12. Statistical analysis

1- Using a t-test, evaluate whether there are any significant differences between the means of the control and test values for each concentration after 24 hours, 48 hours, and 72 hours. Applying that, the difference between the means of amastigote and promastigote has been evaluated at ($p \leq 0.05$) and reported as (Mean± SEM).

2- In the study conducted by Hagiya *et al*. (2012), IC50 values were determined for cell viability using an interpolation method. The x-axis represents the logarithm of the concentration of the tested compound, while the y-axis represents the viability of the cells. By employing the interpolation method, the IC50 value can be found.

3- Data is analyzed using SPSS (version 26, SPSS Inc. Chicago, Illinois, USA). Descriptive statistics (mean, standard deviation) and statistical analysis were carried out using two-way ANOVA at ($p \leq 0.05$), and differences are compared by least significant difference (LSD). Pearson's correlation coefficient (r) determined the relationship between the studied parameters. The sigma plot program was used to draw the curves. (Quinn and Keough , 2002) .

Chapter Four

Results and Discussion

4. Results and Discussion

4.1. Growth of *L. Tropica* Promastigote in NNN Medium and RBMI 1640 Medium

The amastigote stage of the *L.tropica* parasite is observed after 5 days of culture on NNN medium at 25°C. *Leishmania* parasites can be remaining in NNN medium for six months at - 4°C The results of this study were consistent with Aboud (2020).

The promastigote phase appears in RBMI 1640 media when incubated at 4 °C, while the amastigote phase appears when incubated at 37°C. The given *Leishmania* strains were inoculated on NNN Medium and incubated at 24°C. After mass developing promastigotes in NNN media, they were moved to cell culture flasks containing RPMI 1640 (Sigma R8755-1L, Germany) supplemented with 10% Fetal Bovine Serum, HEPES, and 80 g/mL gentamicin. Under an inverted microscope (Leica S40/0.45, Germany), the vitality of promastigotes was examined in flasks (Balikçi *et al*., 2021).

4.2. Diagnosis of the Parasite

4.2.1. Microscopic Examination

There is found to be a cutaneous leishmaniasis infection. A case that had been discovered in the Mosul province was clinically and laboratory confirmed in October 2023.

In addition to being grown in RPMI 1640 medium and examined under a 20X inverted microscope, it was also grown in NNN medium and examined with a 100X light microscope The diagnosis of *Leishmania* species depends on the microscopic examination of smears stained with Gemsa stain and parasite samples isolated from skin lesions of suspected patients with CL and

cultured directly on semi-solid culture media and NNN and confirm the diagnosis of this activity-dependent isolate motile flagellar phase and this agrees with Pourmohammadi *et al*. (2010).

4.2.2. Detection of *Leishmania* by Polymerase Chain Reaction (PCR)

Conventional PCR technique is used to determine the type of parasite that causes cutaneous leishmaniasis, DNA was extracted The DNA of the *Leishmania* kDNA parasite was extracted from the sample and electrophoretically transmitted to ensure its presence in the eye Then it was kept in the necessary refrigeration until use The reactions were carried out by using a PCR machine to copy and amplify the targeted part of the DNA This part was numerically migrated, the products of the PCR reaction are transferred to an agarose gel with a concentration of 2%, and the expoeted bands of 325 bp length belonging to the species *L. tropica*.

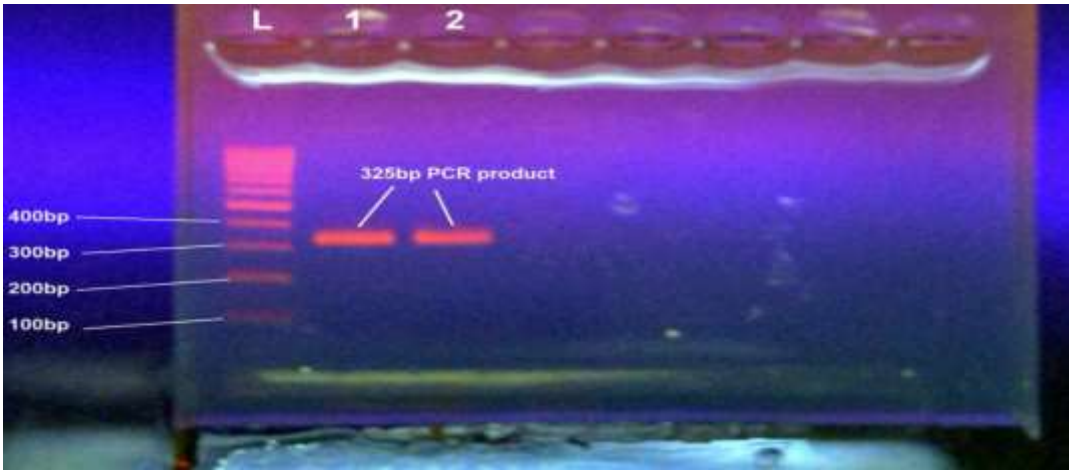


Figure (4.1). Image of agarose gel electrophoresis of PCR products analysis of (ITS1,5.8S,ITS2) for *leishmania* isolates using conventional PCR technique 2% agarose gel concentration and the electric current was done at 10volt and 80 Amp for 45 minutes

*the letter “L” refers to ladder marke

* Lanes 1 and 2 refer to the nember of sample

4.2.3. Sequence Outputs

Within the targeted locus, only one sample is included in the present study. This sample was screened to partially amplify the ribosomal sequences of the *L. tropica*. This is due to the fact that the variation of the ribosomal sequences can be used for *L. tropica* genotyping due to its possible ability to adapt to variable genetic diversity due to many features associated with its biological activity. The sequencing reactions indicated the exact identity after performing NCBI blastn for these PCR amplicons (Iyer *et al*., 2012).

Concerning the 325 bp amplicons, the NCBI BLASTn engine showed about 99% sequence similarities between the sequenced samples and the intended reference target sequences. By comparing the observed nucleic acid sequences of these investigated samples with the retrieved nucleic acid sequences (GenBank acc. OP811448.1), the accurate positions and other details of the retrieved PCR fragments were identified. The total length of the targeted locus was localized in the NCBI server, and the positions of the start and end of the targeted locus are also confirmed within the most homologous *L. tropica* target Figure (4.2).

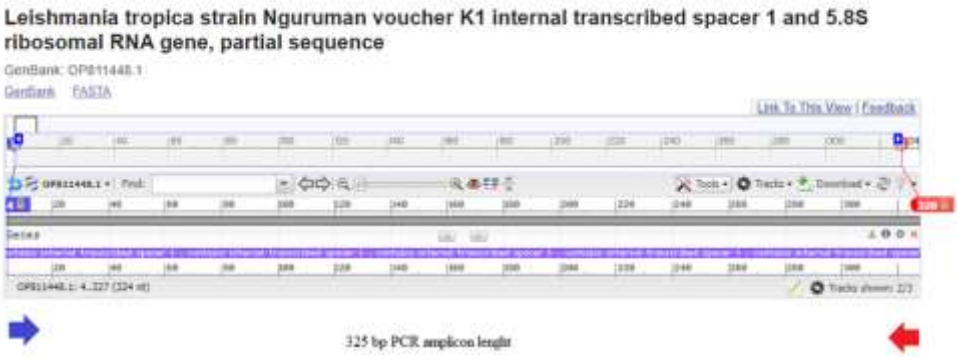


Figure (4.2). The exact position of the retrieved 325 bp amplicon partially covered the ribosomal portions of the L. *tropica* genomic sequences (GenBank acc. no. OP811448.1). The blue arrow refers to the starting point of this amplicon while the red arrow refers to its endpoint.

After positioning the 325 bp amplicons' sequences within the genomic sequences of the *L. tropica*, the details of its sequences were highlighted, and the total length of the amplified amplicons was also determined (Table 4.1).

Table (4.1) The position and length of the 325 bp PCR amplicons that are used to partially amplify the ITS1, 5.8S, and ITS2 ribosomal sequences within *L. tropica* genomic sequences (GenBank acc. no. OP811448.1).

	Reference locus sequences (5′ - 3′)	length
Ribosomal sequences of the *Leishmania tropica*	*TGATACCACTTATCGCACTTTACTGCGTTCTTCAACGAAATAAAGC CAAGTCATCCATCGCGACACGTTATGTGAGCCGTTATCCACACACA CGCACCCCCCGCCAACAAAACCGAAACGCCGTATATTTGTATAAAC ACATTTTTGCTTTTGTGTATACTGCGTTATAATCGATCCCTTTTGTTT ACTGCAAACTTCGAGTATAAAAGTTTGCTGTGTATGTGTGAAACCT ATATAATGTATAATATATACCTCCCCGAGTTTTGTATATGTTTTTTTT GTGTAATCATC AAAATGATCCAG**	325 bp

* refers to forward primer placed in the forward direction

**refers to reverse primer placed in the reverse complement direction

Interestingly, the alignment results of the 325 bp sample revealed the presence of one nucleic acid variation represented by the deletion of two nucleotides in the analyzed sample in comparison with the most similar referring reference nucleic acid sequences (GenBank acc. no. OP811448.1). In this deletion adenine nucleotide and its adjacent cytosine nucleotide were deleted from positions 94th and 95th in the amplified PCR product (Fig. 4.3).

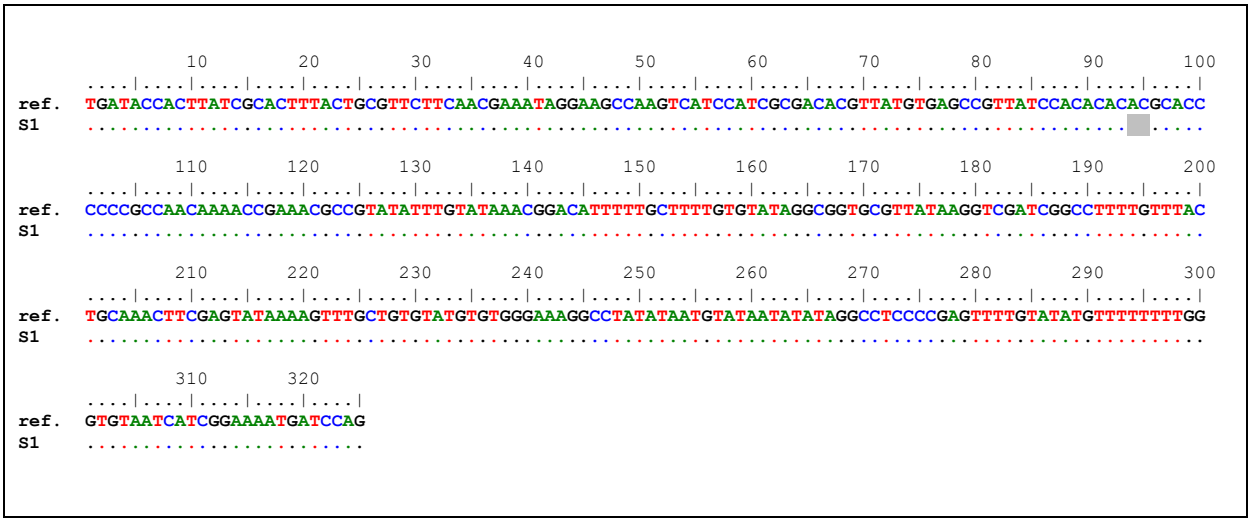


Figure (4.3) Nucleic acid sequences alignment of one sample with its corresponding reference sequences of the 325 bp amplicons of the *L.tropica* ribosomal sequences. The symbol "ref" refers to the NCBI referring sequence (GenBank acc. no. OP811448.1), a nd the letter "S#" refers to the sample number.

Our results showed the identification of nucleic acid deletion observed in the investigated samples, namely 94-95ACdel in the investigated S1 sample. To confirm this deletion, the sequencing chromatogram of the investigated sample, as well as its detailed annotation, are verified and documented. Furthermore, the chromatogram of its this variant is shown according to its position in the PCR amplicons. The presence of this variant is confirmed in their original chromatograms and numbered according to their positions in the amplified PCR products (Fig. 4.3).

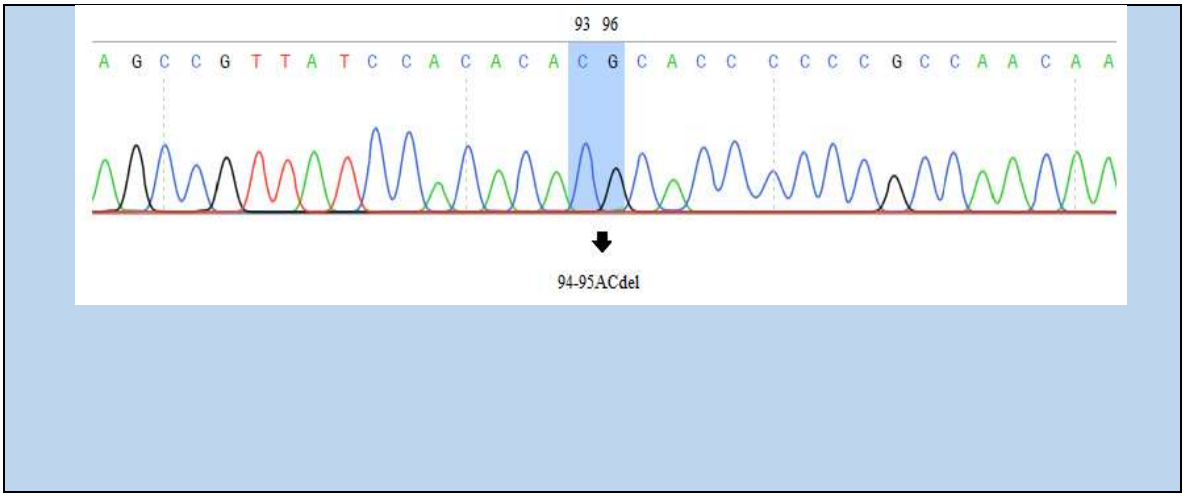


Figure (4.4). The chromatogram of the investigated. *L.tropica*. The letter "del" refers to the deletion of nucleotides in the investigated sample in this study.

The investigated sample is deposited in the NCBI web server, and a unique accession number is obtained for the analyzed S1 sequences. The deposited sequences received the GenBank accession number OQ653468 to represent the S1 sample.

To give a phylogenetic understanding of the actual distances between our investigated sample and the most relative reference strains of *L.tropica*, a comprehensive phylogenetic tree is generated in the present study according to nucleic acid sequences observed in the amplified 325 bp of the ribosomal amplicons. This phylogenetic tree contained the S1 sample alongside other relative nucleic acid sequences of *L.tropica.*

The total number of aligned nucleic acid sequences in this comprehensive tree was twenty-seven. Two types of cladograms are generated to explain two different representations of the incorporated *L.tropica* sequences, a circular cladogram Fig. (4.5). in both cases of the constructed cladogram, the incorporated sequences are clustered into two clades. The most interesting fact observed in our investigated *L. tropica* isolate is correlated with the

positioning of our sample into a distinct phylogenetic position within the clade of *L. tropica*.

This sort of diversity was reflected by the observed phylogenetic effects of the observed nucleic acid substitutions of 94-95ACdel due to their ability to cause obvious alterations in the generated clade. Thus, these variations have constituted important deviations within the same species of *L.tropica*. However, it is found that the investigated S1 sample was positioned in the vicinity of two strains (GenBanks FN677341.1 and OL413428.1) that were isolated from Palestinian and Malaysian sources, respectively. Accordingly, the Asian source of the S1 sample is confirmed. Distinct phylogenetic distances are observed between the clade of *L.tropica* and the nearest clade of the other *Leishmania* sequences.

This adjacent clade is attributed to *L. donovani* sequences, in which five closely related strains were incorporated. Thus, it was inferred from this tree that the clade of *L.donovani* shared a relatively high level of homology compared with the clade of *L. tropica*, which occupied a distinct phylogenetic position away from them. Due to its positioning toward the roots of the tree, it was inferred from this tree that the sequences of *L.tropica* have occupied ancestral positions compared with *L. donovani* sequences.

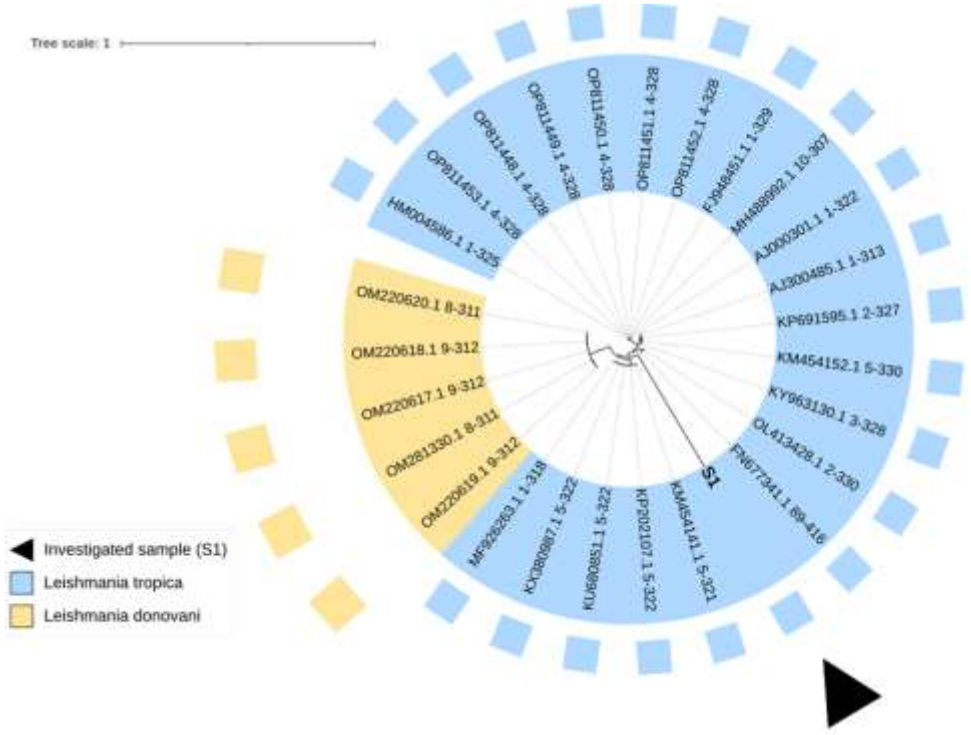


Figure (4.5). The comprehensive circular cladogram phylogenetic tree of ribosomal sequences of one sample of *L.tropica*. The black-colored triangle refers to the analyzed *L. tropica* sample. All the mentioned numbers referred to GenBank accession number of each referring species. The number "1" at the top portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms. The letter "S#" refers to the code of the investigated sample.

4.3. Cytotoxicity assay (MTT assay)

The drug cytotoxicity is tested against the *L.tropica* on promastigotes , in order to determine its cytotoxicity before ex-vivo infection and its effects on the viability of the *Leishmania* parasite which is used to examine cytotoxicity for Sodium Stibogluconate (Pentostam) concentrations, the results were plotted and compared to the control group. Cytotoxicity was measured using data of microtiter-plate reader absorbance and computed as mean± standard deviation SD, the cytotoxicity result showed pentostam ability to inhibit parasite development and proliferation,

as well as a difference in colorimetric absorption. After 24 hours follow-up was performed for promastigotes at all concentrations: 2000,1500, 1000, 500, 250, 125.5 and 62.5, mg), and was found out that there was a statistically significant ($p \leq 0.05$) difference in microtiter plate reader absorption between test and control. except for the low concentration of the drug, there is no statistically significant difference. this was in agreement with Lanza *et al.* (2022) Research on the effect of pentostam on *L. tropica* amastigotes in human macrophage cell line THP-1.

Table (4.2): Cytotoxicity assay (MTT assay) for parasite grow in RPMI1640 and Treated with different concentration of Pentostam (2000 -62, 5 mg/ml) after incubator at 37c for 24h

Group concentration (mg/ml). normal /24h	Mean+ Std Dev	P value
Control	100.000 + 3.059	
mg /ml Pen2000	40.370 +2.190	($P \leq 0.001$)
mg /ml Pen1000	71.012 +9.702	($P \leq 0.001$)
mg /ml Pen500	82.296 +8.087	($P \leq 0.002$)
mg /ml Pen250	85.895 +2.864	($P \leq 0.001$)
mg ml Pen125	92.996 +2.359	($P \leq 0.011$)
62.5 mg /ml Pen	94.261 +6.066	($P > 0.091$)

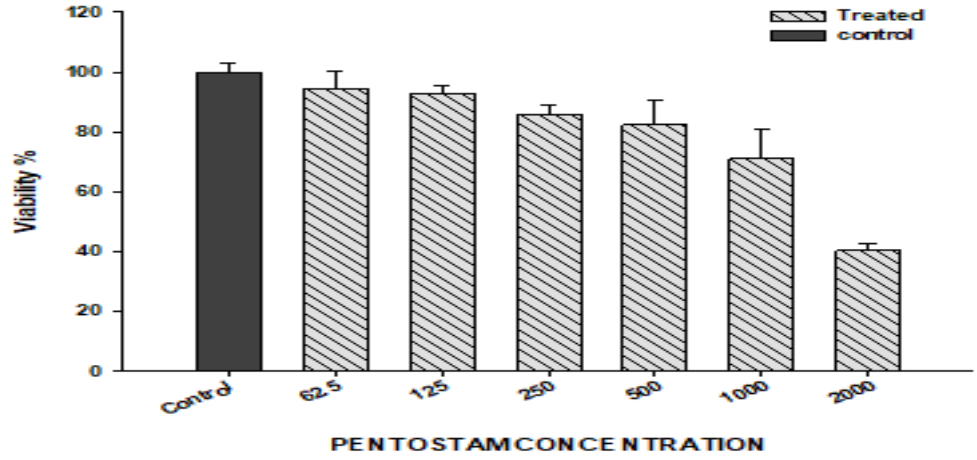


Figure (4. 6) parasite grow in RPMI1640 and Treated with different concentration of drug (2000 -62, 5 mg/ml) after incubator at 37c for 24h

It is found that cutaneous leishmaniasis can infect macrophages at a high rate when it remains without treatment, while when using pentostam treatment, the percentage of viability was found at the highest concentration of 40%, while the lowest concentration was 90%. These results demonstrate that the effect of pentostam on promastigotes infection is greater at higher concentrations, when compared to the control group, these results show that the effect of pentostam on parasitic infection is greater at higher concentrations compared to the control group. The current study agreed with Haydar *et al*. (2018), the survival rate of parasites in the sample remains at approximately 35% of the total number of parasites present at the beginning.

4.4. Ex-vivo infection of U937 cell line with *L.tropica* amastigotes after 24, 48 and 72h (Use different concentrations of Sodium Stibogluconate (Pentostam)

In vitro infection of human macrophage U937 is a widely used method for in vitro evaluation of new anti *leishmania* drugs and for testing the drug sensitivity Investigations have indicated the effect of *Leishmania* parasites on

the immune system of patients with cancer thus tri ering the modulation of anti-cancer immunity.

Kumar *et al. (*2017) highlighted the role of *Leishmania* in mutual modulation of the immune system in a patient with Hodgkin's lymphoma It's clear that in both diseases the host immune response is critical for the disease outcome. In this sense, immune checkpoints are probably essential for regulating immune system homeostasis and metastasis in cancer (Rashidi *et al*., 2021) both diseases are under the control of innate immunity including macrophages and natural killer cells, and of adaptive immunity including CD4/CD8 cells and regulatory T-cells (Schwing *et al*., 2019)

The drug cytotoxicity was tested against the *L.tropica* on culture cell line of promastigotes , as well as the U937 cell line, in order to determine its cytotoxicity before ex-vivo infection and its effects on the viability of the *Leishmania* parasite which was used to examine cytotoxicity and cell viability. for pentostam concentrations, the results were plotted and compared to the control group.

The cytotoxicity was measured by using the data of microtiter-plate reader absorbance and computed as the mean ± standard deviation (SD). The results of cytotoxicity demonstrated that pentostam has the ability to inhibit parasite development and proliferation, as well as a difference in colorimetric absorption. Follow-up was performed for promastigotes at different concentrations (2000, 1500, 1000, 500, 250, 125.5, and 62.5 mg) after 24, 48, and 72 hours. It was found that there was a statistically significant ($p \leq 0.05$) difference % of cell viability between the test and control groups. The results showed a significant decrease in the number of infected macrophages after treatment. The inhibition was found to be dose-dependent. This finding is consistent with the studies by Calvo Alvarez *et al*. (2022) and Hassan & Ali

(2020). Furthermore, the results indicated that artemisinin had a cytotoxic effect on the parasite, with a significant difference ($p \leq 0.05$) observed after 48 hours of treatment. Additionally, the percentage of infectivity of intracellular amastigotes was significantly decreased, as reported by Larraga *et al*.(2022) .

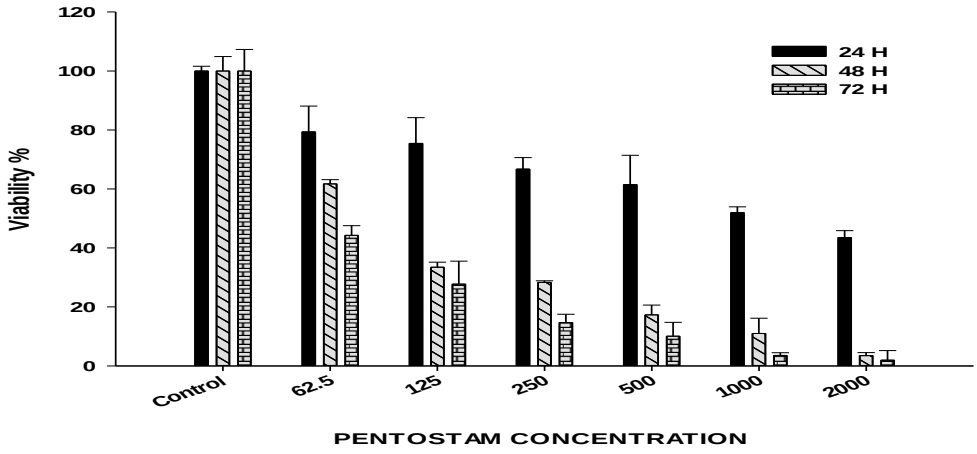


Figure (4.7) : Cytotoxicity of pentostam on U937 Cancer cell infected with *L. tropica* amastigotes treated with different concentrations of pentostam, after 24, 48 and 72 hours of incubation.

Another study (Looker *et al.,* 1986) investigated the impact of pentostam on *L. tropica* amastigotes in the human macrophage cell line THP-1. The researchers examined the cell viability and infectivity of the amastigotes after exposure to different doses of pentostam for 24, 48, and 72 hours. The findings revealed that pentostam exhibited a cytotoxic effect on both the parasites and host cells; however, it was more effective against the parasites than the host cells. By treating U937 cells infected with SSG at different time points (24, 48, and 72 hours), the temporal effect of SSG on parasite growth can be evaluated. This setup mimics the progressive nature of *Leishmania* infection and allows for the examination of the efficacy of SSG at different stages of parasite development. It can provide valuable insights

into whether early intervention with SSG is more effective than late-stage treatment.

4.5. Ex-vivo infection of nourmal cell line with *L.tropica* amastigotes after 24, 48 and 72h (Use different concentrations of Sodium Stibogluconate (Pentostam)

The use of Pentostam as a treatment for the *Leishmania* parasite works by blocking the glycolytic enzymes and the oxidation of the fatty acids of the parasite, which leads to its death. (Looker *et al.*, 1986)

The drug cytotoxicity is tested against the *L.tropica* on normal culture cell line of amastigotes, in order to determine its cytotoxicity before ex-vivo infection and its effects on the viability of the *Leishmania* parasite which is used to examine cytotoxicity and cell viability. for pentostam concentrations, the results are plotted and compared to the control group. cytotoxicity is measured using data of microtiter-plate reader absorbance and computed as mean± standard deviation SD.

The cytotoxicity result showed pentostam ability to inhibit parasite development and proliferation, as well as a difference in colorimetric absorption. After 24, 48 and 72 hours follow-up was performed for amastigotes at all concentrations: 2000,1500, 1000, 500, 250, 125.5 and 62.5 µg), and is found out that there is a statistically significant ($p \leq 0.05$) difference in microtiter plate reader absorption between test and control

MTT assays revealed that the percentage of parasites surviving after 72 hours is also affected by medication concentration, with higher concentrations resulting in lower survival rates .to determine cell vitality, the parasite viability is measured against pentostam concentrations. All pentostam concentrations were compared between the test and the control as

means ± standard deviation (SD). At the greatest concentratione of 2000 µM, the mean of promastigotee cell viability was observed to be 22.383 after 24 hours, (SD± 10.84). Further more, at the lowest concentrations of 62,5 µM, the mean of celle viability was determinede 41.877 after 24 hours, (SD± 8.12). This study showedethat there is a significant difference between the vitality of cells treated with a high concentration of the drug with a low concentration.

It was found that cutaneous leishmaniasis can infect macrophages at a high rate when it remains without treatment, while when using pentostam treatment, the percentage of viability was found at the highest concentration of 40%, while the lowest concentration was 90%. These results demonstrate that the effect of pentostam on promastigotes infection is greater at higher concentrations, when compared to the control group, The results of this study are consistent with the study by Haydar *et al.* (2018), which demonstrated a parasite survival rate of 35%. Our also agree with Nadaf and Haddad (2020), who reported that Pentostam reduced the infection index of *L.tropica* amastigotes in normal cells after 24, 48, and 72 hours of treatment.

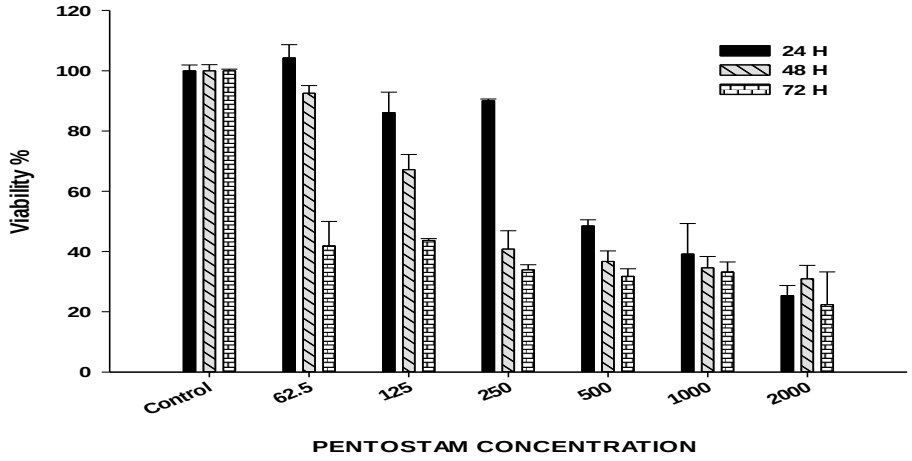


Figure (4.8) : Cytotoxicity of pentostam on Normal cell infected with *L. tropica* amastigotes treated with different concentrations of pentostam, after 24, 48 and 72 hours of incubation

Tabel (4.3): The IC50 values were determined for both normal and cancerous cells at 24, 48, and 72-hour time points. It is clear that 72 hours is the best period for inhibiting natural cells

	IC50	
Time (hours)	Normal cell	Cancer cell
24h	199.0	1426
48h	1967	2678
72h	6414	4384

The 72 hours IC50 values of normal cell and cancer cell are 6414, 4380 respectively. It was found that pentostam was effective on all cancerous and normal cell lines used in this study. Cancer cells were more sensitive than normal cells, while normal cells were not affected by pentostam during the 24-hour period, as shown in the figure(14) ,(15) in the appendix.. This may be due to the effect of treatment over time: antiparasitic substances may take time to fully interact with normal cells. It may have a greater effect after 72 hours than after shorter periods of time. The body's immune system may also develop over time and become more effective in confronting and killing parasites. The immune system may have a role in enhancing the effectiveness of treatment

when normal cells are targeted. . These results are consistent with Sen *et al*. (2007)

The higher IC50 value of normal cells compared to cancer cells infected with the *Leishmania* parasite can be attributed to several reasons. Cancer cells may exhibit altered metabolic pathways compared to normal cells, including increased glucose consumption and altered energy production. These metabolic differences can make cancer cells more susceptible to certain treatments, making cancer cells more sensitive to treatment than normal cells. The immune response to *Leishmania* infection can differ between normal and cancerous cells. The immune system may mount a stronger response against cancer cells, resulting in enhanced elimination of infected cancer cells and a lower IC50 value compared to normal cells. These results are consistent with Zheng *et al*. (2024)

4.6. TGF-β1 Cytokine Assay

Activation of macrophages during the early stages of *Leishmania* infection results in the generation of NO, ROS, and lysosomal enzymes, all of which are essential for parasite destruction. On the other hand, alternative TGF-β stimulation of macrophages creates a favorable environment for *Leishmania* proliferation (Liu & Uzonna, 2012) **.**

In the current study, a significant difference ($p \leq 0.05$) is observed between cancer cells and normal cells. This result is consistent with Tadesse *et al*. (2021) and The results are summarized in Figure (4.10), which illustrates the average differences in TGF-B1 based on various factors, including drug concentration, time period, and a comparison between infected normal cells and infected cancer cells.

Figure (4.9) This part of the figure shows the effect of different drug concentrations on TGF-B1 levels. It indicates the average difference in TGF-B1 levels when different concentrations of the drug are used. By examining this information, we can evaluate the dose-dependent effect of the drug on TGF-B1 levels. It helps determine whether there is an association between drug concentration and changes in TGF-B1 expression. It was found that the concentration of TGF-B1 decreased in normal cells before infection and during infection with the parasite (71.1± 7.1 - 71.1±7.4) respectively compared to its concentration in cancer cells (101.1± 8.2 - 90.7± 2.6)

In addition, TGF-B1 increased after giving SSG treatment There is an inverse relationship between the level of TGF-B1 and the treatment concentration in cancer cells infected with the parasite (r = -.220) . These results are consistent with Resende *et al*. (2016) .

Interestingly, the presence of TGF-β has been associated with inducing immunosuppression characteristics during the course of VL (Kaye *et al.,* 2004) Moreover, the presence of TGF-β in vitro has a protective effect on flagellar macrophages, favoring the maintenance of parasitism (Gantt *et al*., 2003)

In Section B of the figure, the average difference in TGF-B1 levels over different time periods is depicted. This section enables us to assess the temporal changes in TGF-B1 expression throughout the experiment. The results indicated that TGF-B1 concentration increased at 48 hours but decreased at 72 hours (r= .758**, -.134). These findings align with the study conducted by Alves *et al.* (2009), which reported elevated levels of TGF-β associated with an increased parasite load in the lymph nodes of symptomatic dogs.

of Figure (4,11) illustrates the average difference in TGF-B1 levels between the normal cell group and the cancer cell group, indicating the variation in TGF-B1 expression between these two groups. Significant differences in TGF-β1 expression can be expected because TGF-β1 is acytokine involved in regulating various cellular processes, including immune responses and inflammation. It possesses immunosuppressive properties and can inhibit the production of proinflammatory cytokines such as IL-β1. Conversely, IL-β1 is a pro-inflammatory cytokine responsible for initiating and promoting inflammation.

The observed inverse relationship suggests that *Leishmania* infection may alter the balance between TGF-β1 and IL-β1 in normal cells. The parasite might induce an increase in TGF-β1 levels, subsequently inhibiting IL-β1 production. This modification in cytokine levels could be a strategy employed by the parasite to manipulate the host's immune response and establish an environment conducive to its survival and persistence (Adwar, 2021) .

In cancer cells, the positive relationship between TGF-β1 and IL-β1 may be influenced by the unique characteristics of the tumor microenvironment and altered cancer-associated immune responses. TGF-β1 has complex roles in cancer development, and its increased production in the tumor microenvironment can contribute to tumor growth, immune evasion, and inflammation. The presence of the *Leishmania* parasite in this context may further modulatecytokine levels, leading to an elevation in both TGF-β1 and IL-β1.

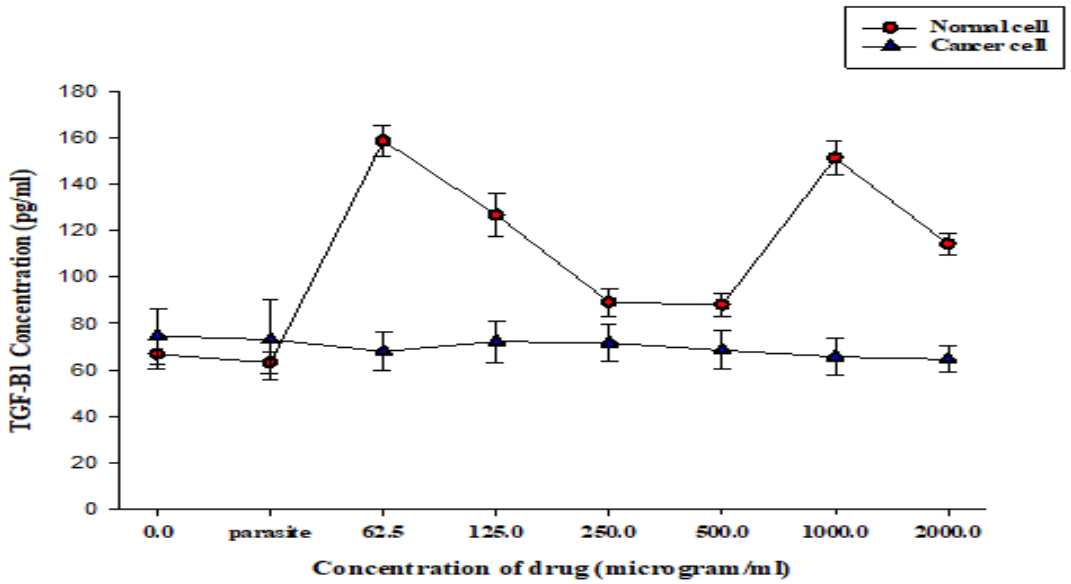


Figure (4.9) : Mean difference TGF-B1 according to concentration of drug.

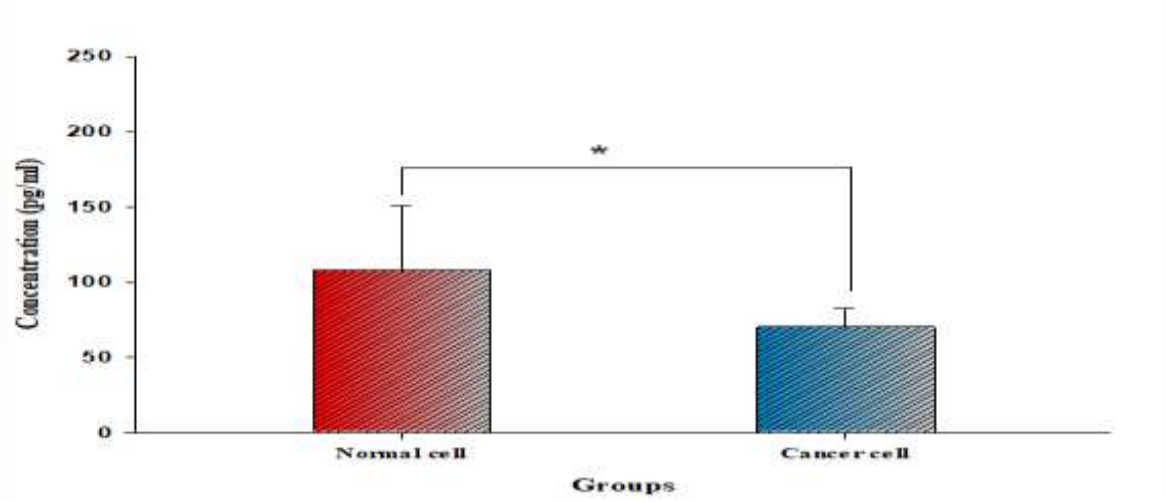


Figure (4.10) : Mean difference of TGF-B1 according to period asterisk (*) on the bar indicate significant difference ($p \leq 0.05$) .

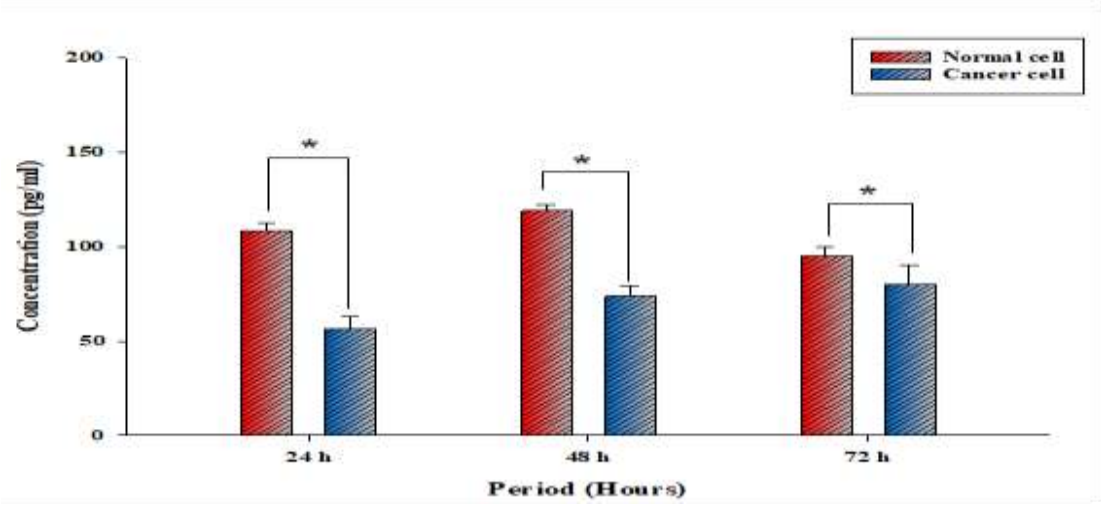


Figure (4.11) : Mean difference of TGF-B1 between normal and cancer cell**,** asterisk (*) on the bar indicate significant difference ($p \leq 0.05$) .

4.7. IL-1β Cytokine Assay

Interleukin-1 beta (IL-1β), a cytokine that promotes inflammation, is crucial to the immune system's defense against *Leishmania* infection. To destroy the intracellular parasites, IL-1β can activate neutrophils and macrophages. It can also trigger the production of other cytokines, including as IL-12, TNF-α, and IFN, which encourage Th1 differentiation and *Leishmania* resistance (Patil *et al*., 2018)

Overall, in this study, during the early stages of *Leishmania* infection, there is an increase in IL-1β production as part of the immune response to combat the parasite, The level of IL-1β decreased with increasing treatment concentrations, and this result is consistent with Santos *et al.* (2018a) **.**

This may be the result of antimony medications like pentostam inhibiting the synthesis of IL-1β and other proinflammatory cytokines. Through the inhibition of IL-1β production, pentostam has the potential to modulate the inflammatory response amongst *Leishmania* infection and avert hyperinflammation that may result in tissue damage (Franca *et al*., 2021).

There is a significant difference between normal and cancerous cells during a period of 24 hours, and there is no significant difference between 48 and 72 hours. As shown in the figure (4-13).

In this study, there is a significant positive relationship between the level of IL-B1 and the time periods in cancer cells infected with the parasite (r= .476**) This means that as the time period of infection increases, the concentration of IL-β1 also increases This result is consistent with Weber *et al*. (2023). This may be because a number of characteristics, such as immune cell infiltration, angiogenesis, and altered cytokine profiles, characterize the tumor microenvironment of cancer cells. *Leishmania* infection inside this milieu may alter the balance of cytokines, eventually increasing IL- β 1 levels. the presence of *Leishmania* parasites within the tumor microenvironment can have several effects on the cytokine balance, potentially leading to an increase in IL-1β levels . *Leishmania* parasites have immunomodulatory properties and can manipulate the host's immune response .they may create an environment that is favorable for their survival and persistence. This could involve suppressing anti-inflammatory cytokines or promoting pro-inflammatory cytokines like IL-1β .

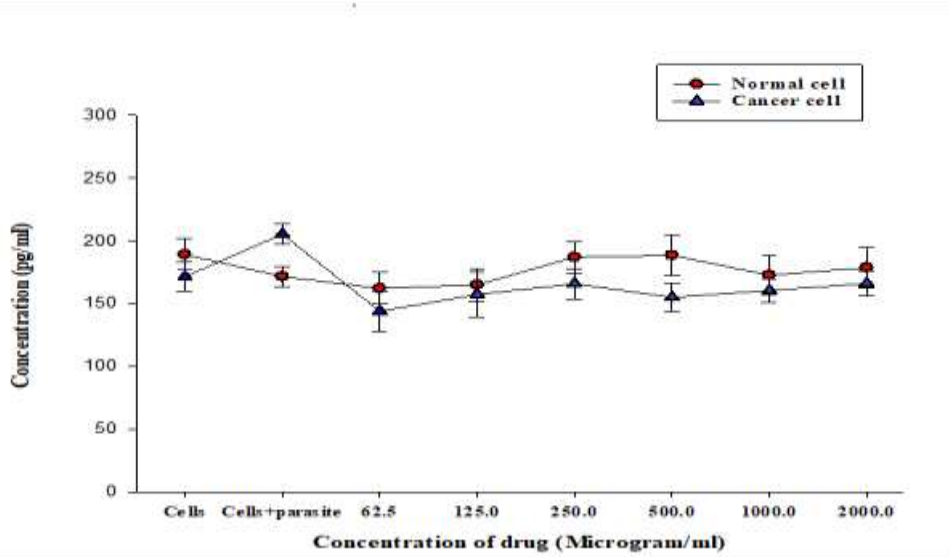


Figure (4-12) : Mean difference in IL-1β according to concentration of drug

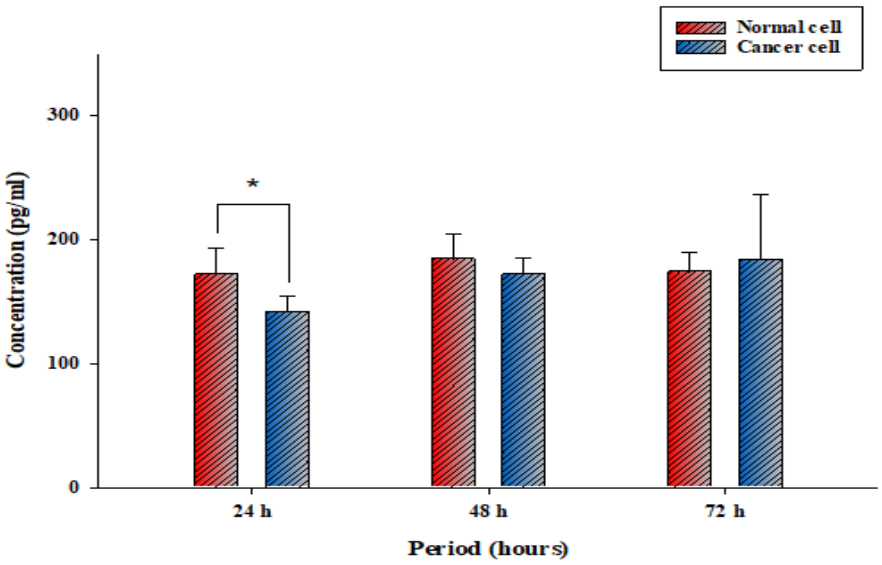


Figure (4-13) : Mean difference of IL-1β according to period. , asterisk (*) on the bar indicate significant difference ($p \leq 0.05$)

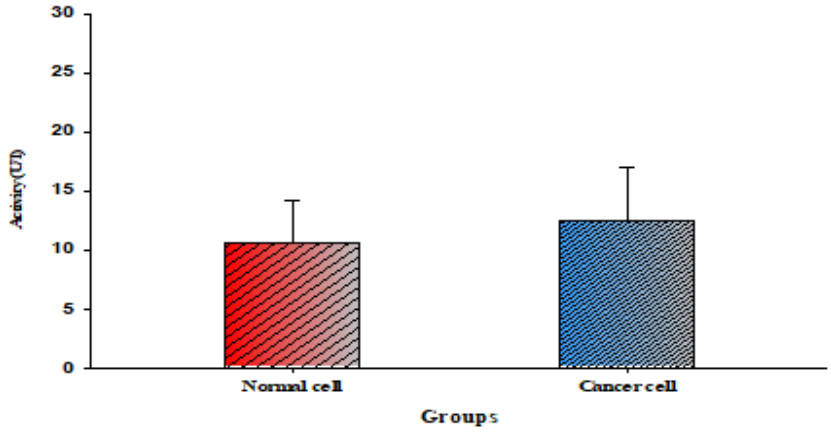


Figure (4-14) : Mean difference of IL-1β between normal and cancer cell , asterisk (*) on the bar indicate significant difference ($p \leq 0.05$)

4.8. Interlukin-10 (IL-10)

IL-10 levels, frequently fall as the infection continues. The ability of the host to mount an effective immune response against the parasite may be responsible for this reduction. As the immune system recognizes and works for control of the infection, IL-10 production may be downregulated to allow for greater pro-inflammatory responses and the activation of effector pathways to battle the parasite. This explains why IL-10 decreased between 48 and 72 hours In this experiment As shown in the figure (4.16). we observed an inverse relationship between IL-10 and both (treatment concentration, time period, and IL-1β) The correlation coefficient was (r=-.280*, -.729**, -.729**)

The negative correlation coefficients indicate that as the treatment concentration and time period increase, or as the IL-1β level increases, the level of IL-10 decreases in the cells infected with *Leishmania*. These findings suest that higher treatment concentrations, longer time periods, and higher IL-1β levels may contribute to a decrease in IL-10 production or release.

Increased IL-10 levels have been linked to tumor development and treatment resistance (Rallis *et al*., 2021)

Therefore, the concentration of IL-10 increased with increasing concentration of treatment in cancer cells and a significant difference between cancer cells and normal cells within 24 hours and 72 hours .This result was agreement with Tadesse *et al.* (2021). who attributed that highe levels of IL-10 are measured prior to treatment Which decreased sharply with treatment to less than the detection limit within 7 days in contrast, our findings indicated that the level of IL-10 was elevated during clactivity. These results differed with Ghalib *et al.*(1993) .

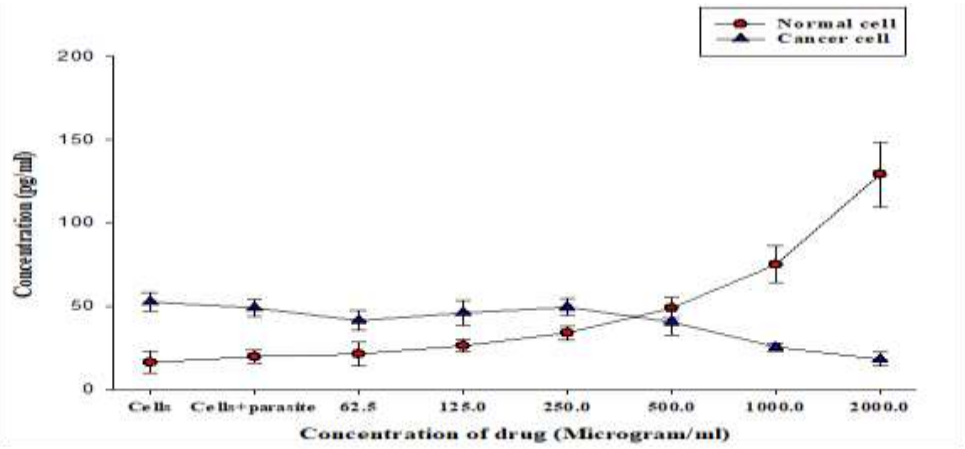


Figure (4-15) : Mean difference in IL-10 according to concentration of drug

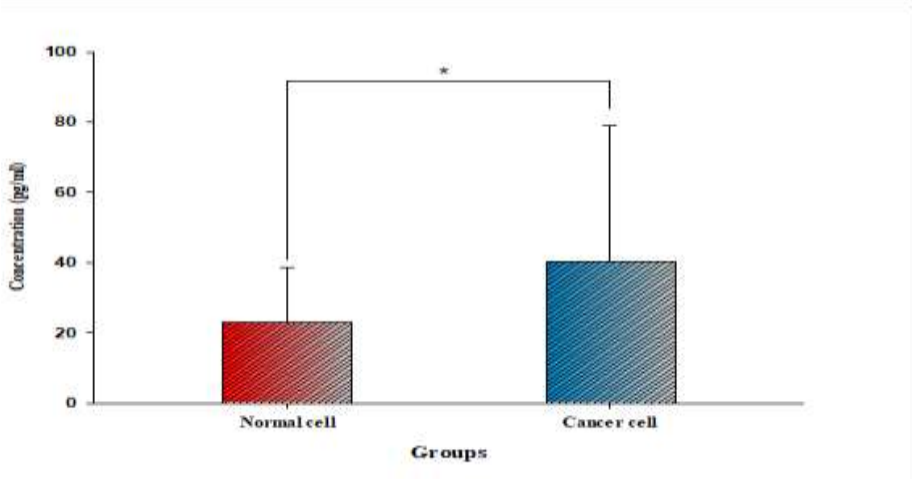


Figure (4-16) : Mean difference of IL-10 according to period. asterisk (*) on the bar indicate significant difference ($p \leq 0.05$)

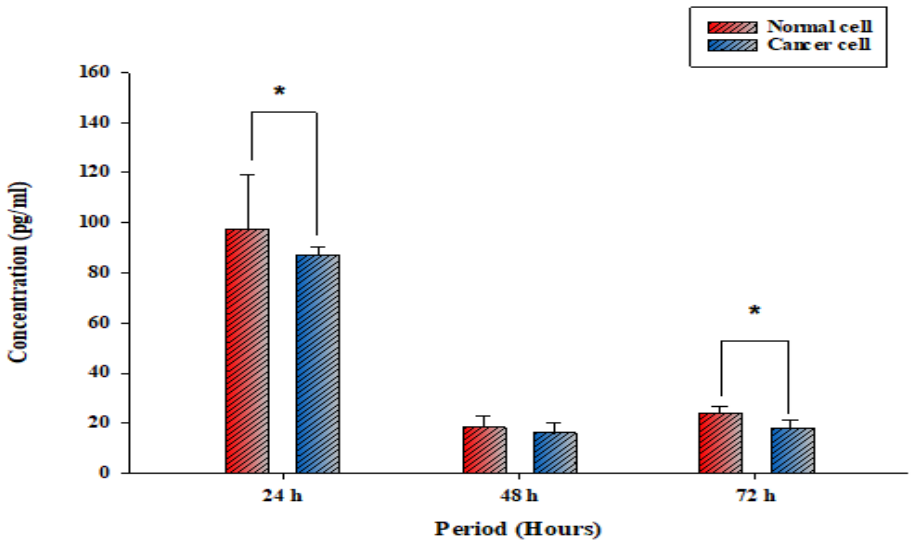


Figure (4-17): Mean difference of IL-10 between normal and cancer cell. asterisk (*) on the bar indicate significant difference ($p \leq 0.05$).

4.9. TNF- α concentration

The present study there is no significant change in TNF- α concentrations between the groups that had varied concentrations of therapy and the control group. This result agreed with Santos *et al*. (2018) .

The mean concentration of total TNF- α in cancer cells was significantlye increased compared to that observed in normal cells, and the study revealed a significant increase ($P \leq 0.05$) between normal and cancer cells, as shown in the figure (4.20) This higher expression could be attributed to Cancerous tissues are known to be invaded with monocytese, T cells, and othere cells capable of generating TNF-α. Tumors and cells in the tumor microenvironment are also known to produce soluble TNF- α receptors (Josephs *et al.*, 2018)

In the figure (4.19), there was no significant difference ($p \leq 0.05$) observed between normal and cancerous cells over a 24-hour period. this is due to the fact that the cytokine TNF- α is not produced at the beginning of leishmaniasis infection and its concentration increases with the increase in

the duration and severity of the infection. As the infection progresses, the immune system recognizes the presence of *Leishmania* parasites and activates immune cells, such as macrophages and dendritic cells, to mount an immune response. These activated immune cells produce TNF-α as part of their effector mechanisms to combat the infection. The correlation coefficient between TNF- α and time was positive ($r = .839^{**}$)

One possible explanation for the decrease in TNF-α levels in cancer cells infected with *Leishmania* is cancer cells themselves can exhibit immunosuppressive properties. Tumor cells often employ mechanisms to suppress immune responses and create an immunosuppressive tumor microenvironment. The interplay between *Leishmania* infection and cancer cells may further contribute to the downregulation of TNF-α production, as both entities can influence immune responses in a way that favors their survival and proliferation.

IL-10 is an immunosuppressive cytokine that can dampen the immune response, while TNF-α is a pro-inflammatory cytokine involved in immune activation (Iyer & Cheng, 2012) . The balance between these two cytokines plays a critical role in shaping the immune response during infection and cancer.

Therefore, in both *Leishmania*-infected cancer cells and cancer in general, the presence of IL-10 can result in decreased TNF-α levels. The immunosuppressive effects of IL-10 can hinder the pro-inflammatory response mediated by TNF-α, leading to an imbalance in the cytokine milieu that favors immune evasion and disease progression (Iyer & Cheng, 2012) . This explains the results obtained there is an inverse relationship between TNF- α and IL10 ($r = -.239^{*}$)

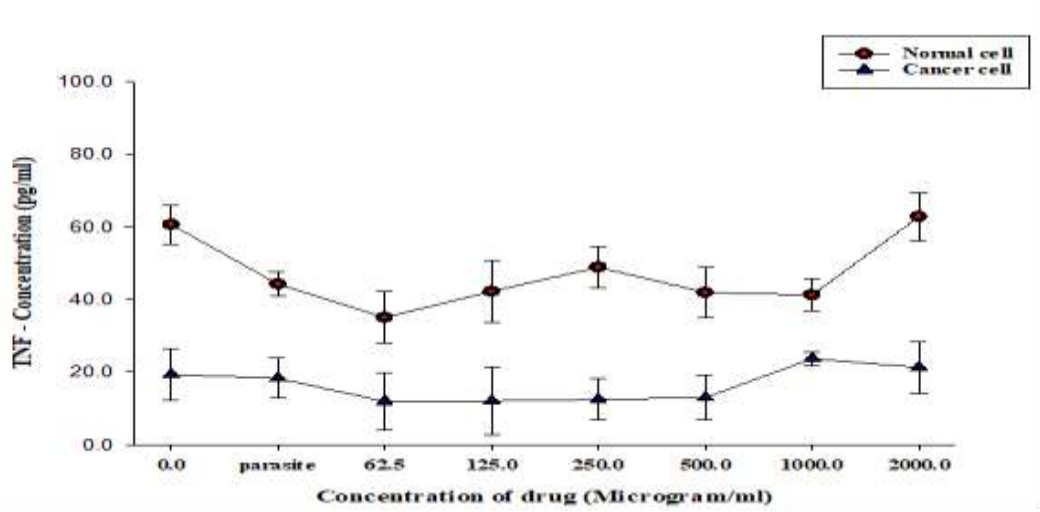


Figure (4-18) : mean differencee TNF- α according to concentration of drug.

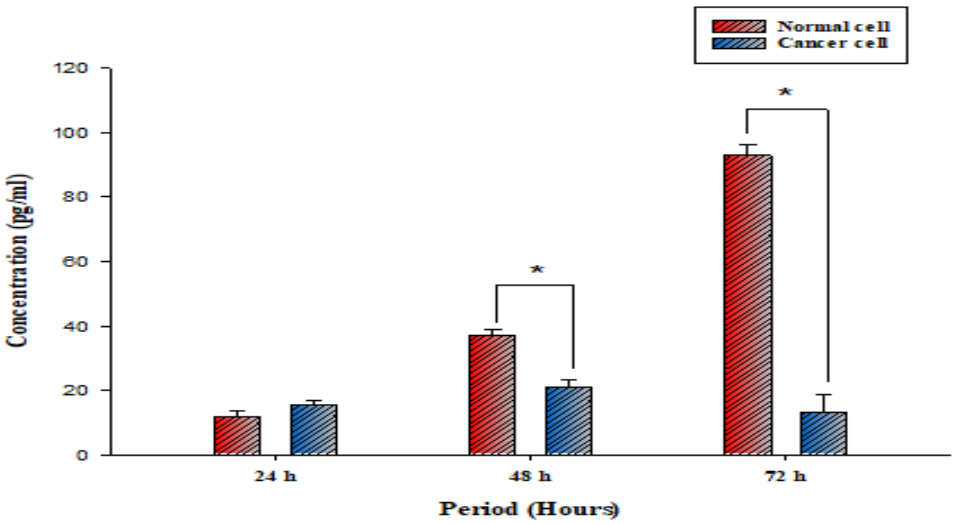


Figure (4-19): Mean differencee of TNF- α according to period asterisk (*) on the bar indicate significant difference ($p \leq 0.05$)

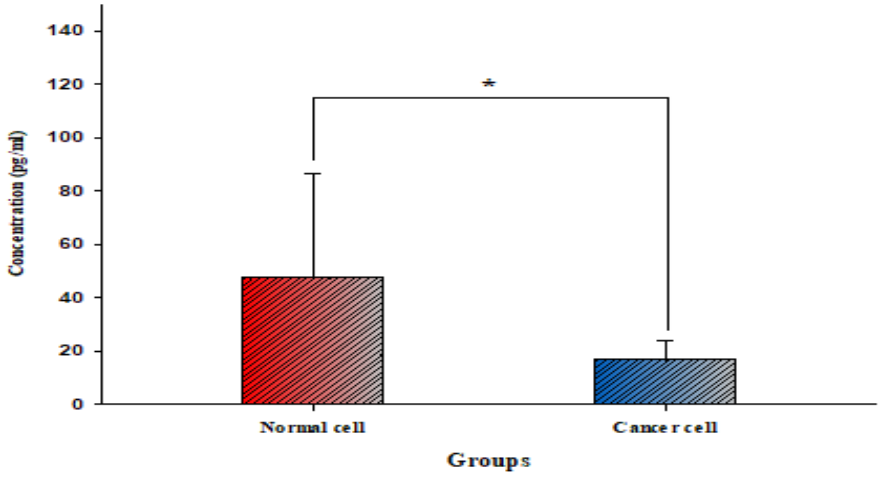


Figure (4-20) : Mean difference of TNF- α between normal and cancer cell, asterisk (*) on the bar indicate significant difference ($p \leq 0.05$)

4.10. Oxidative stress and antioxidant assays

During its life cycle, *Leishmania* encounters and readily adapts to various hostile conditions . such as oxidative stress due to heme digestion in the blood meal, proteases in the sandfly midgut, complement-mediated lysis in the blood upon transmission, and reactive oxygen and nitrogen species (ROS and RNS) generated during phagocytosis by host macrophages (Carneiro *et al*., 2016)

The induction of oxidative stress during parasitic infections triggers the activation of the host's antioxidant response to counteract the damaging effects of the oxidative burst. Macrophages play a crucial role in disease progression or control because the ultimate outcome depends on the interactions between the infecting *Leishmania* species and the type and strength of the host immune response (Reverte *et al*., 2021) .

4.10.1. Measurement of Reactive Oxygen Species (ROS)

Oxidative stress occurs when there is an imbalance between the production of oxidant substances and the antioxidant defenses of the organism . Neutrophils are specialized phagocytic cells with great capacity to produce oxidizing agents, called reactive oxygen species (ROS), which can contribute to oxidative stress (Almeida *et al*., 2013)

ROS act as signal transducers to activate cell proliferation, migration, invasion, and angiogenesis. In contrast, high levels of ROS cause damage to proteins, nucleic acids, lipids, membranes, and organelles, leading to cell death (Nakamura & Takada, 2021) Increased ROS and RNS in response to phagocytosis to destroy the parasite was linked to the ability of activated macrophages to fight infection.(Almohammed *et al*., 2021)

these results showed an increase in the concentration of ROS in the treated cells (16.4 ± 2.2, 15.9 ± 1.6, 15.4 ± 1.4) during 24, 48 and 72 hours. respectively than in untreated cells as shown in the figure (4.22),and these results were consistent with Carneiro *et al*. (2016). the oxidative burst by monocytes from CL patients was higher when compared to monocytes from healthy subjects (HS) It also showed a significant difference between normal cells and cancer cells ,The results also showed a significant difference between normal cells and cancer cells. the concentration of ROS in normal cells As shown in the figure (4.21) .when infected with the parasite without treatment is higher (15.9 ± 2.3, 13.7 ± 1.6, 10.2 ± 2.1) during 24, 48, 72 hours, respectively, Since cancer cells generally have higher levels of ROS compared to normal cells, These results were in agreement with Mazor *et al*. (2008) .

The generation of ROS and RNS by phagocytic cells is dependent on their mitochondrial activity , and because of the differences in the mitochondrial membrane potential between cancer and normal cells (Zaidieh *et al*., 2019) phagocytic cells are the main components of antimicrobial immune responses and tumors, these cells are able to generate a large amount of reactive oxygen andreactive nitrogen types (Hubbard & Rothlein, 2000)

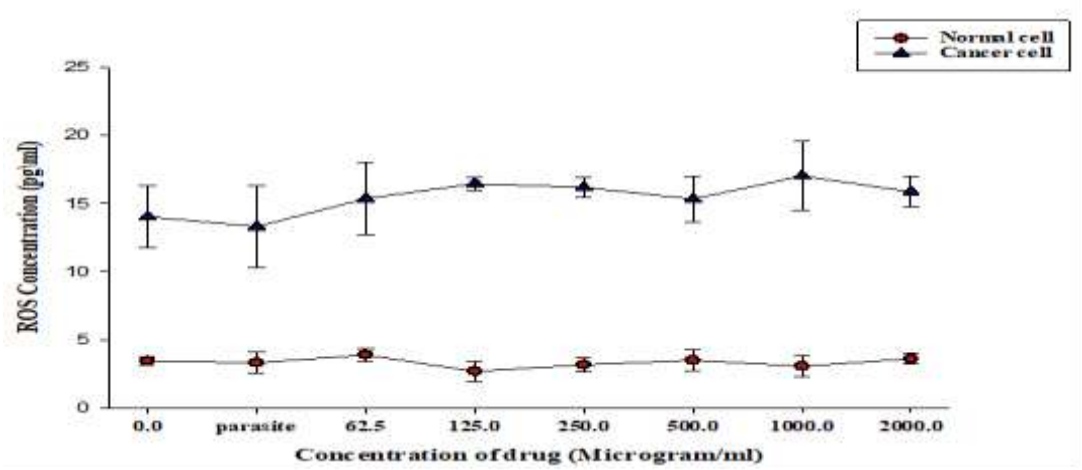


Figure (4-21) : Mean difference in ROS according to concentration of drug

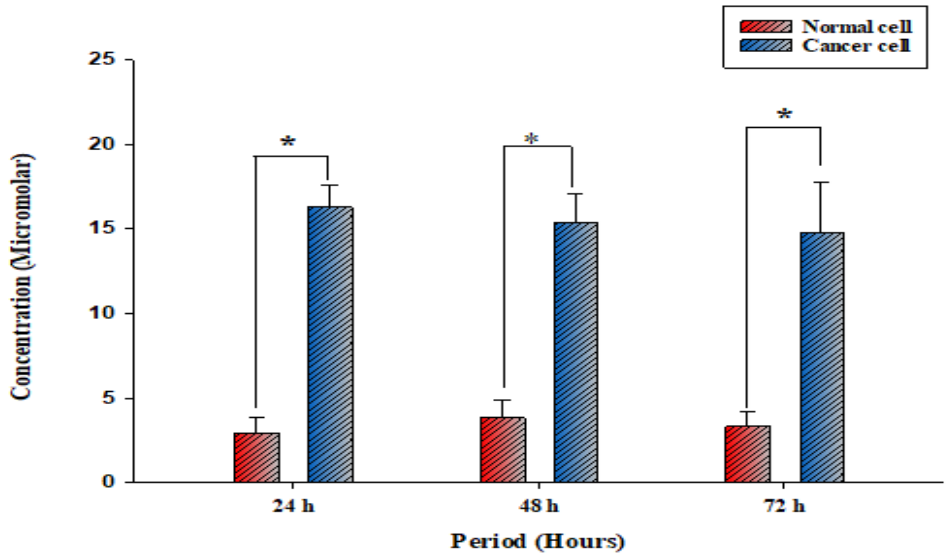


Figure (4-22) : Mean difference of ROS according to period, asterisk (*) on the bar indicate significant difference ($p \leq 0.05$)

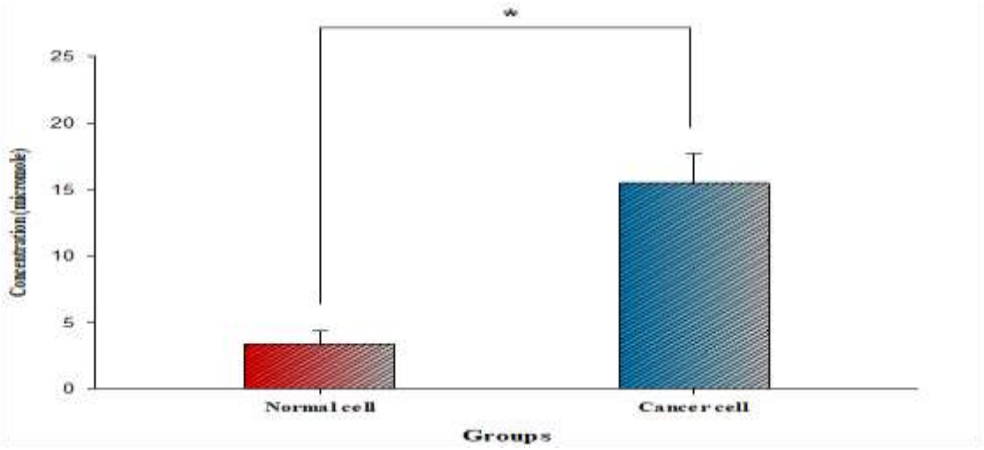


Figure (4-23) :Mean difference of ROS between normel and cancer, asterisk (*) on the bar indicate significant difference ($p \leq 0.05$) as assessed by ANOVA

4.10.2. Glutathione Peroxidase (GPx) activity

GPX stands for glutathione peroxidase, an enzyme that protects cells from oxidative stress by reducing hydrogen peroxide and lipid hydroperoxides. The activity of GPX was studied in leishmaniasis, and the results showed a decrease in the concentration of Gpx in healthy and infected cancer cells within 24 hours, and it decreased more in the time period 48 and 72 hours, The results showed a significant difference in the concentration of Gpx in cancer cells infected with *leishmania* compared with Normal cells infected with the parasite, as shown in the figure (4.25) .

The present results obtained for antioxidant enzyme activities are consistent with the findings of Asma *et al*. (2017) and Mann *et al*. (2021). In their respective studies, they observed a significant reduction in GPx activity in the skin of infected mice and patients with cutaneous leishmaniasis (CL). Both studies attributed these changes to the harmful effects caused by the accumulation of superoxide radicals and hydrogen peroxide..

Conflicting results were found in various studies regarding GPX activity in patients with cutaneous leishmaniasis (CL). Mann *et al.* (2021) reported a significant decrease in GPX activity in CL patients, while Al-hassani & Al-mayali (2020) found a significant decrease ($P \leq 0.05$) in GPX antioxidant activity in affected individuals compared to the control group.

The results revealed several correlations between GPX and other factors. There was an inverse relationship between GPX and period, IL-B1, and TAC, with correlation coefficients of r = -.712**, r = -.414**, and r = -.602** respectively. On the other hand, there was a direct relationship between GPX and IL-10 and between GPX and ROS, with correlation coefficients of r = .637** and r = .308** respectively. As shown in the table (4.4) in cancer cell.

The inverse relationships between GPX and period, IL-B1, and TAC indicate that the presence of the parasite may disrupt the antioxidant capacity and lead to decreased GPX activity. This disruption could be attributed to the harmful effects of *Leishmania*-induced oxidative stress . the direct relationships between GPX and IL-10, as well as GPX and ROS, suggest a possible adaptive response by the host to counteract the effects of the parasite. IL-10 is an anti-inflammatory cytokine that can contribute to regulating the immune response and reducing inflammation. The direct relationship between GPX and IL-10 may indicate a compensatory mechanism to mitigate the oxidative stress induced by the parasite.

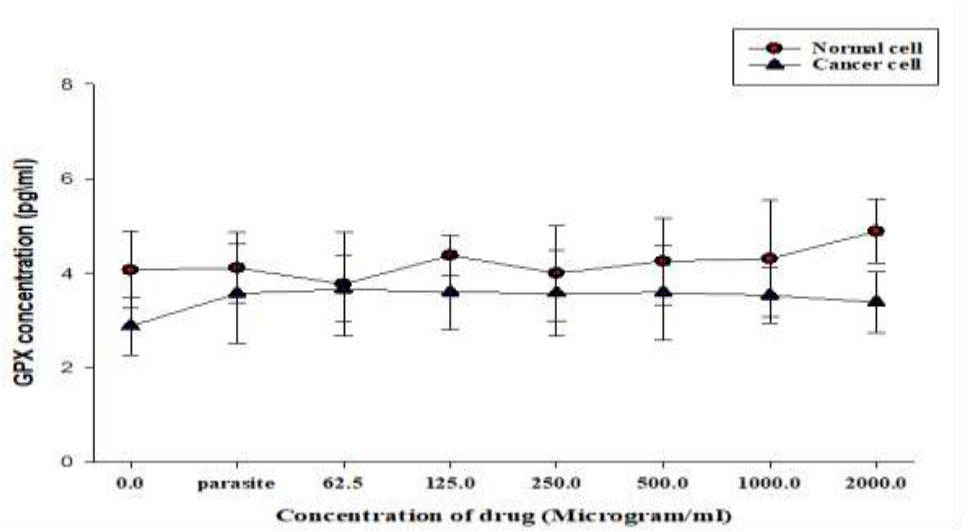


Figure (4-24) : Mean difference in GPX according to concentration of drug.

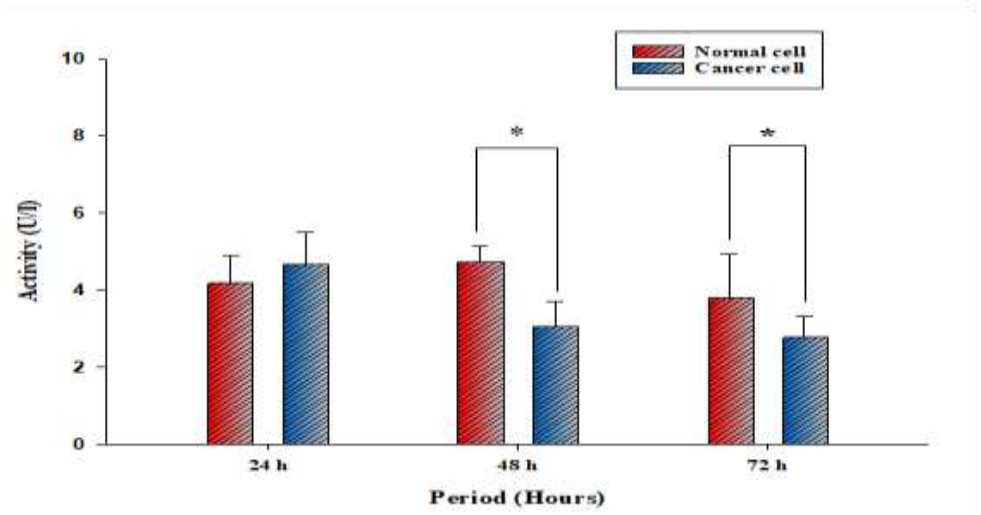


Figure (4-25) Mean difference of GPX according to period وasterisk (*) on the bar indicate significant difference ($p \leq 0.05$)

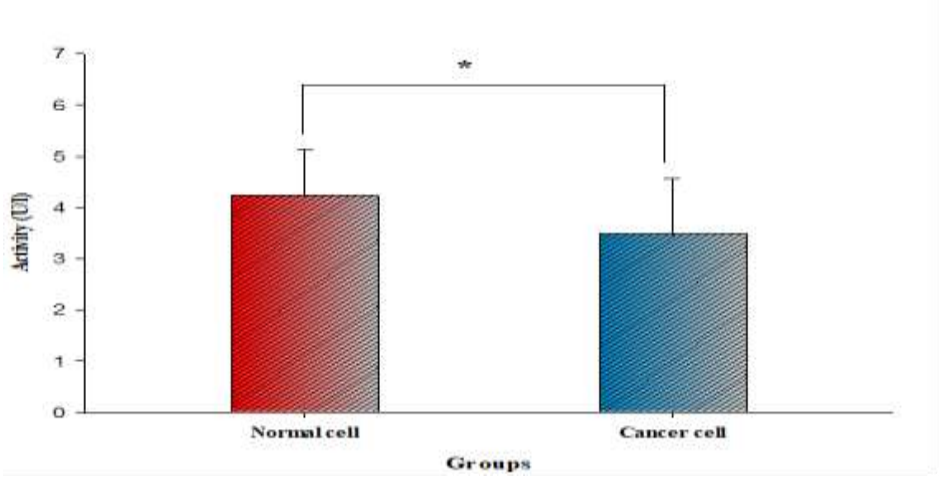


Figure (4-26) : Mean difference of GPX between normel and cancer, asterisk (*) on the bar indicate significant difference ($p \leq 0.05$).

4.10.3. Estimation of Malondialdehyde (MDA):

Lipid peroxidation is a well-recognized mechanism of cellular injury and is used as a marker of oxidative stress in cells and tissues .Polyunsaturated fatty acid derived that are not stable, can decay hence forming many series of complex products They are degraded such as carbonyl compound which are plentiful malondialdehyde (MDA) that is widely used as marker of lipid peroxidation (Asmaa *et al*., 2017)

This study provides clear evidence of the role of MDA (malondialdehyde) as an early biochemical marker of peroxide damage in cells affected by cutaneous leishmaniasis (CL). The concentration of MDA increased in cells treated with drug concentrations of 125 and 250 (18.5 ± 2.7, 18.9 ± 4.1) compared to infected cells without treatment (12.4 ± 2.1) over a 24-hour period. However, the concentration of MDA decreased in cells treated with high concentrations of the drug over the same 24-hour period. as shown in the figure (4.29) The results showed that there is no significant difference between the group of normal and cancerous cells. These findings are

consistent with the study conducted by Bildik *et al.* (2004), which investigated oxidative stress as a mediator of hepatic tissue damage during *Leishmania* (L.) chagasi infection. In their study, they observed a significant increase in Malondialdehyde (MDA) levels, indicating increased lipid peroxidation.

These results also agree with the study conducted by Asmaa *et al*. (2017), which found a significant rise in the levels of free radicals, as represented by lipid peroxidation MDA, in patients with cutaneous leishmaniasis compared to the control group. This indicates that the excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to oxidative stress and increased lipid peroxidation in patients.

Furthermore, Almohammed *et al*. (2021) suggested that the excessive production of ROS, coupled with the depletion of protective antioxidants, may be the cause of the elevated MDA levels in untreated cutaneous leishmaniasis patients in their study. This supports the idea that oxidative damage to lipids and other biomolecules contributes to the observed increase in MDA levels.

Similar results were also observed by Ozbilge *et al*. (2005) and Kocyigit *et al.* (2005), who found a significant decrease in mean catalase activity and an increase in MDA levels in patients with cutaneous leishmaniasis compared to the control group

In these results, the Pearson correlation coefficient for the MDA level showed a positive relationship with ROS in cells infected with *Leishmania*.(r = .379**). the positive relationship between the Malondialdehyde (MDA) level and Reactive Oxygen Species (ROS) in cells infected with *Leishmania* indicates an association between oxidative stress and lipid peroxidation.

Malondialdehyde is a byproduct of lipid peroxidation, which occurs when reactive oxygen species react with lipids in cellular membranes, resulting in the generation of MDA.

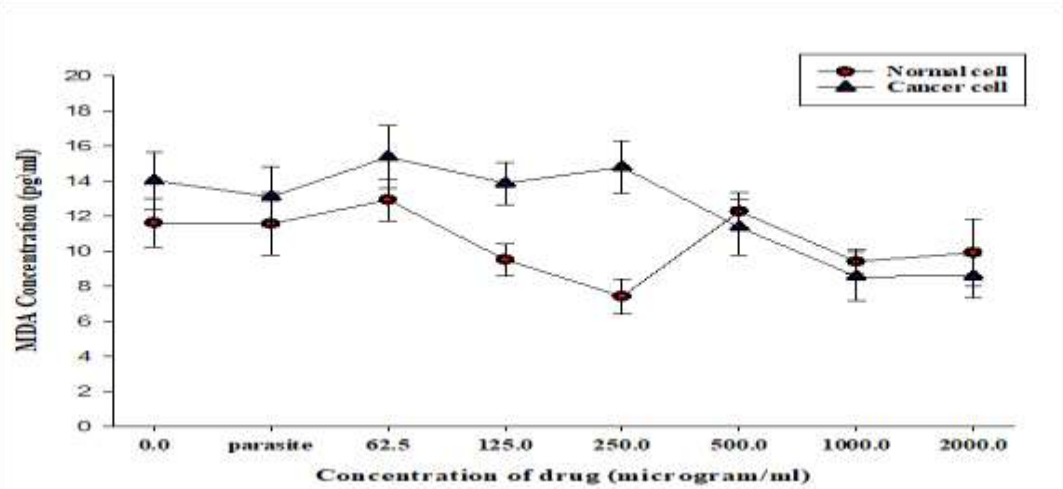


Figure (4-27): Mean difference in MDA according to concentration of drug.

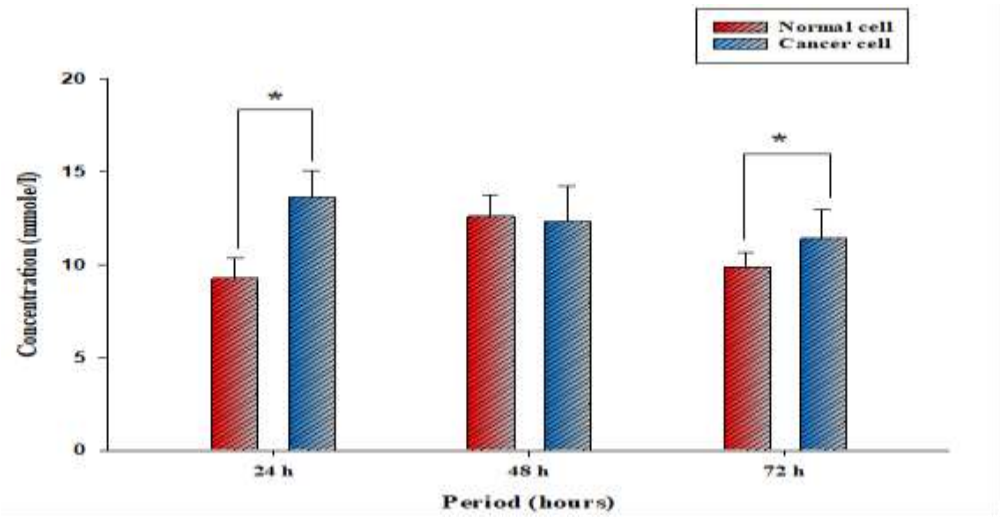


Figure (4-28): Mean difference of MDA according to period, asterisk (*) on the bar indicate significant difference ($p \leq 0.05$) .

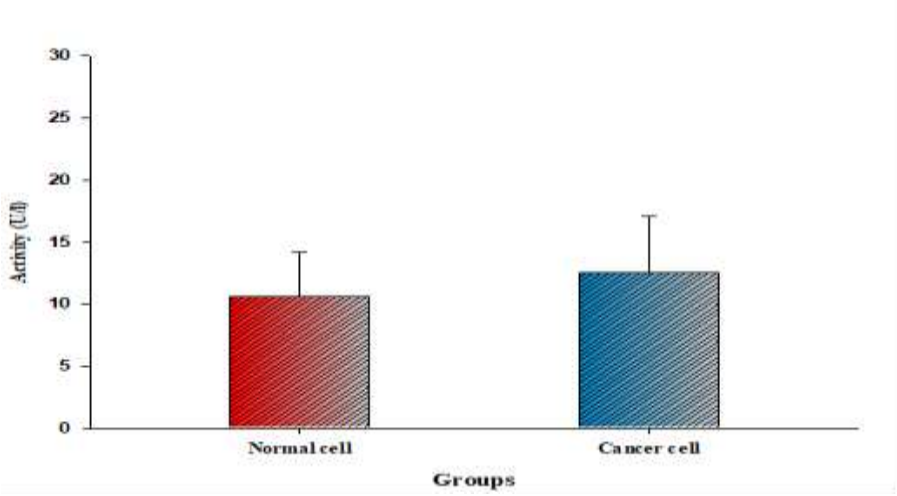


Figure (4-29): Mean difference of MDA between normel and cancer, asterisk (*) on the bar indicate significant difference ($p \leq 0.05$) .

4.10.4. Total Antioxidants Capacity Assay (TAC)

In this study, a decrease in the concentration of TAC is observed in normal cells as well as cancer cells, and this decrease is due to the production of more reactive oxygen species by phagocytes. These results agree with the interpretation that excess ROS leads to damage of vital cellular structures, and increased oxidative stress due to depletion of cellular TAC. It causes lipid peroxidation, which subsequently leads to increased apoptosis (Cendoroglo *et al*., 1999)

The current study also showed a significant increase in the concentration of TAC concentration in cancer cells than it is in normal cells, as shown in the figure (4.31), and this increase continued during three times 24, 48, and 72 hours, with a clear significant difference. These results are in agreement with the results of studies conducted on human neutrophils (Mazor *et al*., 2008) As shown in the figure (4.32).

In this study, a direct relationship was found between the concentration of total antioxidant capacity (TAC) and pro-inflammatory cytokines including IL-1β, TNF, TGF-β1, and the time period. The correlation coefficients were

reported as .496**, .267*, .537**, and -.585** respectively. Conversely, an inverse relationship was observed between TAC and IL-10 (r = -.585**). The positive relationship between TAC and pro-inflammatory cytokines such as IL-1β, TNF, and TGF-β1 suggests that higher levels of TAC are associated with increased production or release of these cytokines, indicating a pro-inflammatory state. TAC represents the overall antioxidant capacity in a system, and higher TAC levels may reflect a greater ability to counteract oxidative stress and inflammation.

On the other hand, the inverse relationship between TAC and IL-10 suggests that higher TAC levels are associated with reduced production of IL-10. IL-10 is an immunosuppressive cytokine that can dampen the immune response. Therefore, a decrease in IL-10 levels may indicate a more pro-inflammatory environment.

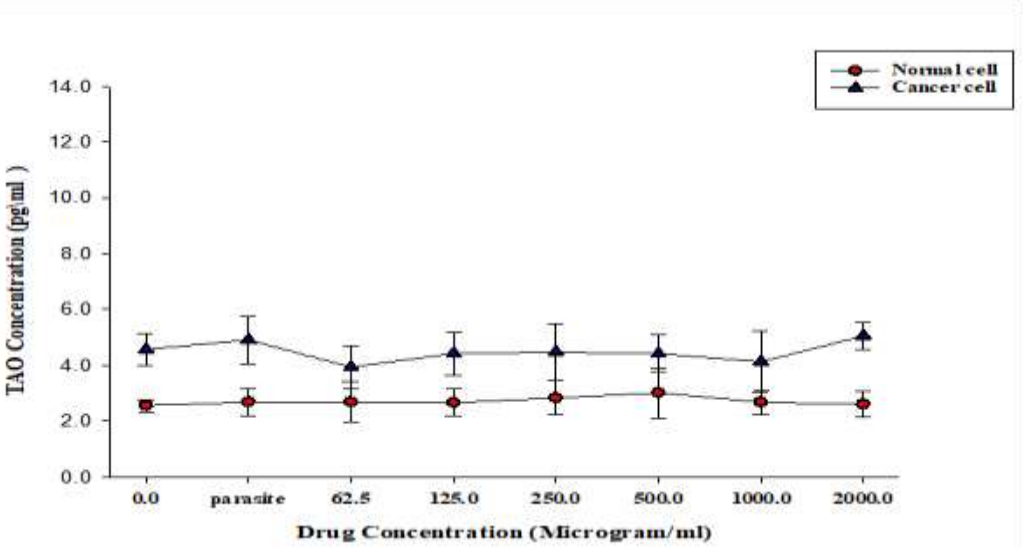


Figure (4-30) : Mean difference in TAC according to concentration of drug

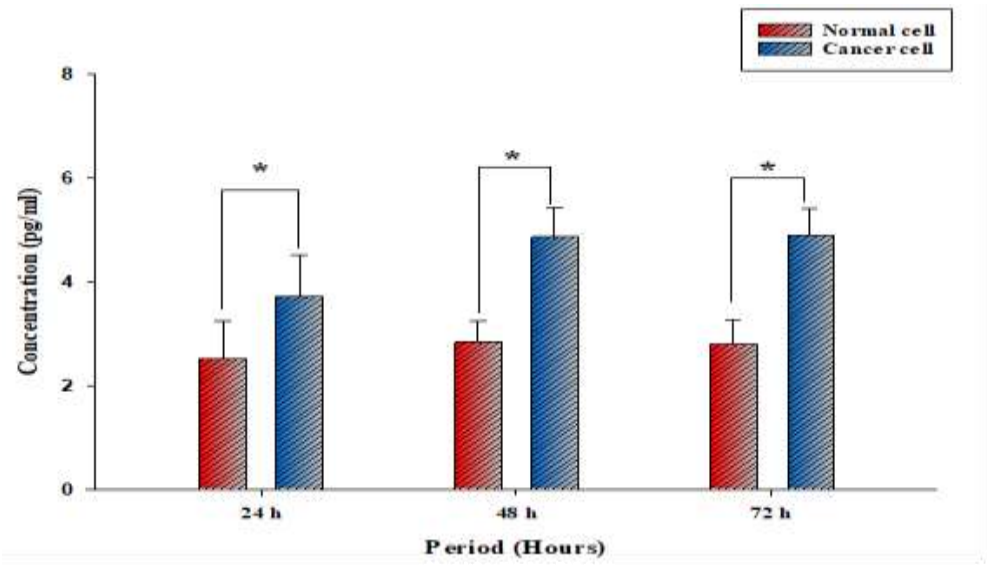


Figure (4-31) : Mean difference of TAC according to , asterisk (*) on the bar indicate significant difference ($p \leq 0.05$) .

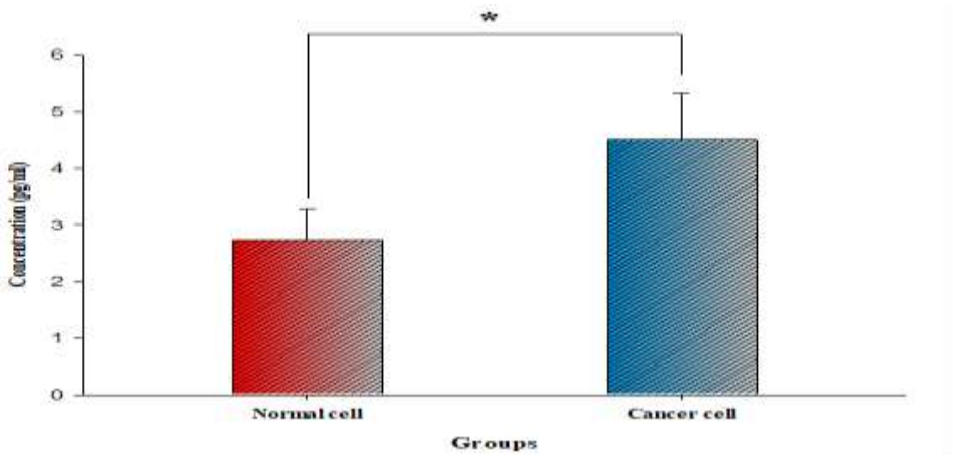


Figure (4-32) : Mean difference of TAC between normel and cancer, asterisk (*) on the bar indicate significant difference ($p \leq 0.05$).

4.11. Correlation between different interleukins and Antioxidant groups in U937 cancer cells infected with the parasite over a period of time and with different doses of treatment

The results of this study showed an inverse relationship between TAC with each of (GPX, MDA, ROS). The correlation coefficient r = (-.602**,-.129, -.322**) , The results of this study showed that there is an inverse relationship between the time factor and each of (GPX, MDA, ROS). These coefficients suggest that as TAC levels decrease, the levels of GPX, MDA, and ROS tend to increase. This finding implies that there may be an association between TAC levels and the presence of GPX, MDA, and ROS in the cancer cells infected with the *Leishmania* parasite.

The results of this study showed an inverse relationship between IL-10 and both (TNF-α and TGF-B1 - IL-B1). The correlation coefficient r = (-.239*, -.558**, -.358**) respectively as shown in Table (4.4). and that the time period is inversely proportional to TNF-α and IL-10 and directly proportional to each of them (IL-B1, TGF-B1) The correlation coefficient r = (TNF-α=-.126, IL-10=-.729**) (IL-B1 =.476**, TGFB1 =.758**) Maybe the reason is due to the cytokine TNF-α is not produced at the beginning of infection with leishmaniasis, and its concentration increases with the increase in the duration and severity of the infection (Melby *et al*., 1994) .This study agreed with a study conducted in Iraq . Decreased levels of TNF-α and IL-β in *Leishmania*-infected macrophages (Al-Mosawi *et al*., 2023)

Table (4.4): correlation coefficient among all biomarkers. (cancer cell)

Biomarkers		Period	IL-1β	IL10	TNF	TGFB	TAO	ROS	MDA	GPX
Con	r	.000	-.064	-.280*	.330**	-.220	.160	.230	-.471**	-.003
	Sig.	1.000	.591	.017	.005	.063	.180	.052	.000	.983
Period	r	1	.476**	-.729**	-.126	.758**	.573**	-.273*	-.197	-.712**
	Sig.		.000	.000	.293	.000	.000	.020	.098	.000
IL-1β	r		1	-.358**	.043	.562**	.496**	-.369**	-.189	-.414**
	Sig.			.002	.718	.000	.000	.001	.111	.000
IL10	r			1	-.239*	-.558**	-.585 **	.190	.206	.637**
	Sig.				.043	.000	.000	.109	.083	.000
TNF	r				1	-.126	.267*	.203	-.153	-.131
	Sig.					.293	.023	.088	.201	.271
TGFB	r					1	.537**	-.395**	-.221	-.567**
	Sig.						.000	.001	.062	.000
TAO	r						1	-.322**	-.129	-.602**
	Sig.							.006	.280	.000
ROS	r							1	-.031-	.308**
	Sig.								.795	.009
MDA	r								1	.110
	Sig.									.357

*. Correlation is significanteat the 0.05 level (2-tailed).

**. Correlationeis significanteat the 0.01 level (2-tailed).

4.12. Correlation between different interleukins and Antioxidant groups in normal cells infected with the parasite over a period of time and with different doses of treatment

The results of this study showed a positive relationship between MDA and Bothe ROS and GPX at the 0.01 level The correlation coefficient r = (.379** , .258*) , The results of this study showed an inverse relationship between TGF- β1 and both (TNF-α and IL-β1-). The correlation coefficient r =(-.350**, -.300*) respectively and the time period is directly proportional to The correlation coefficient r = (TNF-α=.839**-) This modulation of cytokine levels could be a strategy employed by the parasite to manipulate the host's immune response and create an environment favorable for its survival and persistence This result was consistent with Looker *et al*.(1986). Increased

IL-10 levels have been linked to tumor progression and treatment resistance

Table (4.5): correlation coefficient (normal cells)

		Period	IL-β	IL-10	TNF	TGFB	TAO	ROS	MDA	GPX
con	**r**	.000	.026	.266*	.108	.126	-.041	.050	-.113	.302*
	Sig.	1.000	.840	.035	.399	.324	.752	.695	.378	.016
period	**r**	1	.026	-.248	.839**	-.134	.266*	.164	.069	-.124
	Sig.		.838	.050	.000	.294	.035	.200	.591	.332
IL-β	**r**		1	-.083	.104	-.300*	.201	.167	-.181	.115
	Sig.			.519	.416	.017	.114	.191	.156	.370
IL-10	**r**			1	-.170	.088	-.123	.056	-.017	.058
	Sig.				.183	.495	.335	.661	.898	.650
TNF	**r**				1	-.350**	.088	.073	-.158	-.183
	Sig.					.005	.494	.571	.217	.151
TGFB	**r**					1	-.132	.144	.211	.032
	Sig.						.301	.259	.096	.801
TAO	**r**						1	-.077	.108	.062
	Sig.							.549	.401	.632
ROS	**r**							1	.379**	.042
	Sig.								.002	.744
MDA	**r**								1	.258*
	Sig.									.041

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Conclusions

Conclusions:

1. The results demonstrate higher concentrations of certain cytokines in cancer cells compared to normal cells, indicating a specific interaction between cytokines and cancer cells. found that the presence of parasites leads to enhanced immune cell interaction against cancer cells and stimulates the production of anti-tumor cytokines.
2. Specific cytokines, such as IL-10, TGF-B1, and TNF-α , play a role in the immune system's response to the parasite and the development of the disease. this suggests that the immune system regulates the release of cytokines relative
3. Infection with *L.tropica* leads to oxidative stress associated with TAC and GPx activities. Elevated MDA and ROS levels contribute to cell death, while decreased TAC and GPx concentrations indicate antioxidant defense mechanisms
4. The findings reveal that the concentration of reactive oxygen species (ROS) is generally higher in cancer cells infected with *Leishmania* compared to normal cells. this elevated ROS level indicates an upregulation in the activity of dioxygenase within the cells, which is a marker of cellular stress potentially linked to hyperplasia reactions resulting from Leishmania infection
5. Studying the ratios of cytokines in different cell types helps. Understanding the cytokines and immune cells in leishmaniasis can inform new immunotherapy strategies
6. The ribosomal sequences-based comprehensive tree has provided an inclusive tool for the high ability of such genetic fragments to efficiently identify *L. tropica* phylogenetic positioning using the ITS1-ITS2 fragment. This provides a further indication of the capability of the currently utilized rRNA sequences to describe the investigated *L. tropica*

Recommendations:

1. further investigate the levels of cytokines (IL-10, IL-B1, TNF, TGF-B1) in the serum of individuals with cutaneous leishmaniasis. This can provide valuable insights into the immune response and disease development.
2. Further investigation the levels of other cytokines, such as IL-17 and IL-23, in the serum of individuals with cutaneous leishmaniasis. Understanding the involvement of these cytokines can contribute to a more comprehensive learning of the immune response and potential therapeutic targets.
3. liposomal-encapsulated drugs as a treatment option for leishmaniasis. Liposomal formulations can enhance drug penetration into phagocytic cells and prolong its retention at the site of action, potentially improving treatment activity.
4. A larg- scale investigation for *L.tropica* is mandatory to explore further geneti diversity that annot be identified mithont the iclusion of larger number of samples performing DNA sequencing on abroader range of sample mill bronden our insight to more detainls and how *L.tropicalis* avoid the resistant of the host and many other advantages

Refernces

References

Afshar, P. J., Bahrampour, A., & Shahesmaeili, A. (2022). Determination of the trend of incidence of cutaneous leishmaniasis in Kerman province 2014-2020 and forecasting until 2023. A time series study. *PLOS Neglected Tropical Diseases*, *16*(4), e0010250.

Abadías-Granado, I., Diago, A., Cerro, P. A., Palma-Ruiz, A. M., & Gilaberte, Y. (2021). Cutaneous and Mucocutaneous Leishmaniasis. Actas Dermo-Sifiliograficas,112(7),601–618. https://doi.org/10.1016/j.ad.2021.02.008

Abdalla, D., Omer, S., & Dawoud, A. (2018). Comparison Of The Efficacy Of Cryotherapy, Intralesional Sodium Stibogluconate And 7% Sodium Chloride In The Treatment Of Cutaneous Leishmaniasis. *Journal Of Sulaimani Medical College*, *8*(4), 291-298.

Aboud, R. A. (2020). Effects of Different Human Urine Samples in Stimulatulating Growth of Leishmaniaspp. Indian Journal of Public Health Research & Development, 11.(4)

Aransay, A. M., Scoulica, E., & Tselentis, Y. (2000). Detection and identification of Leishmania DNA within naturally infected sand flies by seminested PCR on minicircle kinetoplastic DNA. *Applied and environmental microbiology*, *66*(5), 1933-1938.

Akhoundi, M., Kuhls, K., Cannet, A., Votýpka, J., Marty, P., Delaunay, P., and, Sereno, D. (2016). A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. PLoS neglected tropical diseases, 10(3), e0004349

Al-Bajalan, M. M. M., Niranji, S. S., Al-Jaf, S. M., & Kato, H. (2021). First molecular identification of *Leishmani*a major in Phlebotomus papatasi in an outbreak cutaneous leishmaniasis area in Iraq. Acta Tropica, 215(December2020),105807. https://doi.org/10.1016/j.actatropica.2020.105807

Alemayehu, B., & Alemayehu, M. (2017). Leishmaniasis: A Review on Parasite, Vector, and Reservoir Host. Health Science Journal, 11(4). https://doi.org/10.21767/1791-809x.1000519

Al-Hassani, M. K. K., & Al-Mayali, H. M. H. (2020). Evaluation of some biochemical levels in patients with Cutaneous leishmaniasis serum and their relationship with antioxidant enzymes. *EurAsian J Biosci*, *14*(1), 1999-2006.

Al-Hayali, H., & alkattan, M. (2021). Overview of Epidemiology of Leishmaniasis in Iraq. Rafidain Journal of Science, 30(1), 28–37. https://doi.org/10.33899/rjs.2021.167680

Al-hucheimi, S. N., Sultan, B. A., & Al-Dhalimi, M. A. (2009). A comparative study of the diagnosis of Old World cutaneous leishmaniasis in Iraq by polymerase chain reaction and microbiologic and histopathologic methods. International Journale of Dermatology,e48(4),e404–408. https://doi.org/10.1111/j.1365-4632.2009.03903.x

Ali, L. H. (2019). Dr. Saleem Khudeer Al_Hadraawy (Doctoral dissertation, Babylon University).

Ali, L. H. (2021). Molecular Study For Cutaneous Leishmaniasis and it's Dr. Saleem Khudeer Al_ Hadraawy.June. https://doi.org/10.13140/RG.2.2.30112.71687

Almeida, B. F. M., Narciso, L. G., Melo, L. M., Preve, P. P., Bosco, A. M., Lima, V. M. F., & Ciarlini, P. C. (2013). Leishmaniasis causes oxidative

stress and alteration of oxidative metabolism and viability of neutrophils in dogs.Veterinary Journal,198(3),599–605. https://doi.org/10.1016/j.tvjl.2013.08.024

Almohammed, H. I., Saif, M. A., Albalawi, A. E., & Alanazi, A. D. (2021). Efficacy of pentavalent antimonial injection in improving the oxidative, nitrosative status and immune cellular enzyme activity in treating Saudi patients with cutaneous leishmaniasis. *Journal of the Egyptian Society of Parasitology*, *51*(1), 99-106**.**

Al-Mosawi, A. M. A., Al-Joborae, F. F. M., Al-Joborae, H. F., Al-Saadi, M. A. K., & Al-Charrakh, A. H. (2023). Cytokines Profile in Patients with Hydatidosis in Babylon Province, Iraq. *Medical Journal of Babylon*, *20*(1), 213.

Al-Naimy, A. F., & Al-Waaly, A. B. (2021). Investigation of Cutaneous Leishmaniasis Cases in Baghdad Province, Iraq. *Prof.(Dr) RK Sharma*, *21*(1), 65**.**

Alvar, J., Aparicio, P., Aseffa, A., Den Boer, M., Canavate, C., Dedet, J. P., ... & Moreno, J. (2008). The relationship between leishmaniasis and AIDS: the second 10 years. *Clinical microbiology reviews*, *21*(2), 334-359.

Alvar, J., Vélez, I. D., Bern, C., Herrero, M., Desjeux, P., Cano, J., Jannin, J., & de Boer, M. (2012). Leishmaniasis worldwide and global estimates of its incidence. PLoS ONE, 7(5). https://doi.org/10.1371/journal.pone.0035671

Alves, C. F., de Amorim, I. F. G., Moura, E. P., Ribeiro, R. R., Alves, C. F., Michalick, M. S., Kalapothakis, E., Bruna-Romero, O., Tafuri, W. L., Teixeira, M. M., & Melo, M. N. (2009). Expression of IFN-γ, TNF-α, IL-10, and TGF-β in lymph nodes associates with parasite load and clinical

form of disease in dogs naturally infected with Leishmania (Leishmania) chagasi. Veterinary Immunology and Immunopathology, 128(4), 349–358. https://doi.org/10.1016/j.vetimm.2008.11.020

Al-Warid, H. S., Al-Saqur, I. M., Kadhem, A. J., Al-Tuwaijari, S. B., Al-Zadawi, K. M., & Gompper, M. E. (2019). Spatial and demographic aspects of kala-azar (Visceral leishmaniasis) in iraq durin 2011-2013. Tropical Biomedicine, 36(1), 22–34.

Amorim, C. F., Novais, F. O., Nguyen, B. T., Misic, A. M., Carvalho, L. P., Carvalho, E. M., Beiting, D. P., & Scott, P. (2019). Variable gene expression and parasite load predict treatment outcome in cutaneous leishmaniasis.Science TranslationaleMedicine,11(519),1–10. https://doi.org/10.1126/scitranslmed.aax4204

Amprey, J. L., Im, J. S., Turco, S. J., Murray, H. W., Illarionov, P. A., Besra, G. S., ... & Späth, G. F. (2004). A subset of liver NK T cells is activated during *Leishmania* donovani infection by CD1d-bound lipophosphoglycan. *The Journal of experimental medicine*, *200*(7), 895-904.

Andargie, T. E., & Diro Ejara, E. (2016). Pro- and Anti-inflammatory Cytokines in Visceral Leishmaniasis. Journal of Cell Science & Therapy, 06(02). https://doi.org/10.4172/2157-7013.1000206

Andrés, C. M. C., Pérez de la Lastra, J. M., Juan, C. A., Plou, F. J., & Pérez-Lebeña, E. (2022). The Role of Reactive Species on Innate Immunity. *Vaccines*, *10*(10), 1735.

Arenas, R., Torres-Guerrero, E., Quintanilla-Cedillo, M. R., & Ruiz-Esmenjaud, J. (2017).Leishmaniasis: A review. F1000Research, 6(May), 1–15. https://doi.org/10.12688/f1000research.11120.1

Armitage, E. G., Alqaisi, A. Q. I., Godzien, J., Peña, I., Mbekeani, A. J., Alonso-Herranz, V., López-Gonzálvez, Á., Martín, J., Gabarro, R., Denny, P. W., Barrett, M. P., & Barbas, C. (2018). Complex interplay between sphingolipid and sterol metabolism revealed by perturbations to the *leishmani*a metabolome caused by miltefosine. *Antimicrobial Agents and Chemotherapy*, *62*(5). https://doi.org/10.1128/AAC.02095-17

Asmaa, Q., Al-Shamerii, S., Al-Tag, M., Al-Shamerii, A., Li, Y., & Osman, B. H. (2017). Parasitological and biochemical studies on cutaneous leishmaniasis in Shara'b District, Taiz,
Yemen. Annalseofeclinicalemicrobiologyeandeantimicrobials, 16(1), 1-15.

Azizi, M. H., Bahadori, M., Dabiri, S., Meymandi, S. S., & Azizi, F. (2016). A history of leishmaniasis in Iran from 19th century onward. Archives of Iranian Medicine, 19(2), 153–162. https://doi.org/0161902/AIM.0016

Balikçi, E., Güngör, N., Kolayli, F., & Hökelek, M. (2021). In vitro effect of pelargonium sidoides on promastigote forms of *leishmania* infantum and leishmania tropica. Kafkas Universitesi Veteriner Fakultesi Dergisi, 27(1), 51–56. https://doi.org/10.9775/kvfd.2020.24746

Baek, Y. S., Haas, S., Hackstein, H., Bein, G., Hernandez-Santana, M., Lehrach, H., ... & Seitz, H. (2009). Identification of novel transcriptional regulators involved in macrophage differentiation and activation in U937 cells. *BMC immunology*, *10*(1), 1-15.

Banuls, A. L., Bastien, P., Pomares, C., Arevalo, J., Fisa, R., & Hide, M. (2011). Clinical pleiomorphism in human leishmaniases, with special mention of asymptomatic infection. *Clinical Microbiology and Infection*, *17*(10), 1451-1461.

Barbosa, F. M. C., Dupin, T. V., Toledo, M. D. S., Reis, N. F. D. C., Ribeiro, K., Cronemberger-Andrade, & Xander, P. (2018). Extracellular vesicles released by *Leishmania* (Leishmania) amazonensis promote disease progression and induce the production of different cytokines in macrophages and B-1 cells. *Frontiers in microbiology*, *9*, 3056.

Bayram, D. M., & Ali, H. Z. (2017). Molecular Detection of Suspected Leishmania Isolates Using Polymerase Chain Reaction. *Iraqi Journal of Science*, 2076-2082.

Belkaid, Y., Hoffmann, K. F., Mendez, S., Kamhawi, S., Udey, M. C., Wynn, T. A., & Sacks, D. L. (2001). The role of interleukin (IL)-10 in the persistence of *Leishmania* major in the skin after healing and the therapeutic potential of anti–IL-10 receptor antibody for sterile cure. *The Journal of experimental medicine*, *194*(10), 1497-1506.

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2017). GenBank. *Nucleic acids research*, *45*(Database issue), D37**.**

Berriatua, E., Maia, C., Conceição, C., Özbel, Y., Töz, S., Baneth, G., ... & Gossner, C. M. (2021). Leishmaniases in the European Union and neighboring countries. *Emerging Infectious Diseases*, *27*(6), 1723.

Bhattacharjee, A., & Biswas, A. (2023). Cycling Within a Cell: Cell Cycle of Intracellular Kinetoplastid Parasites. In Pathobiology of Parasitic Protozoa: Dynamics and Dimensions (pp. 95-107). Singapore: Springer Nature Singapore.

Bildik, A., Kargin, F., Seryek, K., Pasa, S., & Özensoy, S. (2004). Oxidative stress and non-enzymatic antioxidative status in dogs with visceral

Leishmaniasis. Research in Veterinary Science,77(1), 63–66. https://doi.org/10.1016/j.rvsc.2004.01.005

Bogdan, C. (2020). Macrophages as host, effector and immunoregulatory cells in leishmaniasis: Impact of tissue micro-environment and metabolism. Cytokine: X, 2(4), 100041. https://doi.org/10.1016/j.cytox.2020.100041

Buege, J. A. and Aust, S. D. (1978). Microsomal Lipid Peroxidation. Methods in Enzymology,52: 302-310.

Calvo Alvarez, E., D'Alessandro, S., Proverbio, D., Spada, E., Perego, R., Taramelli, D., Basilico, N., & Parapini, S. (2022). In Vitro Antiparasitic Activities of Monovalent Ionophore Compounds for Human and Canine Leishmaniases. Animals, 12(18), 1–13. https://doi.org/10.3390/ani12182337

Carregaro, V., Costa, D. L., Brodskyn, C., Barral, A. M., Barral-Netto, M., Cunha, F. Q., & Silva, J. S. (2013). Dual effect of Lutzomyia longipalpis saliva on *Leishmania* braziliensis infection is mediated by distinct saliva-induced cellular recruitment into BALB/c mice ear. *BMC microbiology*, *13*, 1-11.

Carneiro, P. P., Conceição, J., Macedo, M., Magalhães, V., Carvalho, E. M., & Bacellar, O. (2016). The role of nitric oxide and reactive oxygen species in the killing of *Leishmania* braziliensis by monocytes from patients with cutaneous leishmaniasis.PLoSONE,11(2),1–16. https://doi.org/10.1371/journal.pone.0148084

Carta, S., Castellani, P., Delfino, L., Tassi, S., Venè, R., & Rubartelli, A. (2009). DAMPs and inflammatory processes: the role of redox in the different outcomes. Journal of Leukocyte Biology, 86(3), 549–555. https://doi.org/10.1189/jlb.1008598

Cavalcanti, Y. V. N., Brelaz, M. C. A., Neves, J. K. D. A. L., Ferraz, J. C., & Pereira, V. R. A. (2012). Role of TNF-alpha, IFN-gamma, and IL-10 in the development of pulmonary tuberculosis.PulmonaryeMedicine,2012. https://doi.org/10.1155/2012/745483

Cendoroglo, M., Jaber, B. L., Balakrishnan, V. S., Perianayagam, M., King, A. J., & Pereira, B. J. G. (1999). Neutrophil apoptosis and dysfunction in uremia. Journal of the American Society of Nephrology, 10(1), 93–100. https://doi.org/10.1681/asn.v10193

Chang, K. P., & Hendricks, L. D. (1985). Laboratory cultivation and maintenance of *Leishmania*. Leishmaniasis.*(Human Parasitic Diseases Vol. 1.)*, 213-244.

Chanyalew, M., Abebe, M., Endale, B., Girma, S., Tasew, G., van Zandbergen, G., Ritter, U., Gadisa, E., Aseffa, A., & Laskay, T. (2021). Enhanced production of pro-inflammatory cytokines and chemokines in Ethiopian cutaneous leishmaniasis upon exposure to *Leishmani*a aethiopica. Cytokine, 145(May), 155289. https://doi.org/10.1016/j.cyto.2020.155289

Charmoy, M., Megnekou, R., Allenbach, C., Zweifel, C., Perez, C., Monnat, K., ... & Tacchini-Cottier, F. (2007). *Leishmania* major induces distinct neutrophil phenotypes in mice that are resistant or susceptible to infection. *Journal of Leucocyte Biology*, *82*(2), 288-299.

Chen, Y. A., Lien, J. C., Tseng, L. F., Cheng, C. F., Lin, W. Y., Wang, H. Y., & Tsai, K. H. (2019). Effects of indoor residual sprayin and outdoor larval control on Anopheles coluzzii from São Tomé and Príncipe, two islands with pre-eliminated malaria. Malaria Journal, 18(1), 1–16. https://doi.org/10.1186/s12936-019-3037-y

Chiswick, E. L., Duffy, E., Japp, B., & Remick, D. (2012). Detection and quantification of cytokines and other biomarkers. Leucocytes: Methods and Protocols, 15-30.

Christen, J., Bourreau, E., Demar, M., Lightburn, E., Couppié, P., Ginouvès, M., Prévot, G., Gangneux, J., & Savini, H. (2018). Use of the intramuscular route to administer pentamidine isethionate in Leishmania guyanensis cutaneous leishmaniasis increases the risk of treatment failure. Travel Medicine and Infectious Disease, February, 1–6. https://doi.org/10.1016/j.tmaid.2018.02.010

Cruz, A. K., de Toledo, J. S., Falade, M., Terrao, M. C., Kamchonwongpaisan, S., Kyle, D. E., & Uthaipibull, C. (2009). Current treatment and drug discovery against Leishmania spp. and Plasmodium spp.: a review. *Current drug targets*, *10*(3), 178-192.

Cunha, P. R., Flora, T. B., & Kroumpouzos, G. (2019). Travelers' tropical skin diseases: Challenges and interventions. DermatologiceTherapy,32(4),1–9. https://doi.org/10.1111/dth.12665

Davidson, R. N., Boer, M. Den, Ritmeijer, K., Sans, M., Msf, F., Middenlaan, P., & Amsterdam, D. D. (2009). Paromomycin. https://doi.org/10.1016/j.trstmh.2008.09.008

Dayakar, A., Chandrasekaran, S., Kuchipudi, S. V., & Kalangi, S. K. (2019). Cytokines: Key determinants of resistance or disease progression in visceral leishmaniasis: Opportunities for novel diagnostics and immunotherapy. Frontiers in Immunology, 10(APR). https://doi.org/10.3389/fimmu.2019.00670

De Pascali, A. M., Todeschini, R., Baiocchi, S., Ortalli, M., Attard, L., Ibarra-Meneses, A. V., ... & Varani, S. (2022). Test combination to detect

latent Leishmania infection: A prevalence study in a newly endemic area for *L. infantum*, northeastern Italy. *PLOS Neglected Tropical Diseases*, *16*(8), e0010676.

de Gouvêa Viana, L., de Assis, T. S. M., Orsini, M., da Silva, A. R., de Souza, G. F., Caligiorne, R., da Silva, A. C. L., Peruhype-Magalhães, V., Marciano, A. P. V., Martins-Filho, O. A., & Rabello, A. (2008). Combined diagnostic methods identify a remarkable proportion of asymptomatic *Leishmania (Leishmania)* chagasi carriers who present modulated cytokine profiles. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *102*(6), 548–555. https://doi.org/10.1016/j.trstmh.2008.02.007

de Castro, R. B., de Moraes, J. V. B., Bressan, G. C., de Souza Vasconcellos, R., Silva-Júnior, A., & Fietto, J. L. R. (2022). Antigens and their diagnostic performance for canine visceral leishmaniasis: a critical review. *Veterinary Parasitology*, *301*, 109638.

Dey, A., & Singh, S. (2006). Transfusion transmitted leishmaniasis: A case report and review of literature. Indian Journal of Medical Microbiology, 24(3), 165–170. https://doi.org/10.1016/s0255-0857(21)02344-6

Doherty, T. (2002). Principles and practice of clinical parasitology. In Transactions of the Royal Society of Tropical Medicine and Hygiene (Vol.96, Issue 4). https://doi.org/10.1016/s0035-9203(02)90401-3

Dos Santos, P. L., de Oliveira, F. A., Santos, M. L. B., Cunha, L. C. S., Lino, M. T., de Oliveira, M. F., ... & de Almeida, R. P. (2016). The severity of visceral leishmaniasis correlates with elevated levels of serum IL-6, IL-27 and sCD14. *PLoS neglected tropical diseases*, *10*(1), e0004375.

Doyle, J. J. (2022). Cell types as species: Exploring a metaphor. *Frontiers in Plant Science*, *13*, 868565.

Dudek, M. (2013). *Investigations of the Ufm1 pathway and its association with a familial form of hip dysplasia*. The University of Manchester (United Kingdom).

Dubie, T., & Mohammed, Y. (2020). Review on the Role of Host Immune Response in Protection and Immunopathogenesis durin Cutaneous Leishmaniasis Infection. Journal of ImmunologyeResearch,2020. https://doi.org/10.1155/2020/2496713

Edrissian, G., Rokni, M. B., Mohebali, M., Nateghpour, M., Mowlavi, G., & Bahadori, M. (2016). History of Medical Parasitology and Parasitic Infections in Iran. Archives of Iranian medicine, 19(8), 601–607.

Elmahallawy, E. K., Alkhaldi, A. A., & Saleh, A. A. (2021). Host immune response against leishmaniasis and parasite persistence strategies: A review and assessment of recent research. *Biomedicine & Pharmacotherapy*, *139*, 111671.

Efthymiou, A., Mureanu, N., Pemberton, R., Tai-MacArthur, S., Mastronicola, D., Scottà, C., ... & Shangaris, P. (2022). Isolation and freezing of human peripheral blood mononuclear cells from pregnant patients. STAR protocols, 3(1), 101204.

Engwerda, C. R., Murphy, M. L., Cotterell, S. E. J., Smelt, S. C., & Kaye, P. M. (1998). Neutralization of IL-12 demonstrates the existence of discrete organ-specific phases in the control of *Leishmania* donovani. European Journal of Immunology, 28(2), 669–680.https://doi.org/10.1002/(SICI)1521-4141(199802)28:02<669::AID-IMMU669>3.0.CO;2-N

Espinosa, O. A., Serrano, M. G., Camargo, E. P., Teixeira, M. M. G., & Shaw, J. J. (2018). An appraisal of the taxonomy and nomenclature of

trypanosomatids presently classified as *Leishmani*a and Endotrypanum. Parasitology, 145(4), 430–442. https://doi.org/10.1017/S0031182016002092

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical biochemistry*, *38*(12), 1103-1111.

Feijó, D., Tibúrcio, R., Ampuero, M., Brodskyn, C., & Tavares, N. (2016). Dendritic cells and leishmania infection: Addin layers of complexity to a complex disease. Journal of Immunology Research, 2016. https://doi.org/10.1155/2016/3967436

Ferreira, B. A., Coser, E. M., Saborito, C., Yamashiro-Kanashiro, E. H., Lindoso, J. A. L., & Coelho, A. C. (2023). In vitro miltefosine and amphotericin B susceptibility of strains and clinical isolates of Leishmania species endemic in Brazil that cause tegumentary leishmaniasis. *Experimental Parasitology*, *246*, 108462.

Flaih, M. H., Al-Abady, F. A., & Hussein, K. R. (2021). Phylogenetic analysis of kinetoplast DNA: kDNA of Leishmania tropica in Thi-Qar province, Iraq. *Comparative Immunology, Microbiology and Infectious Diseases*, *78*, 101696.

Franca, M., Guimaraes, L. H., Nascimento, M. T., Rocha, P. N., & Carvalho, L. P. (2021). The *Leishmania* antigen-specific pro-inflammatory response in cutaneous leishmaniasis is linked to disease progression but not to the therapeutic failure of pentavalent antimonials. *Microbes and infection*, *23*(9-10), 104866.

Gantt, K. R., Schultz-Cherry, S., Rodriguez, N., Jeronimo, S., Nascimento, E. T., Goldman, T. L., ... & Wilson, M. E. (2003). Activation of TGF-β by *Leishmania* chagasi: importance for parasite survival in macrophages. *The Journal of Immunology*, *170*(5), 2613-2620.

Cunningham, K. T., & Mills, K. H. (2023). Modulation of haematopoiesis by protozoal and helminth parasites. *Parasite Immunology*, e12975.

Ghalib, H. W., Piuvezam, M. R., Skeiky, Y. A. W., Siddig, M., Hashim, F. A., El-Hassan, A. M., Russo, D. M., & Reed, S. G. (1993). Interleukin 10 production correlates with pathology in human Leishmania donovani infections. Journal of Clinical Investigation,92(1), 324–329. https://doi.org/10.1172/JCI116570

Gueirard, P., Laplante, A., Rondeau, C., Milon, G., & Desjardins, M. (2008). Trafficking of *Leishmani*a donovani promastigotes in non-lytic compartments in neutrophils enables the subsequent transfer of parasites to macrophages. Cellular microbiology, 10(1), 100-111.

Ghorbani, M., & Farhoudi, R. (2017). Leishmaniasis in humans: drug or vaccine therapy?. *Drug design, development and therapy*, 25-40.

Giudice, A., Camada, I., Leopoldo, P. T. G., Pereira, J. M. B., Riley, L. W., Wilson, M. E., Ho, J. L., de Jesus, A. R., Carvalho, E. M., & Almeida, R. P. (2007). Resistance of Leishmania (Leishmania) amazonensis and Leishmania (Viannia) braziliensis to nitric oxide correlates with disease severity in Tegumentary Leishmaniasis. BMC Infectious Diseases, 7, 1–12. https://doi.org/10.1186/1471-2334-7-7

Gow, I., Smith, N. C., Stark, D., & Ellis, J. (2022). Laboratory diagnostics for human *Leishmani*a infections: a polymerase chain reaction-focussed review of detection and identification methods. *Parasites & Vectors*, *15*(1), 412.

Gordon, S., & Martinez, F. O. (2010). Alternative activation of macrophages: Mechanism and functions. Immunity, 32(5), 593–604. https://doi.org/10.1016/j.immuni.2010.05.007

Grünebast, J., Lorenzen, S., Zummack, J., & Clos, J. (2021). Life Cycle Stage-Specific Accessibility of Leishmania donovani Chromatin at Transcription Start Regions MSystems, 6(4). https://doi.org/10.1128/msystems.00628-21

Gurel, M. S., Tekin, B., & Uzun, S. (2020). Cutaneous leishmaniasis: A great imitator. Clinics in Dermatology, 38(2), 140–151. https://doi.org/10.1016/j.clindermatol.2019.10.008

Guha, R., Gupta, D., Rastogi, R., Vikram, R., Krishnamurthy, G., Bimal, S., & Mukhopadhyay, A. (2013). Vaccination with leishmania hemoglobin receptor–encoding DNA protects against visceral leishmaniasis. *Science Translational Medicine*, *5*(202), 202ra121-202ra121.

Hafeman, D. G., Sunde, R. A. & Hoekstra, W. G. (1974). Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr. 104: 580-587.

Hagiya, Y., Endo, Y., Yonemura, Y., Takahashi, K., Ishizuka, M., Abe, F., ... & Ogura, S. I. (2012). Pivotal roles of peptide transporter PEPT1 and ATP-binding cassette (ABC) transporter ABCG2 in 5-aminolevulinic acid (ALA)-based photocytotoxicity of gastric cancer cells in vitro. *Photodiagnosis and photodynamic therapy*, *9*(3), 204-214.

Hakim, S., Shah, W., Khan, J., Khattak, J. I., Khan, M. I., & Sohail, M. (2023). PCR-Based Detection of Cutaneous Leishmaniasis. Pakistan Journal of Medical & Health Sciences, 17(01), 631-631

Hameed, A. K., & ; Jihad A. Ahmed, A. H. S. and A. S. A.-Q. (2019). Egyptian Journal of Veterinary Sciences. 51(1), 43–54. https://doi.org/10.21608/ejvs.2019.17122.1098

Hassan, G. M., & Ali, H. Z. (2020). Ex vivo study of Anti-leishmanial activity of Artemisinin against *Leishmania* tropica amastigote. Research Journal of Pharmacy and Technology, 13(8), 3787-3791.

Hassan, H. F., Ibrahim, S. A., & Abass, K. S. (2021). Synergic Effect Of Metronidazole In Combination With Azole Dervatives Against Growth Of Leishmania Tropica Promastigotes. 21(2), 5359–5363.

Hassan, S. M., & Dhumad, B. Q. (2021). Genotyping of Histological Cutaneous Leishmaniasis Isolated by Dental Broach Smear in Iraqi Patients. Indian Journal of Forensic Medicine & Toxicology, 15(4)

Hawn, T. R., Ozinsky, A., Underhill, D. M., Buckner, F. S., Akira, S., & Aderem, A. (2002). *Leishmania* major activates IL-1α expression in macrophages through a MyD88-dependent pathway. *Microbes and Infection*, *4*(8), 763-771.

Haydar, H., Mohammed, S., & Essa, R. H. (2018). Leishmanicidal Activity of Fusarium Silver Nanoparticles Against. Biochemical and Cellular Archives,November.https://www.researchgate.net/profile/Sabaa-Mohammed-2/publication/328790138_LEISHMANICIDAL_ACTIVITY_OF_FUSARIUM_SILVER_NANOPARTICLES_AGAINST_LEISHMANIA_DONOVANI_IN_VITRO_STUDY/links/5be31360a6fdcc3a8dc64193/LEISHMANICIDAL-ACTIVITY-OF-FUSARIUM-SILVER-NANOP

Horta, M. F., Mendes, B. P., Roma, E. H., Noronha, F. S. M., MacDo, J. P., Oliveira, L. S., Duarte, M. M., & Vieira, L. Q. (2012). Reactive oxygen species and nitric oxide in cutaneous leishmaniasis. Journal of Parasitology Research, 2012. https://doi.org/10.1155/2012/203818

Hubbard, A. K., & Rothlein, R. (2000). Intercellular adhesion molecule-1 (ICAM-1) expression and cell signaling cascades. Free Radical Biology and Medicine, 28(9), 1379–1386. https://doi.org/10.1016/S0891-5849(00)00223-9

Hussein, A. M., & Ali, H. Z. (2022). Detection of TNF Alpha Level as Biomarker in Different Stages of Cutaneous Leishmaniasis Infection. Iraqi Journal of Science,63(8),3313–3321. https://doi.org/10.24996/ijs.2022.63.8.6

Ishikawa, H., Hisaeda, H., Taniguchi, M., Nakayama, T., Sakai, T., Maekawa, & Himeno, K. (2000). CD4+ Vα14 NKT cells play a crucial role in an early stage of protective immunity against infection with Leishmania major. *International immunology*, *12*(9), 1267-1274.

Ibrahim Al-Jubori, M., Alrahman Al-Tae, A. A., & Al-Faham, M. A. (2019). Detection of Cutaneouse Leishmaniasis species via PCR in Salah Adeen and Baghdad provences. Tikrit Journal of PureeScience,24(1),2415–1726. http://dx.doi.org/10.25130/tjps.24.2019.009

Ikeogu, N. M., Akaluka, G. N., Edechi, C. A., Salako, E. S., Onyilagha, C., Barazandeh, A. F., & Uzonna, J. E. (2020). *Leishmania* immunity: Advancin immunotherapy and vaccine development. Microorganisms,8(8),1–21. https://doi.org/10.3390/microorganisms8081201

Ilgoutz, S. C., & McConville, M. J. (2001). Function and assembly of the Leishmania surface coat. International Journal for Parasitology, 31(9), 899–908. https://doi.org/10.1016/S0020-7519(01)00197-7

Inceboz, T. (2019). Epidemiology and Ecology of Leishmaniasis. Current Topics in Neglected Tropical Diseases. doi: 10.5772/intechopen.8635

Iwasaki, A., & Medzhitov, R. (2004). Toll-like receptor control of the adaptive immune responses. Nature Immunology, 5(10), 987–995. https://doi.org/10.1038/ni1112

Iyer, S. S., & Cheng, G. (2012). Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Critical Reviews in Immunology,32(1),23–63. https://doi.org/10.1615/critrevimmunol.v32.i1.30

Jadhav, N. (2022). Gallic acid induces reactive oxygen species generation and apoptosis in Leishmania donovani in vitro : Probable antileishmanial cellular mechanism. 1–18.

Jafarzadeh, A., Nemati, M., Chauhan, P., Patidar, A., Sarkar, A., Sharifi, I., & Saha, B. (2020). Interleukin-27 Functional Duality Balances Leishmania Infectivity and Pathogenesis. Frontiers in Immunology,11(August),1–21. https://doi.org/10.3389/fimmu.2020.01573

Jones, C. M., & Welburn, S. C. (2021). Leishmaniasis Beyond East Africa. Frontiers in Veterinary Science, 8(February), 1–10. https://doi.org/10.3389/fvets.2021.618766

Josephs, S. F., Ichim, T. E., Prince, S. M., Kesari, S., Marincola, F. M., Escobedo, A. R., & Jafri, A. (2018). Unleashing endogenous TNF-alpha as a cancer immunotherapeutic. Journal of Translational Medicine, 16(1), 1–8. https://doi.org/10.1186/s12967-018-1611-7

Kadhim, I. K., & Al-Quraishi, P. D. M. A. (2020). Molecular And Diagnostic Study of Cutaneous Leishmaniasis Species by Nested-PCR Technique In Babylon Province/ Iraq. European Journal of Molecular & Clinical Medicine, 7(3), 1190–1198. https://ejmcm.com/article_1597.html

Karampetsou, K., Koutsoni, O., & Dotsika, E. (2019). Quantification of Nitric Oxide and Reactive Oxygen Species in Leishmania-infected J774A.1

Macrophages as a Response to the in vitro Treatment with a Natural Product Compound. Bio-Protocol,9(23),1–14. https://doi.org/10.21769/bioprotoc.3442

Kaye, P. M., Svensson, M., Ato, M., Maroof, A., Polley, R., Stager, S., Zubairi, S., & Engwerda, C. R. (2004). The immunopathology of experimental visceral leishmaniasis. ImmunologicaleReviews,201,239–253. https://doi.org/10.1111/j.0105-2896.2004.00188.x

Keighobadi, M., Emami, S., Fakhar, M., Shokri, A., Mirzaei, H., & Teshnizi, S. H. (2019). Parasitology International Repurposin azole antifungals into antileishmanials : Novel 3- triazolyl fl avanones with promisin in vitro antileishmanial activity against *Leishmania* major. Parasitology International, 69(Novembere2018),103–109. https://doi.org/10.1016/j.parint.2018.12.006

Kenny, G. M., Hardner, G. J., Moore, R. M., & Murphy, G. P. (1972). Current results from treatment of stages C and D bladder tumors at Roswell Park Memorial Institute. *The Journal of Urology*, *107*(1), 56-59.

Khurana, A., Sardana, K., & Chowdhary, A. (2019). Antifungal resistance in dermatophytes: Recent trends and therapeutic implications. Fungal Genetics and Biology, 132(May),103255. https://doi.org/10.1016/j.fgb.2019.103255

Kienle, K., & Lämmermann, T. (2016). Neutrophil swarming: an essential process of the neutrophil tissue response. *Immunological reviews*, *273*(1), 76-93.

Kima, P. E. (2007). The amastigote forms of Leishmania are experts at exploitin host cell processes to establish infection and persist. International

Journal for Parasitology, 37(10),1087–1096. https://doi.org/10.1016/j.ijpara.2007.04.007

Kishore, K., Kumar, V., Kesari, S., Dinesh, D. S., Kumar, A. J., Das, P., & Bhattacharya, S. K. (2006). Vector control in leishmaniasis. *Indian Journal of Medical Research*, *123*(3), 467.

Kocyigit, A., Keles, H., Selek, S., Guzel, S., Celik, H., & Erel, O. (2005). Increased DNA damage and oxidative stress in patients with cutaneous leishmaniasis. Mutation Research - Genetic Toxicology and Environmental Mutagenesis,585(1–2),71–78. https://doi.org/10.1016/j.mrgentox.2005.04.012

Kong, L., Smith, W., & Hao, D. (2019). Overview of RAW264.7 for osteoclastogensis study: Phenotype and stimuli. Journal of Cellular and Molecular Medicine, 23(5), 3077–3087. https://doi.org/10.1111/jcmm.14277

Kumar, R., Chauhan, S. B., Ng, S. S., Sundar, S., & Engwerda, C. R. (2017). Immune checkpoint targets for host-directed therapy to prevent and treat leishmaniasis. Frontiers in immunology, 8, 1492.

Kupani, M., Pandey, R. K., Vashisht, S., Singh, S., Prajapati, V. K., & Mehrotra, S. (2023). Prediction of immunogenic peptide ensemble and multi-subunit vaccine for Visceral leishmaniasis using bioinformatics approaches. *Heliyon*.

Kwon, N., Kim, D., Swamy, K. M. K., & Yoon, J. (2021). Metal-coordinated fluorescent and luminescent probes for reactive oxygen species (ROS) and reactive nitrogen species (RNS). Coordination Chemistry Reviews, 427, 213581. https://doi.org/10.1016/j.ccr.2020.213581

Lanza, J. S., Vallejos, V. M. R., Ramos, G. S., De Oliveira, A. C. B., Demicheli, C., Rivas, L., Pomel, S., Loiseau, P. M., & Frézard, F. (2022).

Nanoassemblies from amphiphilic Sb complexes target infection sites in models of visceral and cutaneous leishmaniases. Pharmaceutics, 14(8), 1743.

Larraga, J., Alcolea, P. J., Alonso, A. M., Martins, L. T. C., Moreno, I., Domínguez, M., & Larraga, V. (2022). Leishmania infantum UBC1 in Metacyclic Promastigotes from Phlebotomus perniciosus, a Vaccine Candidate for Zoonotic Visceral Leishmaniasis. Vaccines, 10(2), 1–19. https://doi.org/10.3390/vaccines10020231

Latifynia, A., Khamesipour, A., & Khansari, N. (2023). Proinflammatory cytokines (TNF-a, IL-1B, IL-6) and antioxidant in different stages of patients with cutaneous Leishmaniasis. *World Journal of Advanced Research and Reviews*, *17*(1), 825-835.

Lazarski, C. A., Ford, J., Katzman, S. D., Rosenberg, A. F., & Fowell, D. J. (2013). IL-4 Attenuates Th1-Associated Chemokine Expression and Th1 Traffickin to Inflamed Tissues and Limits Pathogen Clearance. PLoS ONE, 8(8). https://doi.org/10.1371/journal.pone.0071949

Lemos, M. P., Esquivel, F., Scott, P., & Laufer, T. M. (2004). MHC class II expression restricted to CD8α+ and CD11b+ dendritic cells is sufficient for control of Leishmania major. *The Journal of experimental medicine*, *199*(5), 725-730.

Lidani, K. C. F., Andrade, F. A., Tizzot, M. R. P. A., Costa-Ribeiro, M. C., Beltrame, M. H., Messias-Reason, I. J., ... & Claborn, D. (2017). Visceral leishmaniasis and natural infection rates of Leishmania in Lutzomyia longipalpis in Latin America. The Epidemiology and Ecology of Leishmaniasis. London: Intechopen, 59-77.

Liu, D., & Uzonna, J. E. (2012). The early interaction of Leishmania with macrophages and dendritic cells and its influence on the host immune

response. Frontiers in Cellular and Infection Microbiology, 2(June), 83. https://doi.org/10.3389/fcimb.2012.00083

Looker, D. L., Martinez, S., Horton, J. M., & Marr, J. J. (1986). Growth of Leishmania donovani amastigotes in the continuous human macrophage cell line U937: studies of drug efficacy and metabolism. *Journal of Infectious Diseases*, *154*(2), 323-327.

Lutz, M. B., Schnare, M., Menges, M., Rössner, S., Röllinghoff, M., Schuler, G., & Gessner, A. (2002). Differential Functions of IL-4 Receptor Types I and II for Dendritic Cell Maturation and IL-12 Production and Their Dependency on GM-CSF. The Journal of Immunology, 169(7), 3574–3580. https://doi.org/10.4049/jimmunol.169.7.3574

Machado, P. R., & Penna, G. (2012). Miltefosine and cutaneous leishmaniasis. *Current opinion in infectious diseases*, *25*(2), 141-144.

Mann, S., Frasca, K., Scherrer, S., Henao-Martínez, A. F., Newman, S., Ramanan, P., & Suarez, J. A. (2021). A Review of Leishmaniasis: Current Knowledge and Future Directions. Current Tropical Medicine Reports, 8(2), 121–132. https://doi.org/10.1007/s40475-021-00232-7

Margaroni, M., Agallou, M., Vasilakaki, A., Karagkouni, D., Skoufos, G., Hatzigeorgiou, A. G., & Karagouni, E. (2022). Transcriptional Profilin of Leishmania infantum Infected Dendritic Cells: Insights into the Role of Immunometabolism in Host-Parasite Interaction. Microorganisms, 10(7). https://doi.org/10.3390/microorganisms10071271

Maspi, N., Abdoli, A., & Ghaffarifar, F. (2016). Pro- and anti-inflammatory cytokines in cutaneous leishmaniasis: a review. Pathogens and Globa Health,110(6),247–260. https://doi.org/10.1080/20477724.2016.1232042

Matos, A. P. S., Viçosa, A. L., Ré, M. I., Ricci-Júnior, E., & Holandino, C. (2020). A review of current treatments strategies based on paromomycin for leishmaniasis. *Journal of Drug Delivery Science and Technology*, *57*, 101664.

Mattner, F., Magram, J., Ferrante, J., Launois, P., Padova, K. D., Behin, R., ... & Alber, G. (1996). Genetically resistant mice lacking interleukin-12 are susceptible to infection with Leishmania major and mount a polarized Th2 cell response. *European journal of immunology*, *26*(7), 1553-1559.

Mazor, R., Shurtz-Swirski, R., Farah, R., Kristal, B., Shapiro, G., Dorlechter, F., Cohen-Mazor, M., Meilin, E., Tamara, S., & Sela, S. (2008). Primed polymorphonuclear leukocytes constitute a possible link between inflammation and oxidative stress in hyperlipidemic patients. Atherosclerosis,197(2),937–943. https://doi.org/10.1016/j.atherosclerosis.2007.08.014

Melby, P. C., Andrade-Narvaez, F. J., Darnell, B. J., Valencia-Pacheco, G., Tryon, V. V., & Palomo-Cetina, A. (1994). Increased expression of proinflammatory cytokines in chronic lesions of human cutaneous leishmaniasis. *Infection and immunity*, *62*(3), 837-842.

Mirzaei, A., Maleki, M., Masoumi, E., & Maspi, N. (2021). A historical review of the role of cytokines involved in leishmaniasis. Cytokine, 145, 155297. https://doi.org/https://doi.org/10.1016/j.cyto.2020.155297

Mishra, J., Madhubala, R., & Singh, S. (2013). Visceral and post-Kala-Azar dermal leishmaniasis isolates show significant difference in their in vitro dru susceptibility pattern. Parasitology Research, 112(3), 1001–1009. https://doi.org/10.1007/s00436-012-3222-1

Mitjà, O., Marks, M., Bertran, L., Kollie, K., Argaw, D., Fahal, A. H., Fitzpatrick, C., Fuller, L. C., Garcia Izquierdo, B., Hay, R., Ishii, N., Johnson, C., Lazarus, J. V., Meka, A., Murdoch, M., Ohene, S. A., Small, P., Steer, A., Tabah, E. N., … Asiedu, K. (2017). Integrated Control and Management of Neglected Tropical Skin Diseases. PLoS Neglected TropicaleDiseases,11(1),1–13. https://doi.org/10.1371/journal.pntd.0005136

Michlewska, S. (2011). Macrophage phagocytosis of apoptotic neutrophils is critically regulated by the opposing actions of pro-inflammatory and anti-inflammatory agents: key role for TNF-α.

Mougneau, E., Bihl, F., & Glaichenhaus, N. (2011). Cell biology and immunology of Leishmania. *Immunological reviews*, *240*(1), 286-296.

Mohan, V. P., Scanga, C. A., Yu, K., Scott, H. M., Tanaka, K. E., Tsang, E., ... & Chan, J. (2001). Effects of tumor necrosis factor alpha on host immune response in chronic persistent tuberculosis: possible role for limiting pathology. Infection and immunity, *69*(3), 1847-1855.

Mokni, M. (2019, March). Leishmanioses cutanées. In *Annales de* Dermatologie et de Vénéréologie (Vol. 146, No. 3, pp. 232-246). Elsevier Masson.

Moll, H., Scharner, A., & Kämpgen, E. (2002). Increased interleukin 4 (IL-4) receptor expression and IL-4-induced decrease in IL-12 production by langerhans cells infected with Leishmania major. Infection and Immunity, 70(3), 1627–1630. https://doi.org/10.1128/IAI.70.3.1627-1630.2002

Naman, C. B., Gomes, C. M., & Gupta, G. (2018). Phytodrugs and immunomodulators for the therapy of Leishmaniasis. In *Natural Products and Drug Discovery* (pp. 213-275). Elsevier.

Nakamura, H., & Takada, K. (2021). Reactive oxygen species in cancer: Current findings and future directions. Cancer Science, 112(10), 3945–3952. https://doi.org/10.1111/cas.15068

Naddaf, N., & Haddad, S. (2020). Apigenin effect against Leishmania tropica amastigotes in vitro. *Journal of Parasitic Diseases*, *44*(3), 574-578.

Neamah, S. D., Ali, H. Z., & Al-Halbosiy, M. M. (2023). Artemisinin Efficacy on iNOS Production in U937 Cell-line Infected with Leishmania donovani. *Iraqi Journal of Science*, 525-535.

Neamah, S. D., Ali, H. Z., & Al-Halbosiy, M. M. (2022). Detection of artemisinin effect on macrophage inducible nitric oxide gene expression in macrophage infected with Leishmania donovani. *Annals of Parasitology*, *68*(2), 331-338..

Nogueira, P. M., de Menezes-Neto, A., Borges, V. M., Descoteaux, A., Torrecilhas, A. C., Xander, P., Revach, O. Y., Regev-Rudzki, N., & Soares, R. P. (2020). Immunomodulatory Properties of Leishmania Extracellular Vesicles Durin Host-Parasite Interaction: Differential Activation of TLRs and NF-κB Translocation by Dermotropic and Viscerotropic Species. Frontiers in Cellular and Infection Microbiology,10(July),1–9. https://doi.org/10.3389/fcimb.2020.00380

Noyes, H. A., Reyburn, H., Bailey, J. W., & Smith, D. (1998). A nested-PCR-based schizodeme method for identifying Leishmania kinetoplast minicircle classes directly from clinical samples and its application to the study of the epidemiology of Leishmania tropica in Pakistan. Journal of Clinical Microbiology,36(10),2877–2881. https://doi.org/10.1128/jcm.36.10.2877-2881.1998

Oliveira, L. F., Schubach, A. O., Martins, M. M., Passos, S. L., Oliveira, R. V., Marzochi, M. C., & Andrade, C. A. (2011). Systematic review of the adverse effects of cutaneous leishmaniasis treatment in the New World. Acta Tropica, 118(2), 87–96. https://doi.org/10.1016/j.actatropica.2011.02.007

Olivier, M., & Zamboni, D. S. (2020). Leishmania Viannia guyanensis, LRV1 virus and extracellular vesicles: a dangerous trio influencin the faith of immune response durin muco-cutaneous leishmaniasis. Current Opinion in Immunology, 66, 108–113. https://doi.org/10.1016/j.coi.2020.08.004

Olivier, M., Gregory, D. J., & Forget, G. (2005). Subversion mechanisms by which Leishmania parasites can escape the host immune response: A signalin point of view. Clinical MicrobiologyeReviews,18(2),293–305. https://doi.org/10.1128/CMR.18.2.293-305.2005

Ozbel, Y., Oskam, L., Ozensoy, S., Turgay, N., Alkan, M. Z., Jaffe, C. L., & Ozcel, M. A. (2000). A survey on canine leishmaniasis in western Turkey by parasite, DNA and antibody detection assays. *Acta Tropica*, *74*(1), 1-6.Ozbilge, H., Aksoy, N., Kilic, E., Saraymen, R., Yazar, S., & Vural, H. (2005). Evaluation of oxidative stress in cutaneous leishmaniasis. Journal of Dermatology,32(1),7–11.https://doi.org/10.1111/j.1346-8138.2005.tb00705.x

Pal, M., Paulos Gutama, K., H. D. Steinmetz, C., & Dave, P. (2021). Leishmaniasis: An Emergin and Re-emergin Disease of Global Public Health Concern. American Journal of Infectious Diseases and Microbiology, 10(1), 22–25. https://doi.org/10.12691/ajidm-10-1-4

Patil, T., More, V., Rane, D., Mukherjee, A., Suresh, R., Patidar, A., Bodhale, N., Mosser, D., Dandapat, J., & Sarkar, A. (2018). Pro-inflammatory cytokine Interleukin-1β (IL-1β) controls Leishmania infection. Cytokine, 112(April), 27–31. https://doi.org/10.1016/j.cyto.2018.06.033

Pearson, W. R. (2013). An introduction to sequence similarity ("homology") searching. *Current protocols in bioinformatics*, *42*(1), 3-1.

Pinho, N., Bombaça, A. C., Wiśniewski, J. R., Dias-Lopes, G., Saboia-Vahia, L., Cupolillo, E., de Jesus, J. B., de Almeida, R. P., Padrón, G., Menna-Barreto, R., & Cuervo, P. (2022). Nitric Oxide Resistance in *Leishmania* (Viannia) braziliensis Involves Regulation of Glucose Consumption, Glutathione Metabolism and Abundance of Pentose Phosphate Pathway Enzymes.Antioxidants,11(2). https://doi.org/10.3390/antiox11020277

Pourmohammadi, B., Motazedian, M. H., Hatam, G. R., Kalantari, M., Habibi, P., & Sarkari, B. (2010). Comparison of three methods for diagnosis of cutaneous leishmaniasis. *Iranian journal of parasitology*, *5*(4), 1.

Phumee, A., Siriyasatien, P., & Saksirisampant, W. (2022). Complement-mediated Killing of Leishmania martiniquensis Promastigotes. *South Asian Journal of Parasitology*, *6*(1), 15-23.

Prasad, A., Sedlářová, M., Balukova, A., Ovsii, A., Rác, M., Křupka, M., ... & Pospíšil, P. (2020). Reactive oxygen species imaging in U937 cells. Frontiers in physiology, 11, 552569.

Quinn, G. P., & Keough, M. J. (2002). *Experimental design and data analysis for biologists*. Cambridge university press.

Rabaan, A. A., Bakhrebah, M. A., Mohapatra, R. K., Farahat, R. A., Dhawan, M., Alwarthan, S., Aljeldah, M., Al Shammari, B. R., Al-Najjar, A. H., Alhusayyen, M. A., Al-Absi, G. H., Aldawood, Y., Alsaleh, A. A., Alshamrani, S. A., Almuthree, S. A., Alawfi, A., Alshengeti, A., Alwashmi, A. S. S., Hajissa, K., & Nassar, M. S. (2023). Omics

Approaches in Dru Development against Leishmaniasis: Current Scenario and FutureeProspects.Pathogens,12(1),1–20. https://doi.org/10.3390/pathogens12010039

Raimundo, V. D. (2022). Efeitos dos terpenos no tratamento da leishmaniose visceral: uma revisão sistemática da evidência pré-clínica.

Rallis, K. S., Corrigan, A. E., Dadah, H., George, A. M., Keshwara, S. M., Sideris, M., & Szabados, B. (2021). Cytokine-based cancer immunotherapy: Challenges and opportunities for IL-10. AnticancereResearch,41(7),3247–3252. https://doi.org/10.21873/anticanres.15110

Ramos, G. S., Vallejos, V. M. R., Borges, G. S. M., Almeida, R. M., Alves, I. M., Aguiar, M. M. G., Fernandes, C., Guimarães, P. P. G., Fujiwara, R. T., Loiseau, P. M., Ferreira, L. A. M., & Frézard, F. (2022). Formulation of Amphotericin B in PEGylated Liposomes for Improved Treatment of Cutaneous Leishmaniasis by Parenteral and Oral Routes. Pharmaceutics, 14(5). https://doi.org/10.3390/pharmaceutics14050989

Rashidi, S., Fernández-Rubio, C., Manzano-Román, R., Mansouri, R., Shafiei, R., Ali-Hassanzadeh, M., ... & Nguewa, P. (2021). Potential therapeutic targets shared between leishmaniasis and cancer. *Parasitology*, *148*(6), 655-671.

Rather, S., Yaseen, A., Shah, F. Y., Wani, M., Krishan, K., Zirak, S., ... & Rishi, R. (2021). Pediatric cutaneous leishmaniasis: A clinico-epidemiological study from North India. *Indian Dermatology Online Journal*, *12*(6), 852.

Reguera, R. M., Pérez-Pertejo, Y., Gutiérrez-Corbo, C., Domínguez-Asenjo, B., Ordóñez, C., García-Estrada, C., Martínez-Valladares, M.,

& Balaña-Fouce, R. (2019). Current and promisin novel dru candidates against visceral leishmaniasis. Pure and Applied Chemistry, 91(8), 1385–1404. https://doi.org/10.1515/pac-2018-1102

Reithinger, R., Dujardin, J. C., Louzir, H., Pirmez, C., Alexander, B., & Brooker, S. (2007). Cutaneous leishmaniasis. Lancet Infectious Diseases, 7(9), 581–596. https://doi.org/10.1016/S1473-3099(07)70209-8

Resende, L. A., Aguiar-Soares, R. D. D. O., Gama-Ker, H., Roatt, B. M., De Mendonça, L. Z., Alves, M. L. R., Da Silveira-Lemos, D., Corrêa-Oliveira, R., Martins-Filho, O. A., Araújo, M. S. S., Fujiwara, R. T., Gontijo, N. F., Reis, A. B., & Giunchetti, R. C. (2016). Impact of LbSapSal vaccine in canine immunological and parasitological features before and after leishmania chagasi-challenge. PLoS ONE, 11(8), 1–21. https://doi.org/10.1371/journal.pone.0161169

Rodrigues, A. V., Valério-Bolas, A., Alexandre-Pires, G., Aires Pereira, M., Nunes, T., Ligeiro, D., ... & Santos-Gomes, G. (2022). Zoonotic visceral leishmaniasis: new insights on innate immune response by blood macrophages and liver Kupffer cells to Leishmania infantum parasites. *Biology*, *11*(1), 100.

Roles, B. (2021). Department of Biology and Evolution of Marine Organisms Doctor of Philosophy. 0–23. https://doi.org/10.21954/ou.ro.00012914

Rostami, M. N., & Khamesipour, A. (2021). Potential biomarkers of immune protection in human leishmaniasis. Medical Microbiology and Immunology, 210(2–3), 81–100. https://doi.org/10.1007/s00430-021-00703-8

Reverte, M., Snäkä, T., & Fasel, N. (2022). The dangerous liaisons in the oxidative stress response to leishmania infection. *Pathogens*, *11*(4), 409.

Real, F., Florentino, P. T. V., Reis, L. C., Ramos-Sanchez, E. M., Veras, P. S. T., Goto, H., & Mortara, R. A. (2014). Cell-to-cell transfer of L eishmania amazonensis amastigotes is mediated by immunomodulatory LAMP-rich parasitophorous extrusions. *Cellular microbiology*, *16*(10), 1549-1564.

Roy, M., Sarkar, D., & Chatterjee, M. (2022). Quantitative monitorin of experimental and human leishmaniasis employin amastigote-specific genes. Parasitology, 149(8), 1085-1093. doi:10.1017/S0031182022000610

Ruiz-Postigo, J. A., Jain, S., Mikhailov, A., Maia-Elkhoury, A. N., Valadas, S., Warusavithana, S., & Gasimov, E. (2021). Global leishmaniasis surveillance: 2019-2020, a baseline for the 2030 roadmap/Surveillance mondiale de la leishmaniose: 2019-2020, une periode de reference pour la feuille de route a l'horizon 2030. *Weekly epidemiological record*, *96*(35), 401-420.

Sabzevari, S., Mohebali, M., & Hashemi, S. A. (2020). Mucosal and mucocutaneous leishmaniasis in Iran from 1968 to 2018: a narrative review of clinical features, treatments, and outcomes. International Journal of Dermatology, 59(5), 606–612. https://doi.org/10.1111/ijd.14762

Sadhu, A. (2023). Enhancement Of The Culturin Efficiency Of Hela Cells : An Optimization-Based Approach.Efebruary,0–51. https://doi.org/10.13140/RG.2.2.26306.86726

Saha, B., & Silvestre, R. (2021). Cytokines in the immunity and immunopathogenesis in leishmaniases. Cytokine, 145(September), 155320. https://doi.org/10.1016/j.cyto.2020.155320

Sarhan, S. R., Hashim, H. O., & Al-Shuhaib, M. B. S. (2019). The Gly152Val mutation possibly confers resistance to beta-lactam antibiotics in ovine Staphylococcus aureus isolates. *Open Veterinary Journal*, *9*(4), 339-348.

Salam, N., Al-Shaqha, W. M., & Azzi, A. (2014). Leishmaniasis in the Middle East: Incidence ande pidemiology.PLoSeNeglectedeTropicaleDiseases,8(10),1–8. https://doi.org/10.1371/journal.pntd.0003208

Santos, D., Campos, T. M., Saldanha, M., Oliveira, S. C., Nascimento, M., Zamboni, D. S., Machado, P. R., Arruda, S., Scott, P., Carvalho, E. M., & Carvalho, L. P. (2018). IL-1β Production by Intermediate Monocytes Is Associated with Immunopathology in Cutaneous Leishmaniasis. Journal of Investigative Dermatology,138(5),1107–1115. https://doi.org/10.1016/j.jid.2017.11.029

Santarém, N., Tavares, J., & Cordeiro-da-Silva, A. (2019). In vitro infections of macrophage-like cell lines with Leishmania infantum for drug screening. *Leishmania: Methods and Protocols*, 265-277.

Sauter, I. P., Madrid, K. G., de Assis, J. B., Sá-Nunes, A., Torrecilhas, A. C., Staquicini, D. I., Pasqualini, R., Arap, W., & Cortez, M. (2019). TLR9/MyD88/TRIF signalin activates host immune inhibitory CD200 in Leishmania infection. JCI Insight,4(10). https://doi.org/10.1172/jci.insight.126207

Sen, R., Bandyopadhyay, S., Dutta, A., Mandal, G., Ganguly, S., Saha, P., & Chatterjee, M. (2007). Artemisinin triggers induction of cell-cycle arrest and apoptosis in Leishmania donovani promastigotes. *Journal of medical microbiology*, *56*(9), 1213-1218.

Sunter, J., & Gull, K. (2017). Shape, form, function and Leishmania pathogenicity: from textbook descriptions to biological understanding. Open Biol 7: 170165.

Sundström, C., & Nilsson, K. (1976). Establishment and characterization of a human histiocytic lymphoma cell line (U-937). *International journal of cancer*, *17*(5), 565-577.

Scarpini, S., Dondi, A., Totaro, C., Biagi, C., Melchionda, F., Zama, D., Pierantoni, L., Gennari, M., Campagna, C., Prete, A., & Lanari, M. (2022). Visceral Leishmaniasis: Epidemiology, Diagnosis, and Treatment Regimens in Different Geographical Areas with a Focus on Pediatrics. Microorganisms, 10(10). https://doi.org/10.3390/microorganisms10101887

Schmidt, G.D. and Roberts, LS. (2000). Order kinetoplastidae Leishmaniasis. In: Foundation of Parasitology, 6th ed.; McGraw Hill, United state: 70-80.

Schwing, A., Pomares, C., Majoor, A., Boyer, L., Marty, P., & Michel, G. (2019). Leishmania infection: Misdiagnosis as cancer and tumor-promoting potential. Acta tropica, 197, 104855.

Severino, P., Santana, W., Lisboa, E. S., Santos, V. L. S. do., Lima, E. T. do. S., Cardoso, J. C., Albuquerque-Junior, R. L. C. d., Naveros, B. C., Santini, A., Souto, E. B., & Jain, S. (2022). Cutaneous/Mucocutaneous Leishmaniasis Treatment for Wound Healing: Classical versus New Treatment Approaches. Microbiology Research,13(4),836–852. https://doi.org/10.3390/microbiolres13040059

Shaddad, S. (2012). *Identification and in Vitro Evaluation of New Antileishmanial Drugs Using Bioinformatics* (Doctoral dissertation, Faculty of Pharmacy, University of Khartoum**)**..

Shah, P. T., Tufail, M., Wu, C., & Xing, L. (2022). THP-1 cell line model for tuberculosis: A platform for in vitro macrophage manipulation. Tuberculosis,136,102243. https://doi.org/https://doi.org/10.1016/j.tube.2022.102243

Sharma, U., & Singh, S. (2008). Insect vectors of Leishmania: Distribution, physiology and their control. Journal of Vector Borne Diseases, 45(4), 255–272.

Sherif El Shanat, M. B. (2015). Leishmaniasis: Epidemiology, Control and Future Perspectives with Special Emphasis on Egypt. Journal of Tropical Diseases, 03(01), 1–10. https://doi.org/10.4172/2329-891x.1000153

Silva, H., Liyanage, A., Deerasinghe, T., Chandrasekara, V., Chellappan, K., & Karunaweera, N. D. (2021). Treatment failure to sodium stibogluconate in cutaneous leishmaniasis: A challenge to infection control and disease elimination. PLoS ONE,16(10October),1–16. https://doi.org/10.1371/journal.pone.0259009

Singh, S. (2006). New developments in diagnosis of leishmaniasis. Indian Journal of Medical Research, 123(3), 311–330.

Singh, S., & Sivakumar, R. (2004). Challenges and new discoveries in the treatment of leishmaniasis. Journal of Infection and Chemotherapy, 10(6), 307–315. https://doi.org/10.1007/s10156-004-0348-9

Singh, S., Sharma, U., & Mishra, J. (2011). Post-kala-azar dermal leishmaniasis: Recent developments. International Journal of Dermatology, 50(9), 1099–1108. https://doi.org/10.1111/j.1365-4632.2011.04925.x

Snyder, L. M., Doherty, C. M., Mercer, H. L., & Denkers, E. Y. (2021). Induction of IL-12p40 and type 1 immunity by Toxoplasma gondii in the

absence of the TLR-MyD88 signaling cascade. *PLoS Pathogens*, *17*(10), e1009970**.**

Soong, L., Henard, C. A., & Melby, P. C. (2012). Immunopathogenesis of non-healin American cutaneous leishmaniasis and progressive visceral leishmaniasis. Seminars in Immunopathology, 34(6), 735–751. https://doi.org/10.1007/s00281-012-0350-8

Steverding, D. (2017). The history of leishmaniasis. Parasites and Vectors, 10(1), 1–10. https://doi.org/10.1186/s13071-017-2028-5

Steffen, R., DuPont, H. L., & Wilder-Smith, A. (2007). *Manual of travel medicine and health*. PMPH-USA.

Stockdale, L., & Newton, R. (2013). A Review of Preventative Methods against Human Leishmaniasis Infection. PLoS NeglectedeTropicaleDiseas es,7(6). https://doi.org/10.1371/journal.pntd.0002278

Swihart, K., Fruth, U., Messmer, N., Hug, K., Behin, R., Huang, S., ... & Louis, J. A. (1995). Mice from a genetically resistant background lacking the interferon γ receptor are susceptible to infection with Leishmania major but mount a polarized T helper cell 1-type CD4+ T cell response. *The Journal of experimental medicine*, *181*(3), 961-971.

Tadesse, D., Abdissa, A., Mekonnen, M., Belay, T., & Hailu, A. (2021). Antibody and cytokine levels in visceral leishmaniasis patients with varied parasitemia before, during, and after treatment in patients admitted to arba minch general hospital, southern ethiopia. PLoS Neglected Tropical Diseases, 15(8), 1–16. https://doi.org/10.1371/journal.pntd.0009632

Takahashi, H., Hatta, Y., Iriyama, N., Hasegawa, Y., Uchida, H., Nakagawa, M., ... & Takei, M. (2014). Induced differentiation of human

myeloid leukemia cells into M2 macrophages by combined treatment with retinoic acid and 1α, 25-dihydroxyvitamin D3. *PloS one*, *9*(11), e113722.

Tahir, M., Bashir, U., Hafeez, J., & Ghafoor, R. (2019). Safety and efficacy of miltefosine in cutaneous leishmaniasis: An open label, non-comparative study from Balochistan. Pakistan Journal of Medical Sciences, 35(2), 495–499. https://doi.org/10.12669/pjms.35.2.54

Ticha, L., Sadlova, J., Bates, P., & Volf, P. (2022). Experimental infections of sand flies and geckos with Leishmania (Sauroleishmania) adleri and Leishmania (S.) hoogstraali. Parasites and Vectors, 15(1), 1–11. https://doi.org/10.1186/s13071-022-05417-1

Trinchieri, G. (2003). Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nature Reviews Immunology, 3(2), 133–146. https://doi.org/10.1038/nri1001

Tsigankov, P., Gherardini, P. F., Helmer-Citterich, M., Späth, G. F., Myler, P. J., & Zilberstein, D. (2014). Regulation dynamics of leishmania differentiation: Deconvolutin signals and identifyin phosphorylation trends. Molecular and Cellular Proteomics, 13(7), 1787–1799. https://doi.org/10.1074/mcp.M114.037705

Tuon, F. F., Amato Neto, V., & Sabbaga Amato, V. (2008). Leishmania: Origin, evolution and future since the Precambrian. FEMS Immunology and Medical Microbiology,54(2),158–166.https://doi.org/10.1111/j.1574-695X.2008.00455.x

Ullah, I., Shinwari, Z. K., & Khalil, A. T. (2017). Investigation of the cytotoxic and antileishmanial effects of Fagonia indica L. extract and extract mediated silver nanoparticles (AgNPs). *Pakistan journal of botany*, *49*(4), 1561-1568.

Ul Bari, A., & Rahman, S. B. (2008). Cutaneous leishmaniasis: An overview of parasitology and host-parasite-vector inter relationship. Journal of Pakistan Association of Dermatologists, 18(1), 42–48.

von Stebut, E., & Tenzer, S. (2018). Cutaneous leishmaniasis: Distinct functions of dendritic cells and macrophages in the interaction of the host immune system with Leishmania major. International Journal of Medical Microbiology, 308(1), 206–214. https://doi.org/10.1016/j.ijmm.2017.11.002

Weber, J. I., Rodrigues, A. V., Valério-Bolas, A., Nunes, T., Carvalheiro, M., Antunes, W., ... & Santos-Gomes, G. (2023). Insights on Host–Parasite Immunomodulation Mediated by Extracellular Vesicles of Cutaneous Leishmania shawi and Leishmania guyanensis. *Cells*, *12*(8), 1101.

World Health Organization. (2010). Control of the leishmaniases. World Health Organization Technical Report Series, 949, 22–26.128.

World Health Organization. (2010). Report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, Switzerland, 22-26 March 2010. *WHO technical report series*, (949).

World Health Organization. (2021). Health Topics/Leishmaniasis.

Wynick, C., Petes, C., & Gee, K. (2014). Interleukin-27 mediates inflammation durin chronic disease. Journal of Interferon and Cytokine Research, 34(10), 741–749. https://doi.org/10.1089/jir.2013.0154

Yasmin, H., Adhikary, A., Al-Ahdal, M. N., Roy, S., & Kishore, U. (2022). Host–Pathogen Interaction in Leishmaniasis: Immune Response and Vaccination Strategies. Immuno, 2(1), 218–254. https://doi.org/10.3390/immuno2010015

Yentur Doni, N., Gurses, G., Dikme, R., Aksoy, M., Yildiz Zeyrek, F., Simsek, Z., Satoskar, A. R., Varikuty, S., & Yesilova, Y. (2020). Cutaneous Leishmaniasis due to Three Leishmania Species Amon Syrian Refugees in Sanliurfa, Southeastern Turkey. Acta Parasitologica, 65(4), 936–948. https://doi.org/10.2478/s11686-020-00227-w

Zaidieh, T., Smith, J. R., Ball, K. E., & An, Q. (2019). ROS as a novel indicator to predict anticancer drug efficacy. BMC Cancer, 19(1), 1–14. https://doi.org/10.1186/s12885-019-6438-y

Zamorano, J., Rivas, M. D., & Perez, G. M. (2003). Interleukin-4: A multifunctional cytokine. *Immunologia*, *22*(2),215-224

Zheng, Z., Lu, X., Zhou, D., Deng, X. F., Liu, Q. X., Liu, X. B., ... & Dai, J. G. (2024). A novel enemy of cancer: recent investigations into protozoan anti-tumor properties. *Frontiers in Cellular and Infection Microbiology*, *13*, 1325144.

Zhang, W. F., He, Y. L., Zhang, M. S., Yin, Z., & Chen, Q. (2000). Raman scattering study on anatase TiO2 nanocrystals. *Journal of Physics D: Applied Physics*, *33*(8), 912.

Zeng, J., Chen, Q. W., Yu, Z. Y., Zhang, J. R., Chen, D. L., Song, C., ... & Chen, J. P. (2016). Regulation of intrinsic apoptosis in cycloheximide-treated macrophages by the Sichuan human strain of Chinese Leishmania isolates. *Acta tropica*, *153*,101-110.

Appendices

Table (1): Effect of study group, period and drug concentration on IL-1β concentration.

Group(A)	Period (hour) (B)	Concentration of drug (µg/ml) (C)								$LSD_{(0.05)}$ (A*B*C)
		cell	Parasite+cell	62.5	125	250	500	1000	2000	
		Mean±S.D								
nornal	24	155.9± 11.2	162.2± 4.6	126.5± 7.6	133.4± 3.7	139.0± 12.3	140.7± 16.4	139.2± 4.4	136.6± 11.2	12.364
	48	158.9± 8.9	±1180. 13.5	159.4± 6.9	169.0± 9.2	191.7± 14.4	160.9± 15.3	168.4± 9.3	184.8± 10.4	
	72	199.6± 16.3	273.9± 20.1	146.0± 11.3	169.4± 10.1	166.7± 8.4	163.3± 11.3	173.9± 16.2	176.9± 7.8	
cancer	24	184.0± 9.6	170.8± 6.8	169.3± 8.4	151.9± 15.4	183.5± 4.6	185.7± 7.6	160.1± 15.3	170.2± 11.3	
	48	198.1± 17.2	163.5± 7.7	±6153. 5.5	195.6± 5.5	199.7± 11.4	197.3± 5.1	188.5± 22.3	177.3± 9.6	
	72	185.4± 12.4	180.3± 10.0	163. 5±6.1	147.2± 8.8	177.7± 9.6	182.5± 8.4	169.5± 6.4	188.3± 8.2	

Table (2): Effect of study group, period and drug concentration on IL-10 concentration.

Group(A)	Period (hour) (B)	Concentration of drug (µg/ml) (C)								$LSD_{(0.05)}$ (A*B*C)
		cell	Parasite+cell	62.5	125	250	500	1000	2000	
		Mean±S.D								
Normal	24	129.5± 7.6	±3111. 10.3	±596. 5.6	±7101. 11.3	106.1± 9.3	79.9± 10.3	47.4± 7.6	23.2± 3.7	6.822
	48	11.3± 1.9	16.6± 3.4	10.9± 1.2	18.4± 2.4	19.7± 4.1	20.1± 5.6	16.4± 2.3	14.1± 1.2	
	72	16.8± 2.2	19.17± 2.8	16.8± 1.7	17.8± 3.4	22.3± 3.9	21.4± 3.7	12.6± 4.4	16.5± 3.1	
Cancer	24	±916. 3.4	16.9± 2.7	23.8± 4.2	±535. 6.1	±157. 4.3	97.9± 4.6	180.3± 10.2	348.4± 13.2	
	48	9.3± 1.8	17.5± 1.8	20.2± 2.6	21.2± 1.5	16.9± 3.5	24.2± 5.8	18.6± 3.4	17.5± 3.3	
	72	22.5± 2.1	24.6± 3.3	19.8± 3.1	22.1± 1.6	27.8± 5.1	24.5± 5.6	26.4± 1.9	21.9± 3.5	

Table (3): Effect of study group, period and drug concentration on TNF concentration

Group(A)	Period (hour) (B)	Concentration of drug (µg/ml) (C)								$LSD_{(0.05)}$ (A*B*C)
		cell	Parasite+cell	62.5	125	250	500	1000	2000	
		Mean±S.D								
Normal	24	25.2± 3.4	24.4± 3.7	5.0± 1.1	4.4± 0.7	5.9± 0.7	6.8± 1.2	22.1± 1.8	30.0± 2.9	5.441
	48	21.4± 2.9	18.4± 2.6	22.1± 3.1	23.7± 1.9	18.7± 2.6	20.7± 3.3	22.9± 1.4	19.0± 3.8	
	72	11.3± 1.6	12.2± 1.7	8.6± 1.7	7.9± 2.3	12.8± 1.6	11.5± 2.7	26.0± 4.4	14.8± 2.2	
Cancer	24	13.3± 1.8	±710. 2.2	12.5± 1.4	9.5± 1.6	9.5± 1.1	11.0± 2.0	12.5± 1.3	14.5± 1.6	
	48	37.4± 5.2	41.9± 3.6	27.8± 3.7	36.1± 2.5	46.6± 5.3	49.3± 2.4	29.8± 3.3	27.4± 3.3	
	72	131.4± 9.8	80.2± 12.3	64.9± 5.8	81.2± 4.4	±890. 10.1	65.6± 7.7	81.6± 5.5	146.7± 12.2	

4):Effect of study group, period and drug concentration on TGFB1 concentration

Group(A)	Period (hour) (B)	Concentration of drug (µg/ml) (C)								$LSD_{(0.05)}$ (A*B*C)
		cell	Parasite+cell	62.5	125	250	500	1000	2000	
		Mean±S.D								
Normal	24	65.3± 9.2	±554. 11.2	±858. 9.1	±751. 6.1	57.2± 6.6	59.9± 10.1	55.7± 6.6	45.7± 3.3	10.228
	48	71.1± 7.1	71.1± 7.4	69.8± 7.2	75.6± 12.3	82.3± 5.2	75.9± 8.2	69.9± 3.4	71.1± 5.6	
	72	87.3± 6.9	93.2± 6.1	75.0± 6.3	89.1± 8.4	75.2± 6.4	69.2± 7.1	71.1± 6.2	76.6± 4.9	
Cancer	24	52.9± 10.3	54.2± 5.3	157.9± 5.5	145.8± 7.2	85.0± 7.4	69.9± 7.3	165.0± 12.2	133.4± 9.2	
	48	101.1± 8.2	90.7± 2.6	143.4± 5.8	115.3± 5.6	69.8± 4.9	95.9± 5.7	183.2± 9.7	150.9± 13.4	
	72	45.9± 6.7	44.3± 3.6	174.4± 6.1	118.8± 4.2	112.5± 13.3	98.1± 6.4	105.4± 10.3	58.3± 7.7	

Table (5): Effect of study group, period and drug concentration on TAC concentration.

Group(A)	Period (hour) (B)	Concentration of drug (µg/ml) (C)								$LSD_{(0.05)}$ (A*B*C)
		cell	Parasite+cell	62.5	125	250	500	1000	2000	
		Mean±S.D								
Normal	24	4.4± 1.1	3.9± 0.4	3.2± 0.4	3.5± 1.1	3.1± 0.8	3.7± 0.6	2.9± 0.1	4.9± 1.2	1.225
	48	4.4± 1.3	5.2± 1.1	4.1± 0.3	4.9± 0.2	5.0± 0.2	5.1± 0.4	4.7± 0.3	5.4± 0.8	
	72	4.9± 1.5	5.6± 1.4	4.5± 0.4	4.8± 0.5	5.3± 0.6	4.4± 0.2	4.8± 1.1	4.8± 0.6	
Cancer	24	2.6± 0.8	2.8± 0.8	2.0± 0.3	2.1± 0.1	2.2± 0.1	4.2± 0.3	2.2± 0.4	2.1± 0.4	
	48	6.0± 0.2	2.8± 0.8	2.5± 0.6	3.2± 0.3	3.3± 0.8	2.3± 0.1	2.8± 0.3	3.2± 0.5	
	72	2.5± 1.0	2.4± 0.7	3.6± 0.9	2.7± 0.4	3.0± 0.2	2.5± 0.1	3.1± 0.6	2.5± 0.1	

Table (6): Effect of study group, period and drug concentration on ROS concentration.

Group(A)	Period (hour) (B)	Concentration of drug (µg/ml) (C)								$LSD_{(0.05)}$ (A*B*C)
		cell	Parasite+cell	62.5	125	250	500	1000	2000	
		Mean±S.D								
Normal	24	14.9± 2.6	15.9± 2.3	17.9± 2.2	16.4± 3.2	16.5± 2.2	15.6± 1.7	16.4± 1.5	16.4± 2.2	0.982
	48	14.7± 2.4	13.7± 1.6	15.9± 3.3	16.8± 3.3	15.8± 3.7	14.4± 2.2	15.4± 2.2	15.9± 1.6	
	72	12.5± 3.1	10.2± 2.1	12.2± 2.4	16.2± 3.0	16.3± 1.6	16.1± 1.8	19.2± 1.3	15.4± 1.4	
Cancer	24	1.9± 0.2	3.4± 0.7	2.8± 0.7	2.1± 0.4	3.1± 0.4	2.5± 0.3	2.9± 0.2	4.4± 0.8	

	48	4.9± 0.3	3.1± 0.6	4.3± 1.1	3.2± 0.8	3.5± 0.6	4.9± 0.7	3.9± 0.4	2.6± 0.1	
	72	3.5± 0.8	3.5± 0.4	4.5± 1.2	2.7± 0.6	2.8± 0.3	3.1± 0.5	2.4± 0.6	3.8± 0.2	

Table (7): Effect of study group, period and drug concentration on MDA concentration.

Group(A)	**Period (hour) (B)**	**Concentration of drug (µg/ml) (C)**								**$LSD_{(0.05)}$ (A*B*C)**
		cell	**Parasite+cell**	**62.5**	**125**	**250**	**500**	**1000**	**2000**	
		Mean±S.D								
Normal	**24**	14.7± 2.9	12.4± 2.1	9.5± 0.3	18.5± 2.7	18.9± 4.1	11.7± 1.3	10.2± 0.8	12.8± 1.6	**1.528**
	48	17.4± 3.1	15.7± 1.4	19.2± 4.3	10.1± 2.2	11.0± 2.2	11.7± 1.3	7.3± 0.2	6.3± 0.1	
	72	9.9± 1.2	11.2± 1.3	17.4± 2.8	12.9± 3.3	14.4± 1.6	10.6± 1.4	8.1± 0.6	6.5± 0.7	
Cancer	**24**	9.4± 1.1	9.4± 0.7	9.7± 2.1	7.8± 2.4	6.5± 0.8	11.4± 0.7	8.5± 0.5	11.3± 1.1	
	48	14.0± 2.2	15.3± 1.2	15.2± 4.3	7.5± 1.6	7.1± 1.2	15.1± 2.2	13.3± 1.9	13.1± 2.9	
	72	11.3± 1.7	10.0± 0.5	13.8± 2.6	13.1± 2.3	8.7± 0.6	10.2± 1.6	6.3± 1.2	5.3± 0.7	

Table (8): Effect of study group, period and drug concentration on GPX activity.

Group(A)	**Period (hour) (B)**	**Concentration of drug (µg/ml) (C)**								**$LSD_{(0.05)}$ (A*B*C)**
		cell	**Parasite+cell**	**62.5**	**125**	**250**	**500**	**1000**	**2000**	
		Mean±S.D								
normal	**24**	3.5± 0.4	±64. 0.9	5.8± 1.0	4.7± 0.2	5.2± 1.1	4.3± 0.2	4.9± 0.7	4.0± 0.8	**1.328**
	48	2.3± 0.5	±23. 0.6	3.1± 0.2	3.7± 0.4	2.9± 0.3	3.8± 0.8	2.6± 0.3	2.7± 0.4	
	72	2.9± 0.3	2.9± 0.4	2.1± 0.4	2.4± 0.3	2.6± 0.4	2.7± 0.3	3.0± 0.5	3.4± 0.2	
cancer	**24**	4.3± 0.8	4.6± 0.3	3.8± 0.6	4.1± 1.0	2.8± 0.5	4.3± 0.5	4.7± 0.6	4.7± 0.6	
	48	4.6± 0.6	4.2± 0.1	4.5± 1.2	4.4± 0.8	4.9± 0.7	4.9± 0.2	4.9± 0.3	5.4± 1.2	
	72	3.2± 0.1	3.5± 0.2	3.0± 0.7	4.6± 0.2	4.4± 0.3	3.6± 0.1	3.3± 0.4	4.5± 0.6	

NCBI acceptance letter for GenBank OQ653468

Dear GenBank Submitter:

We have provided GenBank accession number(s) for your nucleotide sequence(s):

SUB12969081 Seq1 OQ653468

A copy of your revised files can be viewed at

https://submit.ncbi.nlm.nih.gov/subs/?search=SUB12969081

The accession number link in the Submission Portal will not be active until a few days after the public release of the sequences.

Changes may have been made to your original submission in order to conform to database annotation conventions. See the following for a list of possible modifications:
https://ncbi.nlm.nih.gov/genbank/flatfile_changes/

If you need to revise your record(s), follow these directions to format your update request: https://www.ncbi.nlm.nih.gov/Genbank/update.html
Send properly formatted updates to: gb-admin@ncbi.nlm.nih.gov
Do not make a new submission for an update request.

Based on the data submitted to us, the scheduled release date for your submission is:

Mar 24, 2023

The entire sequence will be released when the article citing this accession number(s) is published or on the above release date, whichever comes first. If this date is not correct, please let us know as soon as possible, otherwise this submission will be released on the date indicated above. The data will become available from our different servers within a few days of release and are simultaneously made available to other INSDC databases, the European Nucleotide Archive (ENA) and the DNA Data Bank of Japan (DDBJ).

Thank you for your submission of sequence data to GenBank, a contribution which will benefit the scientific community.

Sincerely,

GenBank Direct Submission Staff
gb-admin@ncbi.nlm.nih.gov

Preliminary sequences (flat files) submitted to NCBI for the GenBank OQ653468

```
LOCUS       OQ653468                323 bp    DNA     linear   INV 24-MAR-
2023
DEFINITION  Leishmania tropica isolate Rasha-S1 internal transcribed spacer 1
            and 5.8S ribosomal RNA gene, partial sequence.
ACCESSION   OQ653468
VERSION     OQ653468.1
KEYWORDS    .
SOURCE      Leishmania tropica
  ORGANISM  Leishmania tropica
            Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina;
            Trypanosomatida; Trypanosomatidae; Leishmaniinae; Leishmania.
REFERENCE   1  (bases 1 to 323)
  AUTHORS   Al-Quraishi,M.A. and Jawad,R.R.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-MAR-2023) Department of Life Sciences, College of
            Science, University of Babylon, Al-Tagia, Hilla, Babil 51001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..323
                     /organism="Leishmania tropica"
                     /mol_type="genomic DNA"
                     /isolate="Rasha-S1"
                     /isolation_source="tissue"
                     /host="Sandfly"
                     /db_xref="taxon:5666"
                     /country="Iraq"
                     /collection_date="Jan-2022"
                     /collected_by="Rasha Riyadh Jawad"
     misc_RNA        <1..>323
                     /note="contains internal transcribed spacer 1 and 5.8S
                     ribosomal RNA"
ORIGIN
        1 ctggatcatt ttccgatgat tacacccaaa aaaaacatat acaaaactcg gggaggccta
       61 tatattatac attatatagg cctttcccac acatacacag caaacttta tactcgaagt
      121 ttgcagtaaa caaaaggccg atcgacctta taacgcaccg cctatacaca aaagcaaaaa
      181 tgtccgttta tacaaatata cggcgtttcg gttttgttgg cgggggtgc gtgtgtggat
      241 aacggctcac ataacgtgtc gcgatggatg acttggcttc ctatttcgtt gaagaacgca
      301 gtaaagtgcg ataagtggta tca
//
```

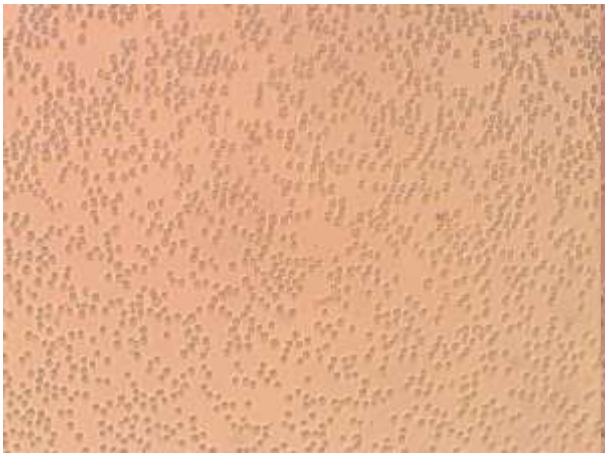


Figure (1) U937 CELL 10X

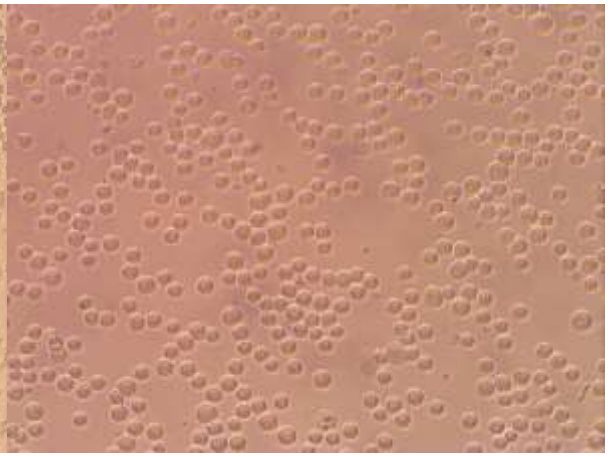


Figure (2) U793 CELL 20X

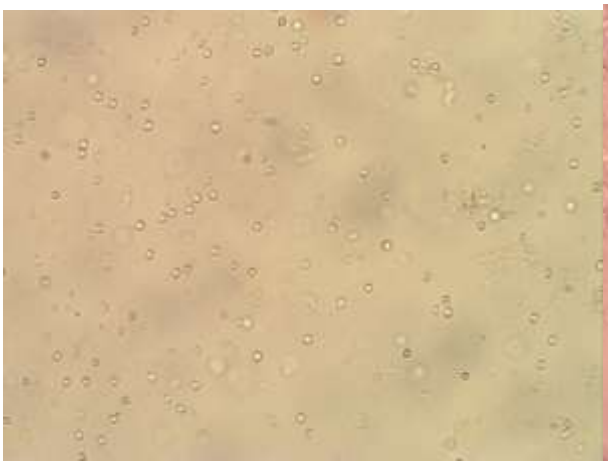


Figure (3) Normal Cell 20X

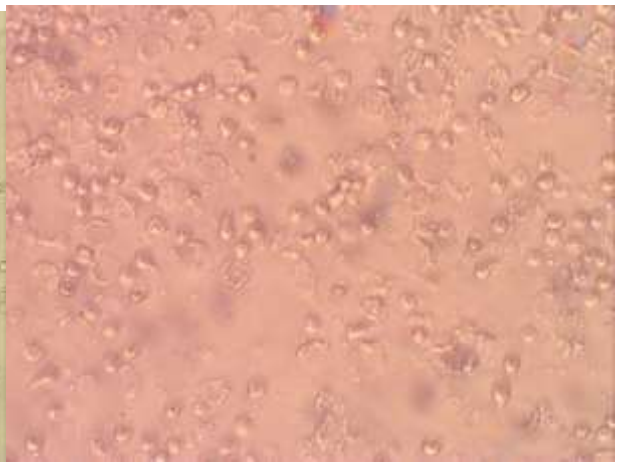


Figure (4) Normal Macrophag Cell 40 X

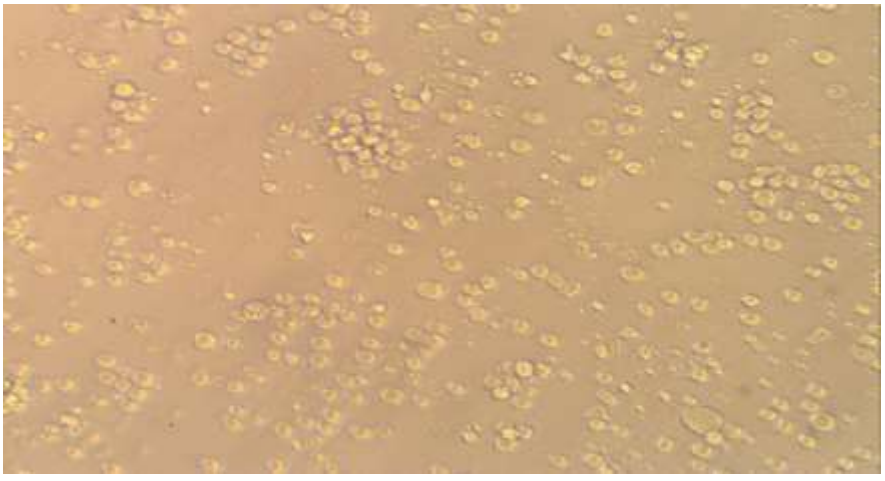


Figure (5)U793 Cell After Add 500ng/ml PMA 20x and add PHA-K (Phytohemagglutinin-K)

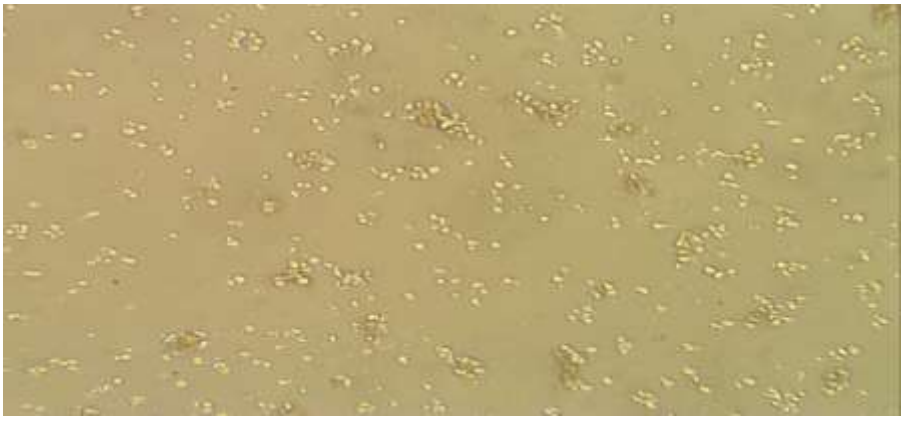


Figure (6)U793 Cell After Add 1000Pma 20x and add PHA-K (Phytohemagglutinin-K

Figure (7) parasite enter cell 20x

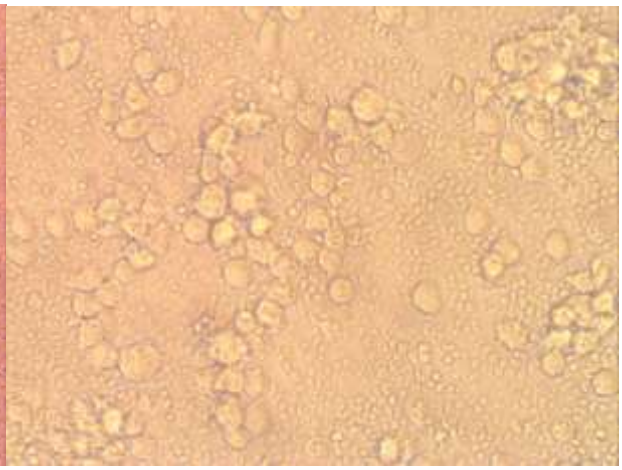


Figure 8) parasite enter cell 40x

Figure (9) parasite with cells 20x

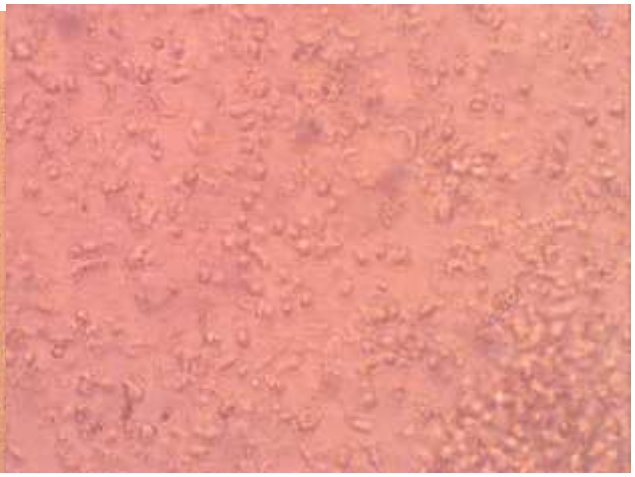


Figure (10) parasite with cells 40 x

Figure (11): Tubes containing N. N. N. medium

Figure (12): RPMI 1640 Medium

Figure (13): Favorgen Plasmid DNA Extraction Mini Kit

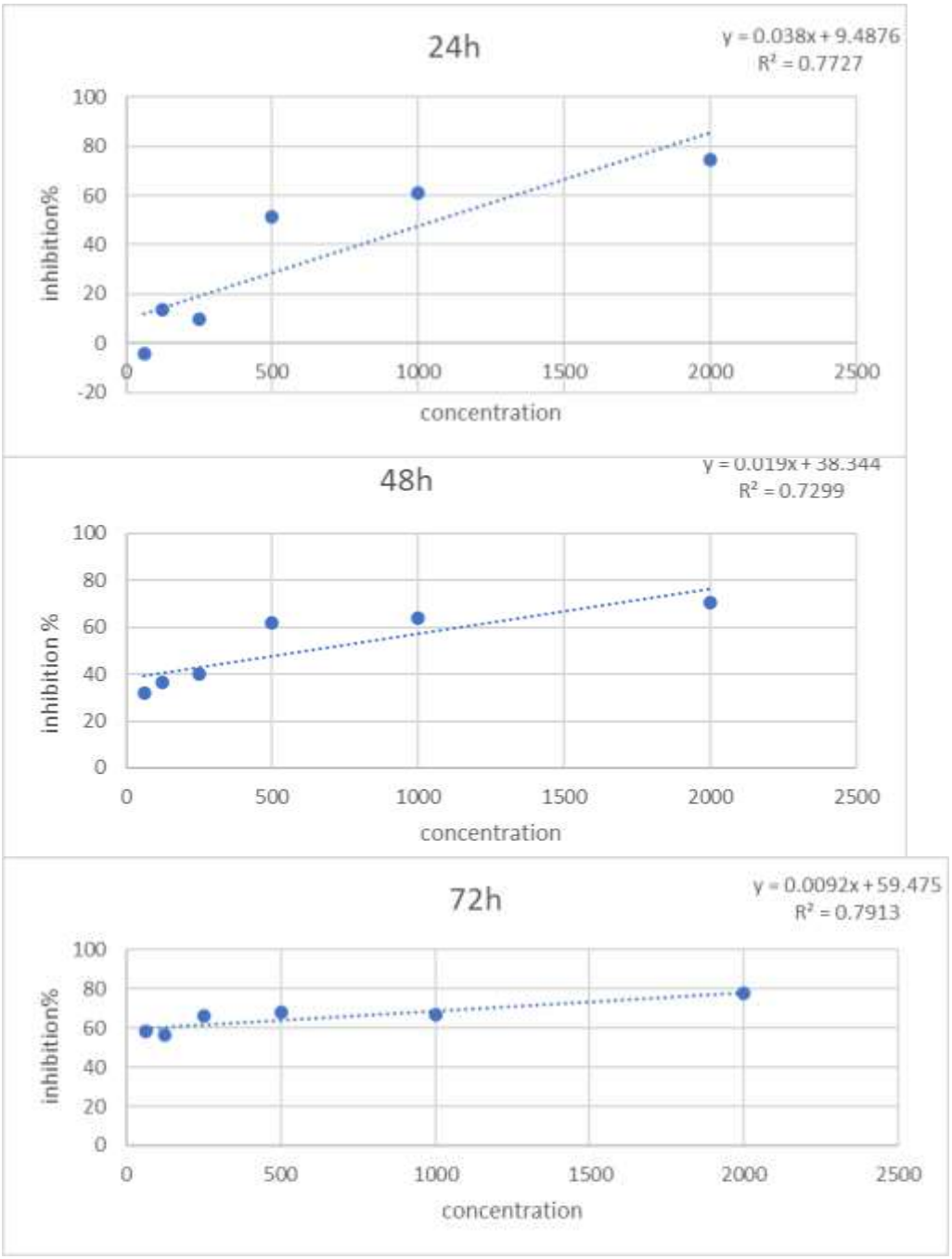


Figure (14): Shows the ic50 of normal cells over a period of time.

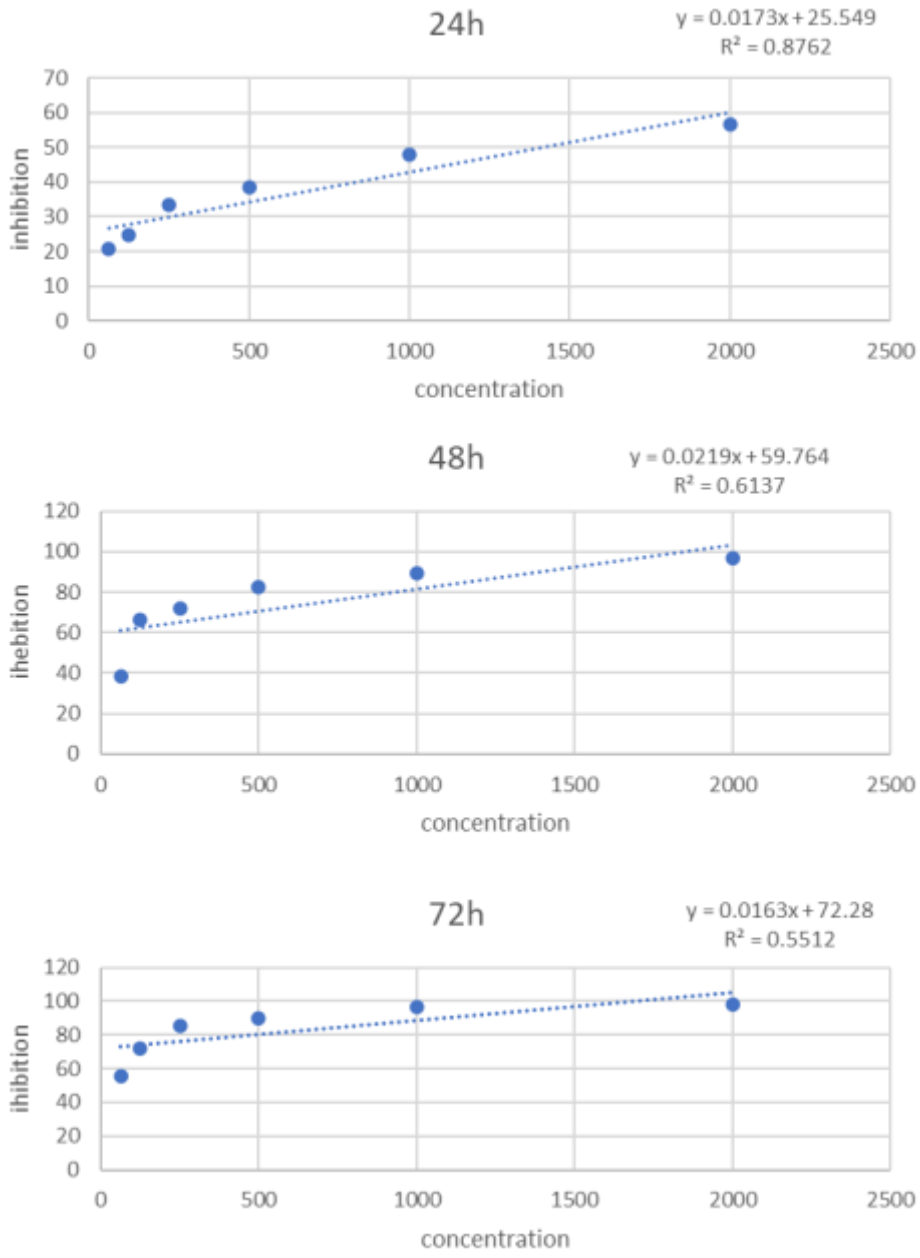


Figure (15): Shows the ic50 of cancer cells over a period of time

الخلاصة :

خلال الفترة من أكتوبر 2022 إلى يونيو 2023، تم دراسة الطفيلي ونموه، وتنفيذ بعض العناصر التجريبية في المختبر المتقدم لعلم الطفيليات في كلية العلوم ومختبر زرع الأنسجة في كلية الطب / جامعة بابل.

الليشمانيا هي مرض يسببه طفيليات البروتوزوا الأولية. يتم نقل هذه الطفيليات إلى البشر عن طريق لدغات الذباب الرملي الأنثوية المصابة. يمكن للطفيلي أن يحفز الجهاز المناعي لإنتاج سايتوكينات التهابية مثل عامل نخر الورم ألفا (TNF-α) وإنترلوكين-1 (IL-1) وإنترلوكين-6 (IL-6) وإنترلوكين-12 (IL-12) وإنترفيرون جاما (IFN-γ). تلعب هذه السايتوكينات دورًا في تنشيط الخلايا المناعية وتعزيز الالتهاب المحلي. بالإضافة إلى ذلك، يمكن لليشمانيا أيضًا أن تحفز إنتاج سايتوكينات مضادة للالتهاب مثل إنترلوكين-10 (IL-10) وعامل تحويل النمو بيتا (TGF-β)، والتي تنظم الاستجابة المناعية وتساعد في تقليل الالتهاب. يمكن أن تختلف استجابة السايتوكينات خلال عدوى الليشمانيا اعتمادًا على نوع الليشمانيا واستجابة الجهاز المناعي للمضيف.

في هذه الدراسة، تم أخذ عينات من مريض يشتبه بإصابته بعدوى ليشمانيا في عيادة الأمراض الجلدية في منطقة سنجار بمحافظة الموصل. تم زرع الطفيلي في وسط ثقافة NNN ووسط RBMI 1640. بعد ذلك، تم تحديد الليشمانيا بواسطة تسلسل الحمض النووي والتحديد الجيني. تم تكبير جزء محدد من الحمض النووي بواسطة تفاعل البلمرة المتسلسل (PCR) باستخدام جزء من فحص IS1 و S rRNA5.8 و ITS rRNA في الليشمانيا التروبيكا. تمت تعريض الشرائح المكبَّرة لتجارب تسلسل سانجر مباشرة لتقييم نمط التشابه الجيني في عينات *L.tropica*. تم بناء شجرة شاملة محددة لتقييم التحديد الجيني الدقيق للمتغيرات المرصودة وتوزيعها الفيلوجيني . وجود متغير واحد لحمض نووي (ACdel95-94) في العينة المدروسة. تشير الشجرة إلى أن العينة المدروسة تحتل نفس الموضع الفيلوجيني داخل تسلسلات ليشمانيا التروبيكا، مع انحراف ملحوظ عن سلالات FN677341.1 و OL413428.1 التي عزلت من مصادر فلسطينية وماليزية على التوالي.

ثم يتم تعريض الطفيلي لتراكيز من بنتوستام بتراكيز 2000 و 1500 و 1000 و 500 و 250 و 62.5 µم لتحديد سميته. يتم اصابة U937 cancer cell وماكروفاج الدم البشرية المشتقة من الكريات البيضاء الأولية (MDMs) بالطفيلي مع تراكيز مختلفة من العلاج العلاج القياسي لليشمانيا ثم يتم اعتبار القدرة الحيوية للخلايا والسمية. يتم قياس مستويات الإنترلوكينات (TNF-α و IL-10 و IL-B1 و TGF-B1) ومستوى (MDA و ROS و GPX و TAC)

في ماكروفاج U937 cancer cell المشتقة وماكروفاج normal cell (MDMs).شملت الدراسة أيضًا تحليل تأثير ستيبوغلوكونات الصوديوم على إفراز السايتوكينات من الخلايا المناعية. أظهرت النتائج أن ستيبوغلوكونات الصوديوم تتناسب مباشرة مع إفراز السايتوكينات الهامة مثل TNF وتتناسب عكسيا مع IL-10 و IL-B1 و TGF-B1. بشكل عام، تشير الدراسة إلى أن ستيبوغلوكونات الصوديوم لها تأثير سام على الليشمانيا الجلدية وتكبح استجابة الجهاز المناعي.

أظهرت نتائج هذه الدراسة انخفاضًا في تركيز إنزيم GPX ومؤشر TAC في الخلايا الطبيعية وكذلك الخلايا السرطانية، ويرجع هذا الانخفاض إلى إنتاج مزيد من أنواع الأكسجين النشط من قبل الخلايا البلعمية. تشير العلاقة العكسية الملاحظة بين مستويات TAC و ROS و MDA ونشاط GPx في الماكروفاج المصابة بالليشمانيا إلى تعطل آليات الدفاع المضادة للأكسدة الطبيعية. قدرة الطفيلي على تعديل استجابة المضيف المناعية والتدخل في الجهاز المضاد للأكسدة يسهم في التوتر الأكسدة وأكسدة الدهون وانخفاض القدرة الكلية للمضادات الأكسدة. يمكن أن تكون هذه التوازنات لها آثار على آلية الإصابة بالليشمانيا الجلدية وقد تؤثر على نتيجة المرض. قد يؤدي الإصابة بالليشمانيا إلى تغييرات في استجابة المناعة وتنشيط الخلايا المناعية المضادة للأورام.

استخدام الخلايا الطبيعية من الخلايا العملاقة والخلايا السرطانية يساعد في فهم استجابات المناعة المختلفة ويوفر للباحثين خيارات لمكافحة الليشمانيا. قد تكون للخلايا الطبيعية مقاومة طبيعية أعلى أو قابلية أقل لتأثيرات العلاج المضاد للطفيليات مقارنة بالخلايا السرطانية. يمكن دمج هذه النتائج في تطوير علاجات جديدة للإصابة بالليشمانيا لتعزيز النتائج العلاجية وتقليل مقاومة الدواء وتحسين رعاية المرضى في مكافحة هذا المرض الطفيلي.