پایان‌نامه جهت اخذ درجه دکتری تخصصی انگل‌شناسی پزشکی

بررسی میزان بیان ژن سایتوکاین‌های غالب در Zoonotic Cutaneous Leishmaniasis مقاوم به گلوکانتیم در محیط آزمایشگاهی و موش Balb/c

طرح تحقیقاتی شماره:۳۹۱۰۴۱

نگارش:

صدیقه صابری

استادان راهنما:

دکتر سیدحسین حجازی، استادانگل‌شناسی

دکتر علی خامسی‌پور، استاد ایمنی‌شناسی

دکترمنصورصالحی، دانشیار ژنتیک

استادان مشاور:

دکترژاله ورشوساز، استاد فارماسیوتیکس

دکتر سیدمحسن حسینی، دانشیار آمارزیستی

دکتر عباس‌علی پالیزبان، دانشیار بیوشیمی

مرداد ماه ۱۳۹۳

چکیده پایان نامه:

ضرورت انجام مطالعه

لیشمانیوز یکی از معضلات بهداشتی مناطق گرمسیری و نیمه‌گرمسیری است که به صورت ضایعات پوستی(سالک)، مخاطی- پوستی و احشایی (کالاآزار) بروز می‌کند. استان اصفهان یکی از مهم‌ترین کانون‌های لیشمانیوز جلدی نوع روستایی در ایران می‌باشد. با توجه به زئونوز بودن این نوع از لیشمانیوز جلدی و عدم وجود واکسنی موثر، در حال حاضر درمان بیماری بسیار مهم می‌باشد. ترکیبات ۵ ظرفیتی آنتی‌موآن هنوز خط مقدم درمان می‌باشد. این ترکیبات باعث فعال شدن سیستم ایمنی در جهت تولید سایتوکاین می‌شوند

هدف مطالعه

در این تحقیق میزان بیان ژن سایتوکاین‌های IL-12, IL-18, IL-27 وTNF-α و میزان IFN-γ و NO که از عوامل بهبود و غلبه بر بیماری لیشمانیوز می‌باشند، با استفاده از داروی طراحی شده‌ی گلوکانتیم بارگذاری‌شده با نانوذرات کیتوزان در پایه ژل به صورت *In vitro* و *In vivo* مورد بررسی قرار گرفته است.

مواد و روش ها:

در این تحقیق ده بیمارآلوده به *L.major* تایید شده با روش Nested PCR که با حداقل یک دوره درمان بهبود نیافته بودند مورد مطالعه قرار گرفتند. در *In vitro* با استفاده از سلول‌های J774 که به ایزوله‌های جدا شده از بیماران همراه با سوش استاندارد (MRHO/IR/75/ER) آلوده شده بودند بیان ژن و یا مقدار سایتوکاین‌های مورد نظر با روش Triplicate Quantitative RT-Real Time PCR تعیین و در گروه‌های درمانی با هم مقایسه گردید. در *In vivo* با استفاده از موش Balb/c ایزوله‌های جدا شده از بیماران و سوش استاندارد در قاعده دم موش‌ها به صورت داخل جلدی تلقیح گردید. ضایعات ایجاد شده تحت درمان روزانه با دو داروی گلوکانتیم معمولی (Sanofi-Aventis) و یا گلوکانتیم بار شده با نانوذره در پایه ژل قرار گرفتند. سپس میزان بیان ژن و یا مقدار سایتوکاین‌های مورد نظر با روشReal Time PCR بررسی گردید.

نتایج:

نتایج حاصل از این مطالعه نشان داد که استفاده از گلوکانتیم بار شده با نانوذره نسبت به گلوکانتیم معمولی و گروه شاهد باعث افزایش معنادار (P-value<۰/۰۰۱) بیان ژن اینترلوکین‌های 12، 18 و 27 در *In vitro* و *In vivo* در گروه‌های تحت درمان با ایزوله‌های بیماران و گونه استاندارد شده است. مقایسه این دو مرحله با آزمون کوواریانس افزایش معنادار را در مرحله‌ی *In vivo* نشان داد. همچنین استفاده از نانو دارو باعث افزایش معنادار بیان ژن فاکتور تومور نکروزان فاکتور (TNF-

α) نسبت به مرحله بدون درمان شد (P-value = ۰/۰۱) در صورتی که افزایش معناداری برای گلوکانتیم معمولی به دست نیامد. همچنین میزان نیتریک‌اکساید (NO) و اینترفرون گاما (IFN-γ) در گروه‌های تحت درمان با نانودارو نسبت به گروه-های بدون درمان و گلوکانتیم معمولی افزایش معنادار نشان داد(P-value < ۰/۰۱). از نظر تحریک و افزایش سایتوکاین‌های مورد بررسی ایزوله‌های بیماران و گونه استاندارد یکسان عمل کردند.

بحث و نتیجه گیری:

در این مطالعه داروی گلوکانتیم بار شده با نانو ذره در مقایسه با گلوکانتیم معمولی باعث افزایش بیان ژن و یا مقدار سایتوکاین‌های موثر در پاسخ Th_1 در شرایط *In vivo* و هم *In vitro* شده است که می‌تواند به دلیل عواملی مانند بالا بودن پایداری زیستی و بیولوژیکی نانوذرات پلیمری نسبت به گلوکانتیم معمولی که مولکول‌های آزاد دارد و احتمالا جذب بهتر و فاگوسیته شدن بیشتر توسط ماکروفاژها باشد. با توجه به این که پاسخ ایمنی هم به سوش‌های جدا شده از بیماران دارای مقاومت کلینیکی به گلوکانتیم و هم سوش استاندارد یکسان بوده است می‌توان هنوز به اثر بخشی و کاربردی بودن داروهای ۵ ظرفیتی انتی‌موآن در درمان سالک با عامل *L.major* امیدوار بود و با توجه به این‌که هنوز خط مقدم درمان بیماری در لیشمانیوز جلدی است و در مقایسه با بسیاری از داروهای گران و یا با سمیت بالا همچنان گزینه مناسبی جهت تحقیق و کاربردی کردن آن به شکلی از دارو که علاوه بر اینکه استفاده آن برای بیمار قابل قبول و آسان باشد بتواند با هزینه کمتر و اثر بخشی بیشتری به عنوان یک درمان موثر در درمان سالک مطرح گردد.

کلید واژه‌ها: لیشمانیوز جلدی، بیان ژن، گلوکانتیم، IL-12، IL-27، IL-18، TNF-α، FN-γ، NO

فهرست مطالب

عنوان **صفحه**

فصل اول: مقدمه و کلیات

فصل دوم: اهداف و فرضیات

فصل سوم : مواد و روش‌ها

فصل چهارم : نتایج

فصل پنجم : بحث و نتیجه گیری

فهرست جداول

عنوان **صفحه**

فهرست نمودار

عنوان **صفحه**

فهرست شکل ها

عنوان **صفحه**

فهرست مراجع:

1- Brent A, Davidson R, Seale A. Oxford handbook of tropical medicine: Oxford University Press; 2014.

2- BL H. Leishmaniasis. Lancet. 1999;354(9185):1191-9.

3- Moreira D, López-García P, Vickerman K. An updated view of kinetoplastid phylogeny using environmental sequences and a closer outgroup: proposal for a new classification of the class Kinetoplastea. International journal of systematic and evolutionary microbiology. 2004;54(5):1861-75.

4- Jose A. Ruiz Postigo. Leishmaniasis in the World Health Organization Eastern Mediterranean Region. International Journal of Antimicrobial Agents.2010; 36s:62s-5s

5- Assembly WH. The World Health Assembly Resolution(WHA60.13) on the ‘‘Control of Leishmaniasis’’. Geneva, Switzerland. 2007.

6- Organization WH. Control of the leishmaniases: report of a meeting of the WHO Expert Commitee on the Control of Leishmaniases, Geneva, 22-26 March 2010.

7- Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PloS one. 2012;7(5):e35671.

8- Hepburn N. Cutaneous leishmaniasis: an overview. Journal of postgraduate medicine. 2003;49(1):50.

9- Hotez PJ, Remme JH, Buss P, George G, Morel C, Breman JG. Combating tropical infectious diseases: report of the Disease Control Priorities in Developing Countries Project. Clinical infectious diseases. 2004;38(6):871-8.

10- Desjeux P. Leishmaniasis: current situation and new perspectives. Comparative immunology, microbiology and infectious diseases. 2004;27(5):305-18.

۱۱- اطهری ع، جلال لو ن. بررسی اپیدمیولوژیک پنج ساله لیشمانیازیس جلدی در ایران . ۱۳۸۴–۱۳۸۰. مجله دانشکده پزشکی اصفهان. ۱۳۸۵;۸۲(۲۴):۱۳-۸.

۱۲- ندیم ا، جوادیان ع، محبعلی م، مومنی ع. انگل لیشمانیا و لیشمانیوزها. چاپ سوم. مرکز نشر دانشگاهی. تهران. ۱۳۸۹

13- Neligan A. Canin leishmaniasis. Journal of tropical Medicine and Hygiene. 1913;156(16).

۱٤- لیشمانیوز جلدی (سالک). وزارت بهداشت ، درمان.و آموزش پزشکی. اداره بیماری‌های قابل انتقال بین انسان و حیوان. انتشارات راز نهان. ۱۳۹۱.

۱۵- صائبی ا. بیماری‌های انگلی در ایران. چاپ چهارم. انتشارات آییژ. تهران ۱۳۸۴.

16- Dowlati Y. Cutaneous leishmaniasis: clinical aspect. Clinics in dermatology. 1996;14(5):425-31.

17- Pulvertaft R, Hoyle G. Stages in the life-cycle of Leishmania donovani. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1960;54(2):191-6.

18- Davidson R N. Leishmaniasis. Protozoal Tropical Infections journal. 2005 ;33(8):43-6.

19- Bryceson AD, Murphy A, Moody AH. Treatment of Old World cutaneous leishmaniasis with aminosidine ointment: results of an on study in London. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1994;88(2):226-8.

20- Palumbo E. Current treatment for cutaneous leishmaniasis: a review. American journal of therapeutics. 2009;16(2):178-82.

21- Golenser J, Frankenburg S, Ehrenfreund T, Domb AJ. Efficacious treatment of experimental leishmaniasis with amphotericin B-arabinogalactan water-soluble derivatives. Antimicrobial agents and chemotherapy. 1999; 43(9):2209-14.

22- Escobar P, Yardley V, Croft SL. Activities of Hexadecylphospho

choline (Miltefosine), AmBisome, and Sodium Stibogluconate (Pentostam) againstLeishmania donovani in Immunodeficient scidMice. Antimicrobial agents and chemotherapy. 2001;45(6):1872-5.

23- Dorlo TP, Balasegaram M, Beijnen JH, de Vries PJ. Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. Journal of antimicrobial chemotherapy. 2012;67(11):2576-97.

24- Arana B, Rizzo N, Diaz A. Chemotherapy of cutaneous leishmaniasis: a review. Medical microbiology and immunology. 2001;190(1-2):93-5.

25- Arana BA, Mendoza CE, Rizzo NR, Kroeger A. Randomized, controlled, double-blind trial of topical treatment of cutaneous leishmaniasis with paromomycin plus methylbenzethonium chloride ointment in Guatemala. The American journal of tropical medicine and hygiene. 2001;65(5):466-70.

26- Fernández MM, Malchiodi EL, Algranati ID. Differential effects of paromomycin on ribosomes of Leishmania mexicana and mammalian cells. Antimicrobial agents and chemotherapy. 2011;55(1):86-93.

27- Ben Salah A, Ben Messaoud N, Guedri E, Zaatour A, Ben Alaya N, Bettaieb J, et al. Topical paromomycin with or without gentamicin for cutaneous leishmaniasis. New England Journal of Medicine. 2013;368(6): 524-32.

28- Mosleh IM, Geith E, Natsheh L, Schönian G, Abotteen N, Kharabsheh Sa. Efficacy of a weekly cryotherapy regimen to treat Leishmania major cutaneous leishmaniasis. Journal of the American Academy of Dermatology. 2008;58(4):617-24.

29- Asilian A, Sadeghinia A, Faghihi G, Momeni A. Comparative study of the efficacy of combined cryotherapy and intralesional meglumine antimoniate (Glucantime®) vs. cryotherapy and intralesional meglumine antimoniate (Glucantime®) alone for the treatment of cutaneous leishmaniasis. International journal of dermatology. 2004;43(4):281-3.

30- Asilian A, Sadeghinia A, Faghihi G, Momeni A, Amini Harandi A. The efficacy of treatment with intralesional meglumine antimoniate alone, compared with that of cryotherapy combined with the meglumine antimoniate or intralesional sodium stibogluconate, in the treatment of cutaneous

leishmaniasis. Annals of tropical medicine and parasitology. 2003;97(5):493-8.

۳۱- خامسی پور ع، گورچی م ح، خاتمی ا، اسکندری س ا، جوادی ا، زرتاب ح. مقایسه میزان اثربخشی تزریق داخل ضایعه ای هفته ای یک بار مگلومین آنتی مونات (گلوکانتیم) در مقایسه با تزریق هفته ای دوبار در درمان سالک آنتروپونوتیک در مشهد: کارآزمایی بالینی تصادفی. مجله پوست و زیبایی. ۲۰۱۱;۲(۱):۱-۸.

32- Sharifi I, Nakhaei N, Aflatoonian MR, Parizi MH, Fekri AR, Safizadeh H, et al. Cutaneous leishmaniasis in Bam: A comparative evaluation of pre- and post-earthquake years (1999â€“2008). Tehran University of Medical Sciences. p. 49.

33- Lobo IMF, Soares MBP, Correia TM, de Freitas LAR, Oliveira MI, Nakatani M, et al. Heat therapy for cutaneous leishmaniasis elicits a systemic cytokine response similar to that of antimonial (Glucantime) therapy. Oxford University Press; 2006. p. 642-9.

34- Bumb R, Prasad N, Khandelwal K, Aara N, Mehta R, Ghiya B, et al. Long-term efficacy of single-dose radiofrequency-induced heat therapy vs. intralesional antimonials for cutaneous leishmaniasis in India. British Journal of Dermatology. 2013;168(5):1114-9.

35- Valencia BM, Miller D, Witzig RS, Boggild AK, Llanos-Cuentas A. Novel Low-Cost Thermotherapy for Cutaneous Leishmaniasis in Peru. PLoS neglected tropical diseases. 2013;7(5):e2196.

36- Asilian A, Iraji F, Hedaiti HR, Siadat AH, Enshaieh S. Carbon dioxide laser for the treatment of lupoid cutaneous leishmaniasis (LCL): a case series of 24 patients. Dermatology online journal. 2006;12(2).

37- Shamsi Meymandi S, Zandi S, Aghaie H, Heshmatkhah A. Efficacy of CO2 laser for treatment of anthroponotic cutaneous leishmaniasis, compared with combination of cryotherapy and intralesional meglumine antimoniate. Journal of the European Academy of Dermatology and Venereology. 2011;25(5):587-91.

38- Nilforoushzadeh M, Shirani-Bidabadi L, Zolfaghari-Baghbaderani A, Saberi S, Siadat A, Mahmoudi M. Comparison of Thymus vulgaris (Thyme), Achillea millefolium (Yarrow) and propolis hydroalcoholic extracts versus

systemic glucantime in the treatment of cutaneous leishmaniasis in balb/c mice. J Vector Borne Dis. 2008;45(4):301-6.

39- Hejazi SH, Shirani-Bidabadi L, Zolfaghari-Baghbaderani A, Saberi S, Nilforoushzadeh M, Moradi S, et al. Comparison effectiveness of extracts of Thyme, Yarrow, Henna and Garlic on cutaneous leishmaniasis caused by L. major in animal model (Balb/c). Journal of Medicinal Plants. 2009;8(30):129-36, 60.

40- Weniger B, Robledo S, Arango GJ, Deharo E, Aragón R, Muñoz V, et al. Antiprotozoal activities of Colombian plants. Journal of ethnopharmacology. 2001;78(2):193-200.

41- Davidson RN, Yardley V, Croft SL, Konecny P, Benjamin N. A topical nitric oxide-generating therapy for cutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2000;94(3):319-22.

42- Nilforoushzadeh MA, Shirani-Bidabadi L, Zolfaghari-Baghbaderani A, Saberi S, Siadat AH, Mahmoudi M. Comparsion Of Proppolis Hydroalcoholic Extract Versus Glucantime In The Ttretment Of Cutaneous Leishmaniasis In Balb/c Mice. Journal of Cell and Tissue Research.2008;8(2)1421-24.

43- Saberi S, Mahmoudi M, Mosavi Z, Arjmand R, Hejazi SH. Study On Inhibitory Activity Of Propolis Alcoholic Extract On Oppotunist Fungi Of Candida In Vitro. Jundishapur Journal of Microbiology. 2013;Especial edition.p.19

44- Shirani-Bidabadi L, Mahmoudi M, Saberi S, Zolfaghari-Baghbaderani A, Nilforoushzadeh M, Abdoli H, et al. The effect of Gel Foam on post laminectomy epidural fibrosis in rabbits. Tehran University Medical Journal. 2009;66(11).

45- Langer R. Biomaterials in drug delivery and tissue engineering: one laboratory's experience. ACS Publications; 2000. p. 94-101.

46- Mohanraj VJ, Chen Y. Nanoparticles-a review. Faculty of Pharmacy, University of Benin; 2007. p. 561-73.

47- Date AA, Joshi MD, Patravale VB. Parasitic diseases: liposomes and polymeric nanoparticles versus lipid nanoparticles. Advanced drug delivery reviews. 2007;59(6):505-21.

48- Chapman Jr WL, Hanson WL, Alving CR, Hendricks LD. Antileishmanial activity of liposome-encapsulated meglumine antimonate in the dog. 1984. p. 1028-30.

49- Davidson RN, Scott A, Maini M, Bryceson ADM, Croft SL. Liposomal amphotericin B in drug-resistant visceral leishmaniasis. Elsevier; 1991. p. 1061-2.

50- Gradoni L, Davidson R, Orsini S, Betto P, Giambenedetti M. Activity of liposomal amphotericin B (AmBisome) against Leishmania infantum and tissue distribution in mice. Informa UK Ltd UK; 1993. p. 311-6.

51- Amato VS, Rabello A, Rotondo-Silva A, Kono A, Maldonado TnPcH, Alves IC, et al. Successful treatment of cutaneous leishmaniasis with lipid formulations of amphotericin B in two immunocompromised patients. Elsevier; 2004. p. 127-32.

52- Yardley V, Croft SL. A comparison of the activities of three amphotericin B lipid formulations against experimental visceral and cutaneous leishmaniasis. Elsevier; 2000. p. 243-8.

53- Ng AW, Wasan KM, Lopez-Berestein G. Development of liposomal polyene antibiotics: an historical perspective. 2003. p. 67-83.

54- Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and mode of action: a state of the art review. Elsevier. p. 51-63.

55- Nagpal K, Singh SK, Mishra DN. Chitosan nanoparticles: a promising system in novel drug delivery. Chem Pharm Bull (Tokyo). 2010; 58(11): 1423-30.

56- Barzegar-Jalali M. Nanovaccine for leishmaniasis: preparation of chitosan nanoparticles containing Leishmania superoxide dismutase and evaluation of its immunogenicity in BALB/c mice. p. 835-42.

57- Katas H, Hussain Z, Ling TC. Chitosan nanoparticles as a percutaneous drug delivery system for hydrocortisone. Hindawi Publishing Corp. p. 45.

58- Mansueto P, Vitale G, Seidita A, Guarneri FP, Pepe I, Rinollo C, et al. Advances In Leishmaniasis Immunopathogenesis. Acta Medica. 2011;27:7.

59- Nilforoushzadeh MA, Shirani-Bidabadi L, Saberi S, Hosseini SM, Jaffary F. Effect of integrated pest management on controlling zoonotic cutaneous leishmaniasis in Emamzadeh Agha Ali Abbas (AS) District, Isfahan province, 2006-2009. Advanced biomedical research. 2014;3.

60- Saberi S, Zamani A, Motamedi N, Nilforoushzadeh MA, Jaffary F, Rahimi E, et al. The knowledge, attitude, and prevention practices of students regarding cutaneous leishmaniasis in the hyperendemic region of the Shahid Babaie Airbase. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA. p. 306-9.

61- Motamedi N, Saberi S, Zamani AR, Hejazi SH, Nilforoshzadeh MA. Effect of education on promoting preventive behavior of coetaneous leishmaniasis in Shahid Babaie airbase. The First International & 4th National Congress on health Education & Promotion, 2011: Tabtiz university of medical sciences.

62- Titus RG, Sherry B, Cerami A. Tumor necrosis factor plays a protective role in experimental murine cutaneous leishmaniasis. The Journal of experimental medicine. 1989;170(6):2097-104.

63- Ghersetich I, Menchini G, Teofoli P, Lotti T. Immune response to Leishmania infection in human skin. Clinics in dermatology. 1999;17(3):333-8.

64- Bogdan C, Röllinghoff M. How do protozoan parasites survive inside macrophages? Parasitology Today. 1999;15(1):22-8.

65- Solbach W, Laskay T. The host response to Leishmania infection. Advances in immunology. 2000;74:275-318.

66- Mosser DM, Edelson PJ. The third component of complement (C3) is responsible for the intracellular survival of Leishmania major. 1987.

67- Wright SD, Silverstein S. Receptors for C3b and C3bi promote phagocytosis but not the release of toxic oxygen from human phagocytes. The Journal of experimental medicine. 1983;158(6):2016-23.

68- Russell DG, Wright S. Complement receptor type 3 (CR3) binds to an Arg-Gly-Asp-containing region of the major surface glycoprotein, gp63, of

Leishmania promastigotes. The Journal of experimental medicine. 1988;168(1):279-92.

69- Guy RA, Belosevic M. Comparison of receptors required for entry of Leishmania major amastigotes into macrophages. Infection and immunity. 1993;61(4):1553-8.

70- Chang K. Cellular and molecular mechanisms of intracellular symbiosis in leishmaniasis. International Review of Cytology. 1983.

71- Beverley SM, Turco SJ. Lipophosphoglycan (LPG) and the identification of virulence genes in the protozoan parasite Leishmania. Trends in microbiology. 1998;6(1):35-40.

72- Brittingham A, Morrison CJ, McMaster WR, McGwire BS, Chang K-P, Mosser DM. Role of the Leishmania surface protease gp63 in complement fixation, cell adhesion, and resistance to complement-mediated lysis. The Journal of Immunology. 1995;155(6):3102-11.

73- Laufs H, Müller K, Fleischer J, Reiling N, Jahnke N, Jensenius JC, et al. Intracellular survival of Leishmania major in neutrophil granulocytes after uptake in the absence of heat-labile serum factors. Infection and immunity. 2002;70(2):826-35.

74- Ilgoutz SC, McConville MJ. Function and assembly of the Leishmania surface coat. International journal for parasitology. 2001;31(9):899-908.

75- Ilg T. Proteophosphoglycans of Leishmania . Parasitology Today. 2000;16(11):489-97.

76- Ilg T, Harbecke D, Overath P. The lysosomal gp63-related protein in Leishmania mexicana amastigotes is a soluble metalloproteinase with an acidic pH optimum. FEBS letters. 1993;327(1):103-7.

77- Medina-Acosta E, Karess RE, Schwartz H, Russell DG. The promastigote surface protease (gp63) of Leishmania is expressed but differentially processed and localized in the amastigote stage. Molecular and biochemical parasitology. 1989;37(2):263-73.

78- Seay MB, Heard PL, Chaudhuri G. Surface Zn-proteinase as a molecule for defense of Leishmania mexicana amazonensis promastigotes against

cytolysis inside macrophage phagolysosomes. Infection and immunity. 1996;64(12):5129-37.

79- Chang K, Chaudhuri G, Fong D. Molecular determinants of Leishmania virulence. Annual Reviews in Microbiology. 1990;44(1):499-529.
80. Turco SJ, Descoteaux A. The lipophosphoglycan of Leishmania parasites. Annual Reviews in Microbiology. 1992;46(1):65-92.

81- Mengeling BJ, Beverley SM, Turco SJ. Designing glycoconjugate biosynthesis for an insidious intent: phosphoglycan assembly in Leishmania parasites. Glycobiology. 1997;7(7):873-80.

82- Glaser TA, Baatz JE, Kreishman GP, Mukkada AJ. pH homeostasis in Leishmania donovani amastigotes and promastigotes. Proceedings of the National Academy of Sciences. 1988;85(20):7602-6.

83- Pupkis MF, Tetley L, Coombs GH. Leishmania mexicana: Amastigote hydrolases in unusual lysosomes. Experimental parasitology. 1986;62(1):29-39.

84- Anthony RL, Sacci Jr JB, Williams KM. Immunodominant Epitopes of the Leishmania Surface Membrane. Leishmaniasis: Springer; 1989. p. 281-93.

85- Peters C, Aebischer T, Stierhof Y-D, Fuchs M, Overath P. The role of macrophage receptors in adhesion and uptake of Leishmania mexicana amastigotes. Journal of cell science. 1995;108(12):3715-24.

86- Wilson ME, Pearson R. Roles of CR3 and mannose receptors in the attachment and ingestion of Leishmania donovani by human mononuclear phagocytes. Infection and immunity. 1988;56(2):363-9.

87- Giorgione JR, Turco SJ, Epand RM. Transbilayer inhibition of protein kinase C by the lipophosphoglycan from Leishmania donovani. Proceedings of the National Academy of Sciences. 1996;93(21):11634-9.

88- Proudfoot L, O'Donnell CA, Liew FY. Glycoinositolphospholipids of Leishmania major inhibit nitric oxide synthesis and reduce leishmanicidal activity in murine macrophages. European journal of immunology. 1995;25(3):745-50.

89- Alexander J, Russell DG. The interaction of Leishmania species with macrophages. Advances in parasitology. 1992;31:175-254.

90- Mosser DM, Wedgwood J, Edelson P. Leishmania amastigotes: resistance to complement-mediated lysis is not due to a failure to fix C3. The Journal of Immunology. 1985;134(6):4128-31.

91- Schmidt GD, Roberts LS. Foundations ofparasitology: CV Mosby, St Louis; 1981.

92- Love DC, Esko JD, Mosser DM. A heparin-binding activity on Leishmania amastigotes which mediates adhesion to cellular proteoglycans. The Journal of cell biology. 1993;123(3):759-66.

93- Straus A, Levery S, Jasiulionis M, Salyan M, Steele S, Travassos L, et al. Stage-specific glycosphingolipids from amastigote forms of Leishmania (L.) amazonensis. Immunogenicity and role in parasite binding and invasion of macrophages. Journal of Biological Chemistry. 1993;268(18):13723-30.

94- Delves PJ, Martin SJ, Burton DR, Roitt IM. Roitt's essential immunology: John Wiley & Sons; 2011.

95- Awasthi A, Mathur RK, Saha B. Immune response to Leishmania infection. Indian Journal of Medical Research. 2004;119:238-58.

96- Handman E, Ceredig R, Mitchell GF. Murine cutaneous leishmaniasis: disease patterns in intact and nude mice of various genotypes and examination of some differences between normal and infected macrophages. Immunology and Cell Biology. 1979;57(1):9-29.

97- Davies EV, Singleton A, Blackwell JM. Differences in Lsh gene control over systemic Leishmania major and Leishmania donovani or Leishmania mexicana mexicana infections are caused by differential targeting to infiltrating and resident liver macrophage populations. Infection and immunity. 1988;56(5):1128-34.

98- Vidal S, Tremblay ML, Govoni G, Gauthier S, Sebastiani G, Malo D, et al. The Ity/Lsh/Bcg locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the Nramp1 gene. The Journal of experimental medicine. 1995;182(3):655-66.

99- Milon G, Del Giudice G, Louis J. Immunobiology of experimental cutaneous leishmaniasis. Parasitology Today. 1995;11(7):244-7.

100- Blackwell J. Genetic susceptibility to leishmanial infections: studies in mice and man. Parasitology. 1996;112(S1):S67-S74.

101- Demant P, Lipoldova M, Svobodova M, Guler M, Murphy K, Gorham J. Resistance to Leishmania major in mice. Science. 1996;274:1392-3.

102- Gorczynski RM. Immunization of susceptible BALB/c mice against Leishmania braziliensis: I. Resistance induced using as immunogen adherent or nonadherent cells from infected mice. Cellular immunology. 1985;94(1):1-10.

103- Hsieh C-S, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science. 1993;260(5107):547-9.

104- Tanaka T, Hu-Li J, Seder RA, de St Groth BF, Paul WE. Interleukin 4 suppresses interleukin 2 and interferon gamma production by naive T cells stimulated by accessory cell-dependent receptor engagement. Proceedings of the National Academy of Sciences. 1993;90(13):5914-8.

105- Kopf M, Brombacher F, Köhler G, Kienzle G, Widmann K-H, Lefrang K, et al. IL-4-deficient Balb/c mice resist infection with Leishmania major. The Journal of experimental medicine. 1996;184(3):1127-36.

106- Himmelrich H, Launois P, Maillard I, Biedermann T, Tacchini-Cottier F, Locksley RM, et al. In BALB/c mice, IL-4 production during the initial phase of infection with Leishmania major is necessary and sufficient to instruct Th2 cell development resulting in progressive disease. The Journal of Immunology. 2000;164(9):4819-25.

107- Noben-Trauth N, Lira R, Nagase H, Paul WE, Sacks DL. The relative contribution of IL-4 receptor signaling and IL-10 to susceptibility to Leishmania major. The Journal of Immunology. 2003;170(10):5152-8.

108- Matthews DJ, Emson CL, McKenzie GJ, Jolin HE, Blackwell JM, McKenzie AN. IL-13 is a susceptibility factor for Leishmania major infection. The Journal of Immunology. 2000;164(3):1458-62.

109- Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to Leishmania major in mice. Nature Reviews Immunology. 2002;2(11):845-58.

110- Sunder-Plassmann R, Breiteneder H, Zimmermann K, Strunk D, Majdic O, Knapp W, et al. Single human T cells stimulated in the absence of feeder cells transcribe interleukin-2 and undergo long-term clonal growth in response to defined monoclonal antibodies and cytokine stimulation. Blood. 1996;87(12):5179-84.

111- Launois P, Maillard I, Pingel S, Swihart KG, Xénarios I, Acha-Orbea H, et al. IL-4 Rapidly Produced by Vβ4 Vα8 CD4 T Cells Instructs Th2 Development and Susceptibility to Leishmania major in BALB/c Mice. Immunity. 1997;6(5):541-9.

112- Soussi N, Saklani-Jusforgues H, Colle J-H, Milon G, Glaichenhaus N, Goossens PL. Effect of intragastric and intraperitoneal immunisation with attenuated and wild-type LACK-expressing Listeria monocytogenes on control of murine Leishmania major infection. Vaccine. 2002;20(21):2702-12.

113- Julia V, Rassoulzadegan M, Glaichenhaus N. Resistance to Leishmania major induced by tolerance to a single antigen. Science. 1996;274(5286):421-3.

114- Aseffa A, Gumy A, Launois P, MacDonald HR, Louis JA, Tacchini-Cottier F. The early IL-4 response to Leishmania major and the resulting Th2 cell maturation steering progressive disease in BALB/c mice are subject to the control of regulatory CD4+ CD25+ T cells. The Journal of Immunology. 2002;169(6):3232-41.

115- Scott P. The role of TH1 and TH2 cells in experimental cutaneous leishmaniasis. Experimental parasitology. 1989;68(3):369-72.

116- Sharma U, Singh S. Immunobiology of leishmaniasis. Indian journal of experimental biology. 2009;47(6):412.

117- Bomfim G, Nascimento C, Costa J, Carvalho EM, Barral-Netto M, Barral A. Variation of cytokine patterns related to therapeutic response in diffuse cutaneous leishmaniasis. Experimental parasitology. 1996;84(2):188-94.

118- Meier CL, Svensson M, Kaye PM. Leishmania-induced inhibition of macrophage antigen presentation analyzed at the single-cell level. The Journal of Immunology. 2003;171(12):6706-13.

119- Kima PE, Soong L, Chicharro C, Ruddle NH, McMahon-Pratt D. Leishmania-infected macrophages sequester endogenously synthesized parasite antigens from presentation to CD4+ T cells. European journal of immunology. 1996;26(12):3163-9.

120- Erb K, Blank C, Ritter U, Bluethmann H, Moll H. Leishmania Major Infection in Major Histocompatibility Complex Class II-Deficient Mice: CD8T Cells Do not Mediate a Protective Immune Response. Immunobiology. 1996;195(2):243-60.

121- Gantt KR, Goldman TL, McCormick ML, Miller MA, Jeronimo SM, Nascimento ET, et al. Oxidative responses of human and murine macrophages during phagocytosis of Leishmania chagasi. The Journal of Immunology. 2001;167(2):893-901.

122- Murray HW, Nathan CF. Macrophage microbicidal mechanisms In vivo: reactive nitrogen versus oxygen intermediates in the killing of intracellular visceral Leishmania donovani. The Journal of experimental medicine. 1999;189(4):741-6.

123- Ding AH, Nathan CF, Stuehr DJ. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. The Journal of Immunology. 1988;141(7):2407-12.

124- Moll H, Binoder K, Bogdan C, Solbach W, Rollinghoff M. Production of tumour necrosis factor during murine cutaneous leishmaniasis. Parasite immunology. 1990;12(4-5):483-94.

125- De Kossodo S, Grau GE, Louis JA, Müller I. Tumor necrosis factor alpha (TNF-alpha) and TNF-beta and their receptors in experimental cutaneous leishmaniasis. Infection and immunity. 1994;62(4):1414-20.

126- Green SJ, Meltzer MS, Hibbs JB, Nacy CA. Activated macrophages destroy intracellular Leishmania major amastigotes by an L-arginine-

dependent killing mechanism. The Journal of Immunology. 1990;144(1):278-83.

127- Green SJ, Crawford RM, Hockmeyer JT, Meltzer MS, Nacy C. Leishmania major amastigotes initiate the L-arginine-dependent killing mechanism in IFN-gamma-stimulated macrophages by induction of tumor necrosis factor-alpha. The Journal of Immunology. 1990;145(12):4290-7.

128- Deng W, Thiel B, Tannenbaum C, Hamilton T, Stuehr D. Synergistic cooperation between T cell lymphokines for induction of the nitric oxide synthase gene in murine peritoneal macrophages. The Journal of Immunology. 1993;151(1):322-9.

129- Wei X-q, Leung BP, Niedbala W, Piedrafita D, Feng G-j, Sweet M, et al. Altered immune responses and susceptibility to Leishmania major and Staphylococcus aureus infection in IL-18-deficient mice. The Journal of Immunology. 1999;163(5):2821-8.

130- Li J, Hunter CA, Farrell JP. Anti-TGF-β treatment promotes rapid healing of Leishmania major infection in mice by enhancing In vivo nitric oxide production. The Journal of Immunology. 1999;162(2):974-9.

131- Liew F, Parkinson C, Millott S, Severn A, Carrier M. Tumour necrosis factor (TNF alpha) in leishmaniasis. I. TNF alpha mediates host protection against cutaneous leishmaniasis. Immunology. 1990;69(4):570.

132- Male D, Brostoff J, Roth D. Immunology Roitt, 2005. Translation by Keyhani AH, Valaei SH, Nazer M, Khodaie M, Fatholahi AR. 7th. Tehran: Arjmand Press; 2007.

133- Campbell KA, Ovendale PJ, Kennedy MK, Fanslow WC, Reed SG, Maliszewski CR. CD40 ligand is required for protective cell-mediated immunity to Leishmania major. Immunity. 1996;4(3):283-9.

134- Andrade RM, Wessendarp M, Subauste CS. CD154 activates macrophage antimicrobial activity in the absence of IFN-γ through a TNF-α-dependent mechanism. The Journal of Immunology. 2003;171(12):6750-6.

135- Liew F, Millott S, Parkinson C, Palmer R, Moncada S. Macrophage killing of Leishmania parasite In vivo is mediated by nitric oxide from L-arginine. The Journal of Immunology. 1990;144(12):4794-7.

136- Pinelli E, Rutten VP, Bruysters M, Moore PF, Ruitenberg EJ. Compensation for decreased expression of B7 molecules on Leishmania infantum-infected canine macrophages results in restoration of parasite-specific T-cell proliferation and gamma interferon production. Infection and immunity. 1999;67(1):237-43.

137- Lertmemongkolchai G, Cai G, Hunter CA, Bancroft GJ. Bystander activation of CD8+ T cells contributes to the rapid production of IFN-γ in response to bacterial pathogens. The Journal of Immunology. 2001;166(2):1097-105.

138- Kamath AT, Sheasby CE, Tough DF. Dendritic cells and NK cells stimulate bystander T cell activation in response to TLR agonists through secretion of IFN-αβ and IFN-γ. The Journal of Immunology. 2005;174(2):767-76.

139- Airoldi I, Gri G, Marshall JD, Corcione A, Facchetti P, Guglielmino R, et al. Expression and function of IL-12 and IL-18 receptors on human tonsillar B cells. The Journal of Immunology. 2000;165(12):6880-8.

140- Ohteki T, Fukao T, Suzue K, Maki C, Ito M, Nakamura M, et al. Interleukin 12–dependent Interferon γ Production by CD8α+ Lymphoid Dendritic Cells. The Journal of experimental medicine. 1999;189(12):1981-6.

141- Scharton-Kersten T, Scott P. The role of the innate immune response in Th1 cell development following Leishmania major infection. Journal of leukocyte biology. 1995;57(4):515-22.

142- Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nature Reviews Immunology. 2003;3(2):133-46.

143- Howard J, Hale C, Chan- Liew W. Immunological regulation of experimental cutaneous leishmaniasis. 1. Immunogenetic aspects of susceptibility to Leishmania tvopica in mice. Parasite immunology. 1980;2(4):303-14.

144- Wang Z-E, Reiner SL, Zheng S, Dalton DK, Locksley RM. CD4+ effector cells default to the Th2 pathway in interferon gamma-deficient mice infected with Leishmania major. The Journal of experimental medicine. 1994;179(4):1367-71.

145- Kobayashi M, Fitz L, Ryan M, Hewick RM, Clark SC, Chan S, et al. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. The Journal of experimental medicine. 1989;170(3):827-45.

146- Abdi K, Singh NJ, Spooner E, Kessler BM, Radaev S, Lantz L, et al. Free IL-12p40 Monomer Is a Polyfunctional Adaptor for Generating Novel IL-12–like Heterodimers Extracellularly. The Journal of Immunology. 2014;192(12):6028-36.

147- Taga T, Kishimoto T. Gp130 and the interleukin-6 family of cytokines. Annual review of immunology. 1997;15(1):797-819.

148- Merberg DM, Wolf SF, Clark SC. Sequence similarity between NKSF and the IL-6/G-CSF family. Immunology today. 1992;13(2):77-8.

149- Hunter CA. New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. Nature Reviews Immunology. 2005;5(7):521-31.

150- Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity. 2000;13(5):715-25.

151- Pflanz S, Timans JC, Cheung J, Rosales R, Kanzler H, Gilbert J, et al. IL-27, a Heterodimeric Cytokine Composed of EBI3 and p28 Protein, Induces Proliferation of Naive CD4 T Cells. Immunity. 2002;16(6):779-90.

152- Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. The Journal of experimental medicine. 2005; 201(2):233-40.

153- Mattner F, Magram J, Ferrante J, Launois P, Padova KD, Behin R, et al. Genetically resistant mice lacking interleukin-12 are susceptible to infection with Leishmania major and mount a polarized Th2 cell response. European journal of immunology. 1996;26(7):1553-9.

154- Shu U, Kiniwa M, Wu CY, Maliszewski C, Vezzio N, Hakimi J, et al. Activated T cells induce interleukin-12 production by monocytes via CD40-CD40 ligand interaction. European journal of immunology. 1995;25 (4):1125-8.

155- Ferlin WG, von der Weid T, Cottrez F, Ferrick DA, Coffman RL, Howard MC. The induction of a protective response in Leishmania major-infected BALB/c mice with anti-CD40 mAb. European journal of immunology. 1998;28(2):525-31.

156- Trinchieri G. Interleukin-12: a cytokine at the interface of inflammation and immunity. Advances in immunology. 1998;70:83-243.

157- Belkaid Y, Mendez S, Lira R, Kadambi N, Milon G, Sacks D. A natural model of Leishmania major infection reveals a prolonged “silent” phase of parasite amplification in the skin before the onset of lesion formation and immunity. The Journal of Immunology. 2000;165(2):969-77.

158- Chehimi J, Trinchieri G. Interleukin-12: a bridge between innate resistance and adaptive immunity with a role in infection and acquired immunodeficiency. Journal of clinical immunology. 1994;14(3):149-61.

159- Murphy EE, Terres G, Macatonia SE, Hsieh C-S, Mattson J, Lanier L, et al. B7 and interleukin 12 cooperate for proliferation and interferon gamma production by mouse T helper clones that are unresponsive to B7 costimulation. The Journal of experimental medicine. 1994;180(1):223-31.

160- Seder RA, Kelsall BL, Jankovic D. Differential roles for IL-12 in the maintenance of immune responses in infectious versus autoimmune disease. The Journal of Immunology. 1996;157(7):2745-8.

161- Park AY, Hondowicz BD, Scott P. IL-12 is required to maintain a Th1 response during Leishmania major infection. The Journal of Immunology. 2000;165(2):896-902.

162- Gazzinelli RT, Wysocka M, Hayashi S, Denkers EY, Hieny S, Caspar P, et al. Parasite-induced IL-12 stimulates early IFN-gamma synthesis and resistance during acute infection with Toxoplasma gondii. The Journal of Immunology. 1994;153(6):2533-43.

163- Ghayur T, Banerjee S, Hugunin M, Butler D, Herzog L, Carter A, et al. Caspase-1 processes IFN-γ-inducing factor and regulates LPS-induced IFN-γ production. 1997.

164- CHAIN S. Induction of IFN-γ production by NK cell stimulatory factor (NKSF): characterization of the responder cells and synergy with other inducers. J Exp Med. 1991;173:869.

165- Chan SH, Kobayashi M, Santoli D, Perussia B, Trinchieri G. Mechanisms of IFN-gamma induction by natural killer cell stimulatory factor (NKSF/IL-12). Role of transcription and mRNA stability in the synergistic interaction between NKSF and IL-2. The Journal of Immunology. 1992;148(1):92-8.

166- Walker W, Aste-Amezaga M, Kastelein RA, Trinchieri G, Hunter CA. IL-18 and CD28 use distinct molecular mechanisms to enhance NK cell production of IL-12-induced IFN-γ. The Journal of Immunology. 1999;162(10):5894-901.

167- Hodge DL, Martinez A, Julias JG, Taylor LS, Young HA. Regulation of nuclear gamma interferon gene expression by interleukin 12 (IL-12) and IL-2 represents a novel form of posttranscriptional control. Molecular and cellular biology. 2002;22(6):1742-53.

168- Macatonia SE, Hsieh C-S, Murphy KM, O'Garra A. Dendritic cells and macrophages are required for Th1 development of CD4+ T cells from αβ TCR transgenic mice: IL-12 substitution for macrophages to stimulate IFN-γ production is IFN-γ-dependent. International Immunology. 1993;5(9):1119-28.

169- Seder RA, Gazzinelli R, Sher A, Paul WE. Interleukin 12 acts directly on CD4+ T cells to enhance priming for interferon gamma production and diminishes interleukin 4 inhibition of such priming. Proceedings of the National Academy of Sciences. 1993;90(21):10188-92.

170- Carrera L, Gazzinelli RT, Badolato R, Hieny S, Muller W, Kuhn R, et al. Leishmania promastigotes selectively inhibit interleukin 12 induction in bone marrow-derived macrophages from susceptible and resistant mice. The Journal of experimental medicine. 1996;183(2):515-26.

171- Hondowicz BD, Scharton-Kersten TM, Jones DE, Scott P. Leishmania major-infected C3H mice treated with anti-IL-12 mAb develop but do not maintain a Th2 response. The Journal of Immunology. 1997;159(10):5024-31.

172- Murray HW. Endogenous interleukin-12 regulates acquired resistance in experimental visceral leishmaniasis. Journal of Infectious Diseases. 1997;175(6):1477-9.

173- Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000;100(6):655-69.

174- Okamura H, Tsutsui H, Kashiwamura S-i, Yoshimoto T, Nakanishi K. Interleukin-18: a novel cytokine that augments both innate and acquired immunity. Advances in immunology. 1998;70:281-312.

175- Chakraborty D, Banerjee S, Sen A, Banerjee KK, Das P, Roy S. Leishmania donovani affects antigen presentation of macrophage by disrupting lipid rafts. The Journal of Immunology. 2005;175(5):3214-24.

176- Kharazmi A, Kemp K, Ismail A, Gasim S, Gaafar A, Kurtzhals J, et al. T-cell response in human leishmaniasis. Immunology letters. 1999;65(1):105-8.

177- Nakamura K, Okamura H, Wada M, Nagata K, Tamura T. Endotoxin-induced serum factor that stimulates gamma interferon production. Infection and immunity. 1989;57(2):590-5.

178- Leung BP, Culshaw S, Gracie JA, Hunter D, Canetti CA, Campbell C, et al. A role for IL-18 in neutrophil activation. The Journal of Immunology. 2001;167(5):2879-86.

179- Bazan JF, Timans JC, Kastelein RA. A newly defined interleukin-1? Nature. 1996;379(6566):591.

180- Nakahira M, Ahn H-J, Park W-R, Gao P, Tomura M, Park C-S, et al. Synergy of IL-12 and IL-18 for IFN-γ gene expression: IL-12-induced STAT4 contributes to IFN-γ promoter activation by up-regulating the binding activity of IL-18-induced activator protein 1. The Journal of Immunology. 2002;168(3):1146-53.

181- Wesche H, Henzel WJ, Shillinglaw W, Li S, Cao Z. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. Immunity. 1997;7(6):837-47.

182- Ohkusu K, Yoshimoto T, Takeda K, Ogura T, Kashiwamura S-i, Iwakura Y, et al. Potentiality of interleukin-18 as a useful reagent for treatment and prevention of Leishmania major infection. Infection and immunity. 2000;68(5):2449-56.

183- Monteforte GM, Takeda K, Rodriguez-Sosa M, Akira S, David JR, Satoskar AR. Genetically resistant mice lacking IL-18 gene develop Th1 response and control cutaneous Leishmania major infection. The Journal of Immunology. 2000;164(11):5890-3.

184- Pollock KG, Conacher M, Wei XQ, Alexander J, Brewer JM. Interleukin-18 plays a role in both the alum-induced T helper 2 response and the T helper 1 response induced by alum-adsorbed interleukin-12. Immunology. 2003;108(2):137-43.

185- Xu D, Trajkovic V, Hunter D, Leung BP, Schulz K, Gracie JA, et al. IL-18 induces the differentiation of Th1 or Th2 cells depending upon cytokine milieu and genetic background. European journal of immunology. 2000;30(11):3147-56.

186- Netea MG, Kullberg BJ, Verschueren I, Van der Meer JW. Interleukin-18 induces production of proinflammatory cytokines in mice: no intermediate role for the cytokines of the tumor necrosis factor family and interleukin-1β. European journal of immunology. 2000;30(10):3057-60.

187- Sher A, Gazzinelli RT, Oswald IP, Clerici M, Kullberg M, Pearce EJ, et al. Role of T-Cell Derived Cytokines in the Downregulation of Immune Responses in Parasitic and Retroviral Infection. Immunological reviews. 1992;127(1):183-204.

188- Reiner SL, Locksley RM. The regulation of immunity to Leishmania major. Annual review of immunology. 1995;13(1):151-77.

189- Kastelein RA, Hunter CA, Cua DJ. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. Annu Rev Immunol. 2007;25:221-42.

190- Yoshida H, Hamano S, Senaldi G, Covey T, Faggioni R, Mu S, et al. WSX-1 Is Required for the Initiation of Th1 Responses and Resistance to< i> L. major</i> Infection. Immunity. 2001;15(4):569-78.

191- Artis D, Johnson LM, Joyce K, Saris C, Villarino A, Hunter CA, et al. Cutting edge: early IL-4 production governs the requirement for IL-27-WSX-1 signaling in the development of protective Th1 cytokine responses following Leishmania major infection. The Journal of Immunology. 2004;172(8):4672-5.

192- Tolouei S, Ghaedi K, Khamesipour A, Akbari M, Baghaei M, Hasheminia S, et al. IL-23 and IL-27 levels in macrophages collected from peripheral blood of patients with healing vs non-healing form of cutaneous leishmaniasis. Iranian journal of parasitology. 2012;7(1):18.

193- Tolouei S, Hejazi SH, Ghaedi K, Khamesipour A, Hasheminia SJ. TLR2 and TLR4 in Cutaneous Leishmaniasis Caused by Leishmania major. Scandinavian journal of immunology. 2013;78(5):478-84.

194- Berman J. Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clinical infectious diseases. 1997;24(4):684-703.

195- Guerin PJ, Olliaro P, Sundar S, Boelaert M, Croft SL, Desjeux P, et al. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. The Lancet infectious diseases. 2002;2(8):494-501.

196- Roberts WL, Rainey PM. Antileishmanial activity of sodium stibogluconate fractions. Antimicrobial agents and chemotherapy. 1993;37(9):1842-6.

197- Kocyigit A, Gur S, Gurel MS, Bulut V, Ulukanligil M. Antimonial therapy induces circulating proinflammatory cytokines in patients with cutaneous leishmaniasis. Infection and immunity. 2002;70(12):6589-91.

198- Belosevic M, Davis C, Meltzer M, Nacy C. Regulation of activated macrophage antimicrobial activities. Identification of lymphokines that cooperate with IFN-gamma for induction of resistance to infection. The Journal of Immunology. 1988;141(3):890-6.

199- Muniz-Junqueira MI, de Paula-Coelho VN. Meglumine antimonate directly increases phagocytosis, superoxide anion and TNF-α production, but only via TNF-α it indirectly increases nitric oxide production by phagocytes of healthy individuals. International immunopharmacology. 2008;8(12):1633-8.

200- Manna L, Reale S, Picillo E, Vitale F, Gravino AE. Interferon-gamma (INF-γ), IL4 expression levels and Leishmania DNA load as prognostic markers for monitoring response to treatment of leishmaniotic dogs with miltefosine and allopurinol. Cytokine. 2008;44(2):288-92.

201- Haldar AK, Sen P, Roy S. Use of antimony in the treatment of leishmaniasis: current status and future directions. Molecular biology international. 2011;2011.

202- Yaghoobi-Ershadi M, Javadian E. Zoonotic cutaneous leishmaniasis to the north of Isfahan. Human infection in 1991. Bulletin de la Societe de pathologie exotique (1990). 1994;88(1):42-5.

203- Noyes HA, Reyburn H, Bailey JW, Smith D. 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. 1998;36(10):2877-81.

204- Overbergh L, Valckx D, Waer M, Mathieu C. Quantification of murine cytokine mRNAs using real time quantitative reverse transcriptase PCR. Cytokine. 1999;11(4):305-12.

205- Maroof A, Kaye PM. Temporal regulation of interleukin-12p70 (IL-12p70) and IL-12-related cytokines in splenic dendritic cell subsets during Leishmania donovani infection. Infection and immunity. 2008;76(1):239-49.

206- Chawla B, Madhubala R. Drug targets in Leishmania. Journal of Parasitic Diseases. 2010;34(1):1-13.

207- Motamedi N, Hejazi S. H, Hazavei S. M. M, Zamani A, Saberi S, Rahimi E. Effect of education based on Health Belief Model on promoting preventive behavior of coetaneous leishmaniasis. Journal of Military Medicine. 2010; 11(4):38.

208- Saberi S, Nilfroushzadeh M, Zamani A, Hejazi S, Siadat A, Motamedi N, et al. Evaluation Of Efficacy Of Deet Repellent Pen In Control Of Leishmaniasis In A Military Area. Electronic Journal of Environmental Sciences Vol. 2011;4:9-11.

209- Hadighi R, Boucher P, Khamesipour A, Meamar A, Roy G, Ouellette M, et al. Glucantime-resistant Leishmania tropica isolated from Iranian patients with cutaneous leishmaniasis are sensitive to alternative antileishmania drugs. Parasitology research. 2007;101(5):1319-22.

210- Croft SL, Yardley V. Chemotherapy of leishmaniasis. Current pharmaceutical design. 2002;8(4):319-42.

211- Shazad B, Abbaszadeh B, Khamesipour A. Comparison of topical paromomycin sulfate (twice/day) with intralesional meglumine antimoniate for the treatment of cutaneous leishmaniasis caused by L. major. European Journal of Dermatology. 2005;15(2):85-7.

212- Sundar S, Chakravarty J. Leishmaniasis: an update of current pharmacotherapy. Expert opinion on pharmacotherapy. 2013;14(1):53-63.

213- Faraut-Gambarelli F, Piarroux R, Deniau M, Giusiano B, Marty P, Michel G, et al. In vitro and In vivo resistance of Leishmania infantum to meglumine antimoniate: a study of 37 strains collected from patients with visceral leishmaniasis. Antimicrobial agents and chemotherapy. 1997;41(4):827-30.

214- Lira R, Sundar S, Makharia A, Kenney R, Gam A, Saraiva E, et al. Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of Leishmania donovani. Journal of Infectious Diseases. 1999;180(2):564-7.

215- Sundar S. Drug resistance in Indian visceral leishmaniasis. Tropical Medicine & International Health. 2001;6(11):849-54.

216- Mishra M, Biswas U, Jha D, Khan A. Amphotericin versus pentamidine in antimony-unresponsive kala-azar. The Lancet. 1992;340 (8830):1256-7.

217- Murray SSaHW. Availability of miltefosine for the treatment of kala-azar in India. Bulletin of the World Health Organization. 2005;83(5):394–5.

218- S. Sundar LBG, M. K.Makharia et al. Oral treatment of visceral leishmaniasis with miltefosine. Annals of Tropical Medicine and Parasitology. 1999; 93(6):589–97.

219- Alving CR, Steck EA, Hanson WL, Loizeaux PS, Chapman Jr WL, Waits VB. Improved therapy of experimental leishmaniasis by use of a liposome-encapsulated antimonial drug. Life sciences. 1978;22(12):1021-5.

220- Alving CR, Swartz Jr GM, Hendricks LD, Chapman Jr WL, Waits VB, Hanson WL. Liposomes in leishmaniasis: effects of parasite virulence on treatment of experimental leishmaniasis in hamsters. Annals of tropical medicine and parasitology. 1984;78(3):279-86.

221- Berman J, Hanson W, Chapman W, Alving C, Lopez-Berestein G. Antileishmanial activity of liposome-encapsulated amphotericin B in hamsters and monkeys. Antimicrobial agents and chemotherapy. 1986;30 (6):847-51.

222- Roden MM, Nelson LD, Knudsen TA, Jarosinski PF, Starling JM, Shiflett SE, et al. Triad of acute infusion-related reactions associated with liposomal amphotericin B: analysis of clinical and epidemiological characteristics. Clinical infectious diseases. 2003;36(10):1213-20.

223- Groot O TR, Girbes A, Swart N, Beishuizen A. Acute refractory hyperkalaemia and fatal cardiac arrest related to administration of liposomal amphotericin B. Neth J Med. 2008;66:433-7.

224- Bagnis CI, Deray G. Amphotericin B nephrotoxicity. Saudi Journal of Kidney Diseases and Transplantation. 2002;13(4):481.

225- Watkins BM. Drugs for the control of parasitic diseases: current status and development. Trends in parasitology. 2003;19(11):477-8.

226- Layegh P, Rajabi O, Jafari MR, Emamgholi Tabar Malekshah P, Moghiman T, Ashraf H, et al. Efficacy of topical liposomal amphotericin B versus intralesional meglumine antimoniate (Glucantime) in the treatment of cutaneous leishmaniasis. Journal of parasitology research. 2011;2011.

227- Pal S, Ravindran R, Ali N. Combination therapy using sodium antimony gluconate in stearylamine-bearing liposomes against established and chronic

Leishmania donovani infection in BALB/c mice. Antimicrobial agents and chemotherapy. 2004;48(9):3591-3.

228- Murray HW, Berman JD, Wright SD. Immunochemotherapy for intracellular Leishmania donovani infection: γ interferon plus pentavalent antimony. Journal of Infectious Diseases. 1988;157(5):973-8.

229- Hejazi R, Amiji M. Chitosan-based gastrointestinal delivery systems. Journal of Controlled Release. 2003;89(2):151-65.

230- Huanbutta K, Luangtana-anan M, Sriamornsak P, Limmatvapirat S, Puttipipatkhachorn S, Nunthanid J. Factors affecting preparations of chitosan microcapsules for colonic drug delivery. Journal of Metals, Materials and Minerals. 2008;18(2):79-83.

231- Babior BM. Oxygen-dependent microbial killing by phagocytes (second of two parts). The New England journal of medicine. 1978; 298(13): 721.

232- Vouldoukis I, Riveros-Moreno V, Dugas B, Ouaaz F, Becherel P, Debre P, et al. The killing of Leishmania major by human macrophages is mediated by nitric oxide induced after ligation of the Fc epsilon RII/CD23 surface antigen. Proceedings of the National Academy of Sciences. 1995;92(17): 7804-8.

233- al-Mofleh I, Mahmoud A, al-Khuwaiter A, al-Tuwaijri A. Effect of Leishmania major on luminol-dependent chemiluminescence of human whole blood phagocytosis. Tropical medicine and parasitology: official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ). 1989;40(3):279-84.

234- Al Tuwaijri A, Al Mofleh I, Mahmoud A. Effect of Leishmania major on human polymorphonuclear leucocyte function In vitro. Journal of medical microbiology. 1990;32(3):189-93.

235- Rais S, Perianin A, Lenoir M, Sadak A, Rivollet D, Paul M, et al. Sodium stibogluconate (Pentostam) potentiates oxidant production in murine visceral leishmaniasis and in human blood. Antimicrobial agents and chemotherapy. 2000;44(9):2406-10.

236- Fernández-Guerrero ML, Aguado J, Buzón L, Barros C, Montalbán C, Martín T, et al. Visceral leishmaniasis in immunocompromised hosts. The American journal of medicine. 1987;83(6):1098-102.

237- Wadhone P, Maiti M, Agarwal R, Kamat V, Martin S, Saha B. Miltefosine promotes IFN-γ-dominated anti-leishmanial immune response. The Journal of Immunology. 2009;182(11):7146-54.

238- Basu JM, Mookerjee A, Sen P, Bhaumik S, Sen P, Banerjee S, et al. Sodium antimony gluconate induces generation of reactive oxygen species and nitric oxide via phosphoinositide 3-kinase and mitogen-activated protein kinase activation in Leishmania donovani-infected macrophages. Antimicrobial agents and chemotherapy. 2006;50(5):1788-97.

239- Dasgupta B, Roychoudhury K, Ganguly S, Kumar Sinha P, Vimal S, Das P, et al. Antileishmanial drugs cause up-regulation of interferon-gamma receptor 1, not only in the monocytes of visceral leishmaniasis cases but also in cultured THP1 cells. Annals of tropical medicine and parasitology. 2003;97(3):245-57.

240- Murray HW, Delph-Etienne S. Roles of endogenous gamma interferon and macrophage microbicidal mechanisms in host response to chemotherapy in experimental visceral leishmaniasis. Infection and immunity. 2000;68(1): 288-93.

241- Murray HW. Kala-azar as an AIDS-related opportunistic infection. AIDS Patient Care and STDs. 1999;13(8):459–65.

242- Alvar J, Cañavate C, Gutierrez-Solar B, Jiménez M, Laguna F, Lopez-Velez R, et al. Leishmania and human immunodeficiency virus coinfection: the first 10 years. Clinical Microbiology Reviews. 1997;10(2):298-319.

243- De Jimenez G, Ercoli N. Effect of drugs on various Leishmania isolates and succinic dehydrogenase inhibition. Experimental parasitology. 1965;17(3):302-8.

244- Romani L, Puccetti P, Bistoni F. Interleukin-12 in infectious diseases. Clinical Microbiology Reviews. 1997;10(4):611-36.

245- Neal R, Croft S. An in-vitro system for determining the activity of compounds against the intracellular amastigote form of Leishmania donovani. Journal of Antimicrobial Chemotherapy. 1984;14(5):463-75.

246- Nabors GS, Afonso L, Farrell JP, Scott P. Switch from a type 2 to a type 1 T helper cell response and cure of established Leishmania major infection in mice is induced by combined therapy with interleukin 12 and Pentostam. Proceedings of the National Academy of Sciences. 1995;92(8):3142-6.

247- Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annual review of immunology. 1995;13(1):251-76.

248- Smith LE, Rodrigues M, Russell DG. The interaction between CD8+ cytotoxic T cells and Leishmania-infected macrophages. The Journal of experimental medicine. 1991;174(3):499-505.

249- Carter K, Hutchison S, Boitelle A, Murray H, Sundar S, Mullen A. Sodium stibogluconate resistance in Leishmania donovani correlates with greater tolerance to macrophage antileishmanial responses and trivalent antimony therapy. Parasitology. 2005;131(06):747-57.

250- Basu JM, Roy S. Sodium antimony gluconate (SAG) mediates antileishmanial effect by stimulating innate and cellular arms of the immune system. Science and Culture. 2007;73(5/6):138.

251- Sypek J, Chung CL, Mayor S, Subramanyam J, Goldman S, Sieburth D, et al. Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. The Journal of experimental medicine. 1993;177(6):1797-802.

252- Engwerda CR, Murphy ML, Cotterell SE, Smelt SC, Kaye PM. Neutralization of IL-12 demonstrates the existence of discrete organ-specific phases in the control of Leishmania donovani. European journal of immunology. 1998;28(2):669-80.

253- Murray HW, Hariprashad J. Interleukin 12 is effective treatment for an established systemic intracellular infection: experimental visceral leishmaniasis. The Journal of experimental medicine. 1995;181(1):387-91.

254- Nabors GS, Farrell JP. Depletion of interleukin-4 in BALB/c mice with established Leishmania major infections increases the efficacy of antimony therapy and promotes Th1-like responses. Infection and immunity. 1994;62(12):5498-504.

255- Murray HW, Jungbluth A, Ritter E, Montelibano C, Marino MW. Visceral leishmaniasis in mice devoid of tumor necrosis factor and response to treatment. Infection and immunity. 2000;68(11):6289-93.

256- Murray HW, Hariprashad J, Fichtl RE. Treatment of experimental visceral leishmaniasis in a T-cell-deficient host: response to amphotericin B and pentamidine. Antimicrobial agents and chemotherapy. 1993;37(7):1504-5.

257- Croft S, Neal R, Thornton E, Herrmann D. Antileishmanial activity of the ether phospholipid ilmofosine. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1993;87(2):217-9.

258- Murray H, Oca M, Granger A, Schreiber R. Requirement for T cells and effect of lymphokines in successful chemotherapy for an intracellular infection. Experimental visceral leishmaniasis. Journal of Clinical Investigation. 1989;83(4):1253.

259- Taylor AP, Murray HW. Intracellular antimicrobial activity in the absence of interferon-γ: effect of interleukin-12 in experimental visceral leishmaniasis in interferon-γ gene-disrupted mice. The Journal of experimental medicine. 1997;185(7):1231-40.

260- Satoskar AR, Rodig S, Telford III SR, Satoskar AA, Ghosh SK, von Lichtenberg F, et al. IL-12 gene-deficient C57BL/6 mice are susceptible to Leishmania donovani but have diminished hepatic immunopathology. European journal of immunology. 2000;30(3):834-9.

261- Ghosh M, Roy K, Roy S. Immunomodulatory effects of antileishmanial drugs. Journal of Antimicrobial Chemotherapy. 2013;68(12):2834-8.

262- Ponte CB, Alves ÉAR, Sampaio RNR, Urdapilleta AAA, Kückelhaus CdS, Muniz-Junqueira MI, et al. Miltefosine enhances phagocytosis but decreases nitric oxide production by peritoneal macrophages of C57BL/6 mice. International immunopharmacology. 2012;13(1):114-9.

263- Murray HW, Montelibano C, Peterson R, Sypek JP. Interleukin-12 regulates the response to chemotherapy in experimental visceral leishmaniasis . Journal of Infectious Diseases. 2000;182(5):1497-502.

264- Munder M, Mallo M, Eichmann K, Modolell M. Murine macrophages secrete interferon γ upon combined stimulation with interleukin (IL)-12 and IL-18: a novel pathway of autocrine macrophage activation. The Journal of experimental medicine. 1998;187(12):2103-8.

265. Schindler H, Lutz MB, Röllinghoff M, Bogdan C. The production of IFN-γ by IL-12/IL-18-activated macrophages requires STAT4 signaling and is inhibited by IL-4. The Journal of Immunology. 2001;166(5):3075-82.

266- Schmitt E, Hoehn P, Huels C, Goedert S, Palm N, Rüde E, et al. T helper type 1 development of naive CD4+ T cells requires the coordinate action of interleukin-12 and interferon-γ and is inhibited by transforming growth factor-β. European journal of immunology. 1994;24(4):793-8.

267- Seder RA, Paul WE. Acquisition of lymphokine-producing phenotype by CD4+ T cells. Annual review of immunology. 1994;12(1):635-73.

268- Watford WT, Hissong BD, Bream JH, Kanno Y, Muul L, O'Shea JJ. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. Immunological reviews. 2004;202(1):139-56.

269- Quinones M, Ahuja SK, Melby PC, Pate L, Reddick RL, Ahuja SS. Preformed membrane-associated stores of interleukin (IL)-12 are a previously unrecognized source of bioactive IL-12 that is mobilized within minutes of contact with an intracellular parasite. The Journal of experimental medicine. 2000;192(4):507-16.

270- Asadpour A, Riazi-Rad F, Khaze V, Ajdary S, Alimohammadian M. Distinct strains of Leishmania major induce different cytokine mRNA expression in draining lymph node of BALB/c mice. Parasite immunology. 2013;35(1):42-50.

271- Launois P, Swihart KG, Milon G, Louis JA. Early production of IL-4 in susceptible mice infected with Leishmania major rapidly induces IL-12 unresponsiveness. The Journal of Immunology. 1997;158(7):3317-24.

272- Launois P, Ohteki T, Swihart K, MacDonald HR, Louis JA. In susceptible mice, Leishmania major induce very rapid interleukin-4 production by CD4+ T cells which are NK1. European journal of immunology. 1995;25(12):3298-307.

273- Gracie JA, Forsey RJ, Chan WL, Gilmour A, Leung BP, Greer MR, et al. A proinflammatory role for IL-18 in rheumatoid arthritis. Journal of Clinical Investigation. 1999;104(10):1393-401.

274- Wei X-Q, Niedbala W, Xu D, Luo Z-X, Pollock KG, Brewer JM. Host genetic background determines whether IL-18 deficiency results in increased susceptibility or resistance to murine Leishmania major infection. Immunology letters. 2004;94(1):35-7.

275- Yoshimoto T, Yoshimoto T, Yasuda K, Mizuguchi J, Nakanishi K. IL-27 suppresses Th2 cell development and Th2 cytokines production from polarized Th2 cells: a novel therapeutic way for Th2-mediated allergic inflammation. The Journal of Immunology. 2007;179(7):4415-23.

276- de Saldanha RR, Martins-Papa MC, Sampaio RNR, Muniz-Junqueira MI. Meglumine antimonate treatment enhances phagocytosis and TNF-α production by monocytes in human cutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2012;106(10):596-603.

277- Yamasaki K, Edington H, McClosky C, Tzeng E, Lizonova A, Kovesdi I, et al. Reversal of impaired wound repair in iNOS-deficient mice by topical adenoviral-mediated iNOS gene transfer. Journal of Clinical Investigation. 1998;101(5):967.

278- Schäffer MR, Tantry U, van Wesep RA, Barbul A. Nitric oxide metabolism in wounds. Journal of Surgical Research. 1997;71(1):25-31.

279- Iuvone T, Carnuccio R, Di Rosa M. Modulation of granuloma formation by endogenous nitric oxide. European journal of pharmacology. 1994;265(1):89-92.

280- Witte MB, Barbul A. Role of nitric oxide in wound repair. The American Journal of Surgery. 2002;183(4):406-12.

281- Brahmachari U. Chemotherapy of antimonial compounds in kala-azar infection. Part I. By UN Brahmachari, 1922. The Indian journal of medical research. 1989;89:492-522.

282- Vouldoukis I, Drapier JC, Nüssler A, Tselentis Y, Da Silva OA, Gentilini M, et al. Canine visceral leishmaniasis: successful chemotherapy induces macrophage antileishmanial activity via the L-arginine nitric oxide pathway. Antimicrobial agents and chemotherapy. 1996;40(1):253-6.

283- Dalton DK, Pitts-Meek S, Keshav S, Figari IS, Bradley A, Stewart TA. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. Science. 1993;259(5102):1739-42.

284- Gajewski TF, Fitch FW. Anti-proliferative effect of IFN-gamma in immune regulation. I. IFN-gamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. The Journal of Immunology. 1988;140(12):4245-52.

285- Kuroda E, Kito T, Yamashita U. Reduced expression of STAT4 and IFN-γ in macrophages from BALB/c mice. The Journal of Immunology. 2002;168(11):5477-82.

Abstract:

Introduction: Isfahan is one of the most important centers of Leishmaniasis in Iran. Due to the nature of this type of zoonotic cutaneous leishmaniasis and lack of an effective vaccine, this is currently under treatment for disease control. Research scientists have shown that 5-valence compounds of antimony are still the first line treatment activates the immune system to produce cytokines. Accordingly, in this study the gene expression levels of cytokines IL-27, IL-18, IL-12, Tumor Necrosis Factor (TNF-α) and the amount of Interferon Gama (IFN-γ) and Nitric oxide (NO), which are the healing and overcoming leishmaniasis factors by using meglumine antimoniate loaded chitosan nanoparticles designed with a gel base *In vitro* and *In vivo* has been studied.

Materials and Methods:

In this study, ten patients that had not recovered with two courses of treatment to *L.major* approved Nested PCR method were enrolled. In vitro Phase by using cultured cell line J774, which were infected with the patient isolates or the standard type (MRHO/IR/75/ER), cytokines gene expression by method of Triplicate Quantitative RT-Real time PCR, NO by Griess kite and IFN-γ by ELISA murine kite were evaluated. After that treatment groups were compared. Phase In vivo using mice Balb/c types isolated from patients and injected into the tail base and Leishman lesion was created and treated daily with either drug-free meglumine antimoniate or meglumine antimoniate were loaded with nanoparticles in a gel (nano drug) base. The level of gene expression or the amount of the cytokines was studied using Real Time PCR.

Results:

The results showed that the use of nano drug than the nono-free meglumine antimoniate and untreated stage significantly increased the expression of interleukin 12, 18 and 27 in both In vitro and In vivo phases in the standard specie and patient isolates groups. Compared with the two-phases ANCOVA showed a significant increase In vivo phase. Significant increase were seen by use of nano medicine in gene expression of (TNF-α) comparing with untreated stage While between the In vitro and In vivo phases as well as for nono-free meglumine antimoniate was not found. The levels of nitric oxide (NO) and

interferon gamma (IFN-γ) in the groups treated with nano-drug compared to the no treatment and meglumine antimoniate significantly increased. Stimulating and increasing cytokines in patient isolates and the standard specie was same.

Discussion:

In this study, treatment with nano drug compared to free form it has been increased the amount of gene expression of effective cytokines in Th_1 response both In vivo and In vitro phases which can be due to factors such as high biological stability of polymeric nanoparticles, better uptake and more phagocytosis by macrophages compared to the free form of drug. According to the response of the patient isolates with clinical resistance to glucantime and standard specie were similar to, the effectiveness and applicability of the drugs can still hope for 5 valence antimony and Because they are still the first line treatment of cutaneous, Compared with many expensive drugs or high toxicity, Still suitable for research and applied it to a form of medication which not only is easy to use and acceptable to the patient but also is lower cost and more effective as an effective drug in the treatment of cutaneous leishmaniasis may be considered.

Key words: Cutaneous leishmaniasis, Glocantime, IL-12, IL-27, IL-18, TNF-α, IFN-γ, NO

PhD Thesis

Title:

The evaluation of gene expression of predominant cytokines in Glucantime resistant Zoonotic Cutaneous Leishmaniasis *in vitro* and Balb/c model

Grant No: 391041

By
Sedigheh Saberi

Supervisors:

Dr. Seyed Hossein Hejazi

Dr. Ali Khamesipour

Dr. Mansour Salehi

Advisors:

Dr. Jaleh Varshosaz

Dr. Seyed mohsen Hosseini

Dr. Abbas Ali Palizban

August 2014