Vestige radiosources radiation on *Leishmania donovanii*

Nebras Rada Mohammed[1]; Abid Ali Genzel[1], Hanaa Salih Sabaa [2] ; Israa Salim Mousa[3]; Khawla Hori Zghair[3]; Suhair Shakir Mageed [4] and Khalid Hadi Mahdi [5]

1) Al- Turath University / Iraq

2) Physics department – College of Science – Al- Mustansiriya University/ Iraq

3) Biology department – College of Science – Baghdad University/ Iraq

4) Biology department – College of Science – Al- Mustansiriya University/ Iraq

5) College of Education Ibn Al Haitham –Baghdad University/ Iraq

and Physics department/ Science Faculty/ Karabuk university/ Karabuk/ Turkey

Abstract

The objective of this treatise in order to determine insinuation of radiosources rays on *Leishmania donovanii*. In this descriptive study design of 10 *Leishmania donovanii* cross-sectional study design, collected from Al-Karamah hospitals in Baghdad from different patients.The parasite was diagnosed based on biochemical tests and depending on its cultural characteristics by utilizing a special medium N199 semi soild medium for growth leishmania.

Study populations of (10) *Leishmania donovanii* in order to treatise vestige radiation by cultivated on M199 media at 25°C for five days to reach the stationary-phase culture, then culture was centrifuged (5000 rpm for 10 min). The pellet was suspended in 150 ml of sterile normal saline ,then 1 ml of this solution was exposed to different radioactive radiations, comparison to control group (without exposure),rediluted and inoculated in M199 media, then cultivated within test tube containing distilled water 5ml, then exposing to an different radiosources including CS^{137}, Sr^{90} and CO^{60}, with activity 1-10 µci with different radiation doses for different peroids 1 hrs.

The results of exposure beta and gamma rays emitted by CS^{137} with Almonium, activity 10 µci, viable cell in 1hrs. 10.8 (97.29%) L1; CS^{137} without Almonium, activity 10 µci, viable cell in 1hrs. 2.7 (89.18%) cells L2,; also exposure L3 to CS^{137} with Almonium, activity 1 µci, viable cell in 1hrs. 2.7(97.29%); exposure L4 to CS^{137} without Almonium, activity 1 µci, viable cell in 1hrs. 8.1(91.89%).

Exhibition Sr^{90} radiosources to L5 with activity 3 µci, viable cell in 1hrs. 2.7(97.29%) and exhibition L6 to CO^{60} with 1 µci. with Almonium for 1hrs.5.4(94.59%), also exposing L7 to CO^{60} with 1 µci. without Almonium for 1hrs. 0(100%).

The Vestige of irradiation on the viability of *L.donovani* appear by count viability with calculate percentage of killing, the number of viable cells of *L. donovani* is fewer compared

before exhibition to irradiation, but percentage of killing is higher compared with before exposure to irradiation, irradiation is efficient for killing *L. donovani* with devoid flagellum. Statistical Analysis System- SAS program all results are significant.

Key words: Impress, rays, *Leishmania spp.*

Introduction

Leishmania donovani is a species of intracellular parasites belonging to the genus *Leishmania*, a group of haemoflagellate kinetoplastids that cause the disease leishmaniasis. It is a human blood parasite responsible for visceral leishmaniasis or *kala-azar*, the most severe form of leishmaniasis. It infects the mononuclear phagocyte system including spleen, liver and bone marrow. Infection is transmitted by species of sandfly belonging to the genus *Phlebotomus*[1,2,3,4].

Pathogenicity of *L. donovani* is the causative agent of visceral leishmaniasis, known as *kala-azar* (black fever). The disease is highly lethal if not treated properly. The incubation period ranges from 3 to 6 months and in some cases may be over a year. The target cells are mononuclear phagocyte system. The two main tissues of infection are spleen and liver. Clinical symptoms include pyrexia (recurring high fever which may be continuous or remittent), enlargement of spleen and liver and heavy skin pigmentation which darkens the physical appearance (the reason for naming "black fever"). Morphological symptoms are noticeable particularly on facial and abdominal regions. Skin becomes coarse and hard,in a fully developed stage, the patient shows emaciation and anaemia.The disease is often accompanied by complicationswith dysentery, tuberculosis, septicaemia andeven HIV infection[5,6,7].

Leishmania protozoa cause cutaneous, mucocutaneous or visceral leishmaniasis (VL). Globally, the disease results in ≈2 million new cases and 2.4 million disability-adjusted life years each year [8,9,10].One of the most dramatic examples is a new focus of cutaneous leishmaniasis (CL) in Sri Lanka [5],from which >400 cases have been reported since 2001[11].

L. donovani typically causes VL, a potentially fatal disease and ongoing public health problem in neighboring India, Bangladesh and Nepal, as well as in East Africa examined a limited number of isolates and used a single technique, MLEE[12,13,14,15].

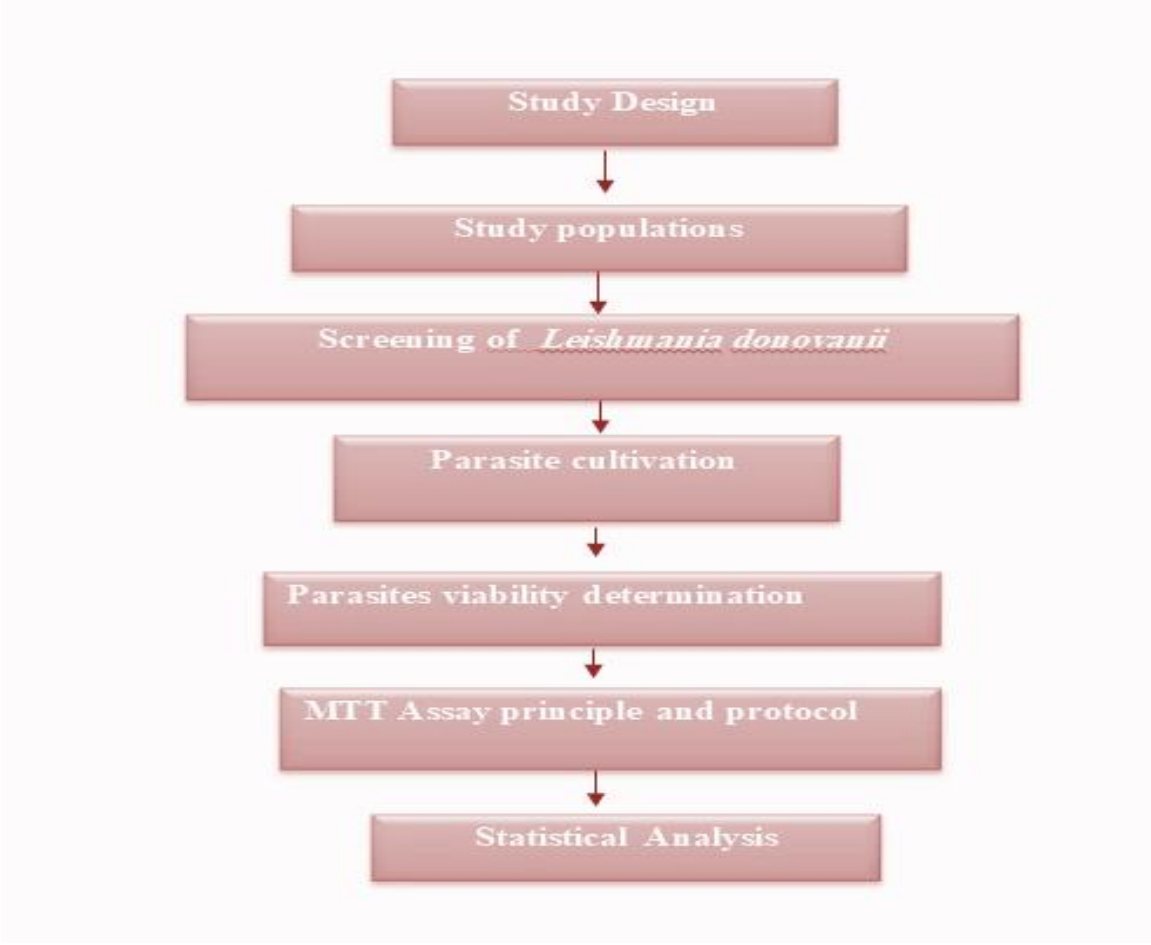
L. donovani was independently discovered by two British medical officers William Boog Leishman in Netley, England and Charles Donovan in Madras, India, in 1903. However, the correct taxonomy was provided by Ronald Ross. The parasite requires two different hosts for a complete life cycle, humans as the definitive host and sandflies as the intermediate host. In some parts of the world other mammals, especially canines, act as reservoir hosts. In human cell they exist as small, spherical and unflagellated amastigote form; while they are elongated with flagellum as promastigote form in sandflies. Unlike other parasitic protists they are unable to directly penetrate the host cell and are dependent upon phagocytosis[16,17]. The whole genome sequence of *L. donovani* obtained from southeastern Nepal was published in 2011[18].

Methodology

Clinical examination include study design, study populations from different patients with different age, screening of *Leishmania donovanii*,parasites cultivation, parasites- viability, MTT assay, Vestige radiosources rays on *Leishmania donovanii* and Statistical analysis [19].

Study design

In this descriptive study design of 10 *Leishmania donovanii* cross-sectional study design, collected from Al-Karamah hospitals in Baghdad from different patients. Study design are specific plan or protocol for direct study which allows the researcher to translate the conceptual hypothesis into an operational one, another define is the formulation of trials and experiments, as well as observational studies in medical, clinical or other types of research (e.g:epidemiological) involving human beings[20].



Scheme (1): Methodology of research project

Study populations

Study populations of (10) *Leishmania donovanii* from different age from male and female. The parasite was diagnosed based on biochemical tests and depending on its cultural characteristics by utilizing a special medium N199 semi soild medium for growth leishmania.

Screening of *Leishmania donovanii*

Parasite cultivation

Parasite cultivation was done as follows: *L. donovani* were cultivated in M199 media at 25° C for five days to reach the stationary-phase culture for activation.

Parasites- Viablitty

Parasites viability determination In vitro parasites viability was determined by using MTT assay. MTT assay principal and protocol MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; thiazolyl blue] is a water soluble tetrazolium salt yielding a yellowish solution. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by dehydrogenase enzymes [21]. This water insoluble formazan can be solubilized using Dimethyl sulfoxide (DMSO), and the dissolved material is measured spectrophoto-metrically yielding absorbance as a function of concentration of converted dye [22].

The equation of calculating the killing of *Leishmania donovanii*:

$$\text{Killing of } \textit{Leishmania donovanii}\ \% = \frac{\text{Control} - \text{Patronize}}{\text{Control}} * 100$$

MTT Assay

Relative numbers of live cells were determined based on the optical absorbance of the treated and untreated samples and blank wells using the formula mentioned below. *L. donovani* was prepared in 96-well plates in a final volume of 100 µl/well and incubated at 25˚C for three days. Ten µl of MTT solution was added per well and then the plate was incubated for 4 h at 25˚C. The media was removed and 100 µl of DMSO solution was added in order to solubilize the formazan crystals. The plate was stirring gently then, left for 15 min. Absorbance was recorded at 490 or 630 nm by microplate reader and viability determined using the formula:

$$\text{Viable cells (\%)} = (AT-AB) / (AC-AB) \times 100)$$

Where AC, AT and AB is the absorbance of the untreated, treated samples and blank respectively [23].

Vestige radiosources rays on *Leishmania donovanii*

Parasite cultivation was done as follows: *L. donovani* promastigotes were cultivated in M199 media at 25˚C for five days to reach the stationary-phase culture for activation. then culture was centrifuged (5000 rpm for 10 min). The pellet was suspended in 150 ml of sterile normal saline ,then 1 ml of this solution was exposed to different radioactive sources comparison to control group (without exposure), rediluted and inoculated in M199 media, then cultivated within test

tube containing distilled water 5ml, then exposing to an different radiosources including CS^{137}, Sr^{90} and CO^{60}, with activity 1-10 µci with different radiation doses for different peroids 1-2-3 hrs. in comparison with control group (without exposure) and re-diluted, inoculated in M199 media.

Statistical analysis :

Statistical analysis Chi-square test was used to assignment the difference between two groups. $P < 0.05$ was considered as significant difference and $P < 0.01$ as highly significant.Statistical Analysis System- SAS .program was used to effect of difference factors in study parameters . Chi-square test was used to significant compare between[24].

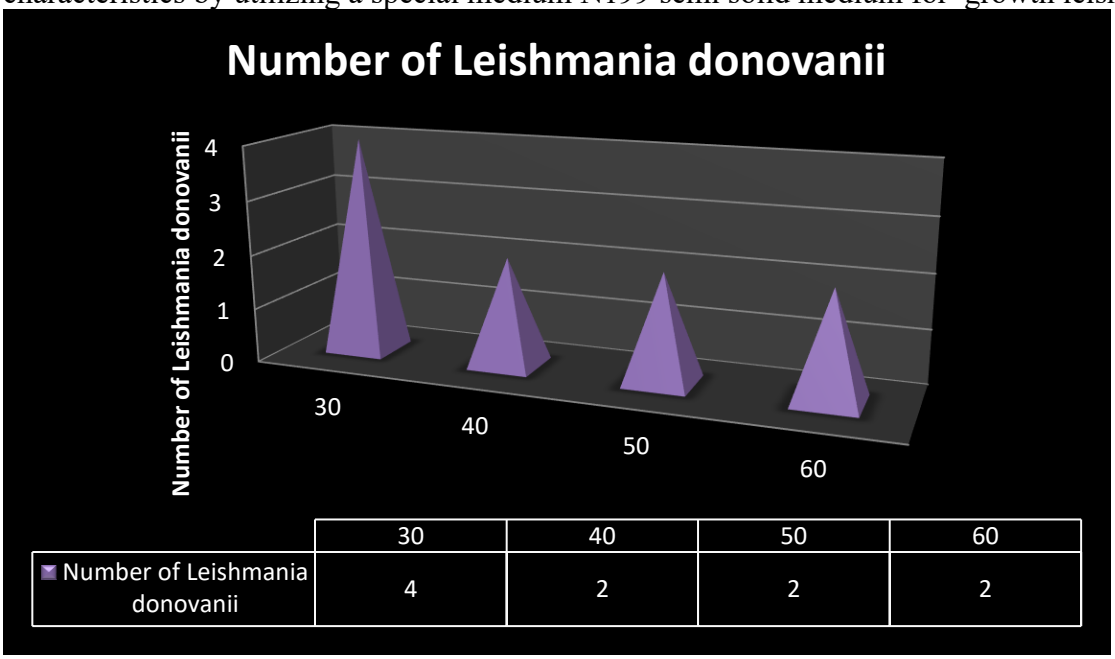
Results and Discussion

Study design

In this descriptive study design of (10) *Leishmania donovanii* by cross-sectional study design collected from Baghdad hospitals.

Study populations

Study populations of (10) *Leishmania donovanii* from different age from male and female inclusive 4 from 30 years, 2 from 40 years, 2 from 50 years and 2 from 60 years as shown in figure(2). The parasite was diagnosed based on biochemical tests and depending on its cultural characteristics by utilizing a special medium N199 semi soild medium for growth leishmania.



Figure(1): Prevelance *Leishmania donovanii* collected from AL-Karamah Hospitals in Baghdad.

Vestige radiosources rays on *Leishmania donovanii*

The results of exposure beta and gamma rays emitted by CS^{137} with Almonium, activity 10 µci, viable cell in 1hrs. 10.8 (97.29%) L1; CS^{137} without Almonium, activity 10 µci, viable cell in 1hrs. 2.7 (89.18%) cells L2,; also exposure L3 to CS^{137} with Almonium, activity 1 µci, viable

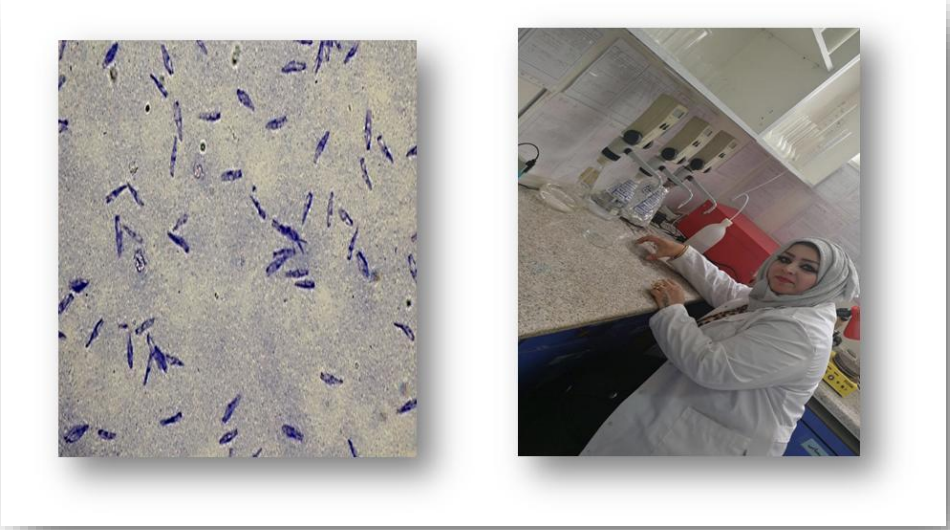
cell in 1hrs. 2.7(97.29%); exposure L4 to CS ^{137} without Almonium, activity 1 µci, viable cell in 1hrs. 8.1(91.89%).

Exhibition Sr^{90} radiosources to L5 with activity 3 µci, viable cell in 1hrs. 2.7(97.29%) and exhibition L6 to CO ^{60} with 1 µci. with Almonium for 1hrs.5.4(94.59%), also exposing L7 to CO ^{60} with 1 µci. without Almonium for 1hrs. 0(100%).

The Vestige of irradiation on the viability of *L.donovani* appear by count viability with calculate percentage of killing, the number of viable cells of *L. donovani* is fewer compared before exhibition to irradiation, but percentage of killing is higher compared with before exposure to irradiation, irradiation is efficient for killing *L. donovani* with devoid flagellum as shown in table(1) and figure(2).

Table(1): Vestige different rays with Proportion viability with percentage of homicide of *L.donovanii* exposed to radioactive radiations.

Percentage of homicide (%)	Viable cells of *L.donovanii* (%)	T-B (Treated-Blank)	C-B (Control-Blank)	Treated (T)	Control (C)	Blank (B)	Radioactive rays	Sample
97.29%	2.7 %	0.001	0.037	0.64	0.1	0.063	CS^{137}/1µci + AL	L1
89.18%	10.8 %	0.004	0.037	0.067	0.1	0.063	CS^{137}/1µci - AL	L2
97.29%	2.7 %	0.001	0.037	0.064	0.1	0.063	CS^{137}/10µci + AL	L3
91.89%	8.1 %	0.003	0.037	0.066	0.1	0.063	CS^{137}/10µci - AL	L4
97.29%	2.7 %	0.001	0.037	0.064	0.1	0.063	Sr^{90}/1µci + AL	L5
94.59%	5.4 %	0.002	0.037	0.065	0.1	0.063	Co^{60}/1µci + AL	L6
100%	0 %	0.0	0.037	0.063	0.1	0.063	Co^{60}/1µci - AL	L7



Figure(2): Affected *Leishmania donovanii* after its exposing to different radioactive rays with her lost the flagellum

A previous study by [25] effect of Beta and Gamma irradiation by 137Cs isotopes , in dose 1.776*10-4 sV of Beta ray (energy 0.514 MeV) and exposure to Gamma ray(energy 0.662 MeV) in dose 96.950 sV in 2hr. , cesium isotopes (137 Cs) and exposure to 90Sr that give one type of decay is Beta rays (energy 0.198 MeV) in dose 63.100 sV, the viability of *L. donovani* promastigote were than control.

Statistical analysis :

Results of Statistical Analysis System- SAS program all results are significant.

Conclusions

1-Cesium, Coblit, Strontium with Almonium shield or without Almonium shield that vestige on *Leishmania donovanii*.

2- Vestige radiosources radiation on *Leishmania donovanii* positively by lost flagellum and impact on movement and infection of this parasites.

Refrences

1- **Johan**,V.G.; Ermias,D.(2012). "Visceral Leishmaniasis". *Infectious Disease Clinics of North America.* **26**(2): 309–322.

2- **Evans**,T.G.(1993)."Leishmaniasis". Infectious Disease Clinics of North America. **7** (3): 527–46. *PMID* 8254158.

3- **Herwaldt**,BL(1999). "Leishmaniasis". *Lancet*. 354 (9185):1191–9. PMID 10513726.

4- **Desjeux**, P.(2004)."Leishmaniasis: current situation and new perspectives". Comparative Immunology, Microbiology and Infectious Diseases. 27 (5): 305–18. *PMID* 15225981.

5-**Chatterjee**, K.D. (2009). Parasitology: Protozoology and Helminthology (13th ed.). New Delhi: CBC Publishers. pp. 67–72. ISBN 9788123918105.

6-**Okwor**, I.; Uzonna, J.E. (2013). "The immunology of Leishmania/HIV co-infection". *Immunologic Research*. 56 (1): 163–71. doi:10.1007/s12026-013-8389-8. PMID 23504228.
7-**Olivier**, M; Badaró, R; Medrano, FJ; Moreno, J (2003). "The pathogenesis of Leishmania/HIV co-infection: cellular and immunological mechanisms". **Annals of Tropical Medicine and Parasitology**. 97 (Suppl 1): 79–98. PMID 14678636.
8- Davies, C.R.; Kaye, P.; Croft, S.L. and Sundhar, S.(2003). Leishmaniasis: new approaches to disease control. *BMJ* ;326:377–82 10.1136/bmj.326.7385.377 .
9- **Ashford**, R.W.(2000). The leishmaniases as emerging and reemerging zoonoses. *Int J Parasitol* .30:1269–81 10.1016/S0020-7519(00)00136-3.
10- **Desjeux,** P.(2001). The increase in risk factors for leishmaniasis worldwide. *Trans R Soc Trop Med Hyg* .;95:239–43 10.1016/S0035-9203(01)90223-8.
11- **Karunaweera**, N.D.; Pratlong, F.;Siriwardana, H.V.Y.D.; Ihalamulla, R.L.; Dedet, J.P. and Sri,L.(2003). Lankan cutaneous leishmaniasis is caused by *Leishmania donovani* MON 37. *Trans R Soc Trop Med Hyg*.97:380–1 10.1016/S0035-9203(03)90061-7.
12- **Mebrahtu**, Y.B.; Van Eys, G.; Guizani, I.; Lawyer, P.G.; Pamba, H.; Koech, D.(1993).Human cutaneous leishmaniasis caused by *Leishmania donovani s.l.* in Kenya. *Trans R Soc Trop Med Hyg*. 87:598–601 10.1016/0035-9203(93)90101-U.
13- **Pratlong**, F.; Bastein, P.; Perello, R.; Lami, P.and Dedet, J.P.(1995). Human cutaneous leishmaniasis caused by *Leishmania donovani* sensu stricto in Yemen. *Trans R Soc Trop Med Hyg*.89:398–9 10.1016/0035-9203(95)90025-X.
14- **Ben-Ami**, R; Schnur, L.F.; Golan, Y.; Jaffe, C.L.; Mardi, T. and Zeltser, D.(2002). Cutaneous involvement in a rare case of adult visceral leishmaniasis acquired in Israel. *J Infect*. ;44:181–4 10.1053/jinf.2002.0953.
15- Sharma, N.L.; Mahajan, V.K.; Kanga, A.; Sood, A.; Katoch, V.M. and Mauricio, I.(2005). Localized cutaneous leishmaniasis due to *Leishmania donovani* and *Leishmania tropica*: preliminary findings of the study of 161 new cases from a new endemic focus in Himachal Pradesh, *India. Am J Trop Med Hyg.*72:819–24.
16- **Engwerda**, CR; Ato, M; Kaye, PM (2004). "Macrophages, pathology and parasite persistence in experimental visceral leishmaniasis". Trends in Parasitology. **20** (11): 524–30. doi:10.1016/j.pt.2004.08.009. PMID 15471704.
17-**Lodge**, R.; Descoteaux,A.(2008). Leishmania invasion and phagosome biogenesis. Subcellular Biochemistry. **47**. pp. 174–81. doi:10.1007/978-0-387-78267-6_14. ISBN 978-0-387-78266-9. PMID 18512351.
18-**Downing,** T.; Imamura, H.; Decuypere, S.; Clark, T.G.; Coombs, G.H.; Cotton, J.A.; Hilley, J.D.; de Doncker, S.; Maes, I.; Mottram, J.C.; Quail, M.A.; Rijal, S.; Sanders, M.; Schönian, G.; Stark, O.; Sundar, S.; Vanaerschot, M.; Hertz-Fowler, C.; Dujardin,J.C. and Berriman,M.(2011). "Whole genome sequencing of multipleLeishmania donovani clinical isolates provides insights into population structure and mechanisms of drug resistance". Genome Research. **21** (12): 2143–56.
19- **Collins** , M.D. and Lawson, P.A. (2000). The genus A biotrophia (Kawamura *et al*.) is not monophiletic: proposal of Granulicatella gen. nov., Granulicatella adiacens comb.nov.,

Granulicatella elegans comb. nov.and Granulicatella balaenopterae comb. nov. *Int. J. Syst. Evol. Microbiol*. ;50:365-369.
20-Porta,M. (2014) "A dictionary of epidemiology", 6th edn, New York: Oxford University Press. ISBN 9780199976737.
21- **Mosmann**, T.(1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Meth*. 65: 55–63.
22- **Verma** ,N.K. ; Singh, G. and Dey, C.S.2007. Miltefosine induces apoptosis in arsenite-resistant Leishmania donovani promastigotes through mitochondrial dysfunction. Exp Parasitol. 116(1):1-13. • Stinson, S. ;Somber, J.R. ; and Blum,J.(1989).Morphology of Leishmania braziliensis changes .*J. Parasitol*.75:400-431.
23- **Riss**, T.L.; Moravec, B.S.R.A.; Niles, M.S. A.L.; Benink, H.A. and Worzella, T.J. (2013). Cell Viability Assays. In; Sittampalam GS, Coussens NP, Nelson H, et al., editors. Assay Guidance Manual. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences .
24-**SAS,(**2020). Statistical Analysis System- SAS.
25- **Abd**, R.S.; Mohammed,N.R.; Zghair,K.H.; Sabaa,h.s. and Mousa,I.M.(2016). The Effect of Semiconductor , He-Ne laser and Beta , Gamma irradiation on Leishmania donovani promastigote. *Advances in Life Science and Technolog*. ISSN 2224-7181 (Paper) ISSN 2225-062X. Vol.41.