

Efficacy of Intralesional D-Penicillamine in the Treatment of Acute Cutaneous Leishmaniasis: A Clinical Study

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ABSTRACT

Background: Cutaneous leishmaniasis (CL) is a parasitic disease prevalent in the Mediterranean and Middle East, including Iraq. This study evaluated the efficacy and safety of intralesional D-penicillamine (D-PA) as a treatment for acute CL, leveraging its potential to enhance the Th1 immune response. **Methods:** A unicentric, inpatient-controlled trial was conducted at Al-Yarmouk Teaching Hospital, Baghdad, from January to August 2023. Forty-nine patients with 144 lesions were divided into two groups: Category A received intralesional 5% D-PA injections weekly for six sessions, and Category B (control) received no treatment. Lesion responses were monitored, and statistical analysis was performed using SPSS (V. 24). **Results:** Of 95 lesions treated in Category A, 87 (91.5%) showed improvement, with 69 (72.6%) achieving complete response and 18 (18.9%) showing marked response after three to six sessions. Category B showed no healing in 49 lesions, with 5 (10.2%) becoming ulcerated. **Conclusion:** Intralesional D-penicillamine is a safe, effective, and affordable option for treating acute CL, achieving high cure rates with minimal side effects.

Keywords: Cutaneous Leishmaniasis, D- Penicillamine, Intralesional Therapy.

INTRODUCTION

Cutaneous leishmaniasis (CL) described as ancient ailment [1]. It caused by parasite that named hemoflagellate *Leishmania*, CL is widespread and may cause serious health problems in communities in the Mediterranean area and the Middle East, including our country, Iraq [2, 3]. Annually, more than 100,000 new cases are reported but real incidence is speculated to be higher by tripled or even up to five times, which related to absence of health documentation for some case [4]. CL is endemic in Iraq and mostly caused by *L. major* and *L. tropica* with the transmission to man (which is an accidental host) occur exclusively by infected female sand fly bite beyond species of *Phlebotomus* and *Lutzomyia*. in our region, acute cutaneous leishmaniasis in most cases diagnosed clinically so that skin biopsy not always done, which account 0.35% from totally 281 skin biopsies [5]. Epidemiologically, CL is unstable disease, so that, the number of reported cases is fluctuated in unpredictable way [6]; based on that, in Iraq, the total rate of incidence varies from 2.3 / 100000 to 45.5 / 100000 [6]. CL is described as disease that self-healing, but that may take many months or years, which leaving permanent scare, causing serious social stigma [7, 8]. Antimony is drug of choice for treatment of CL, but it is an unstable drug and even toxic. with rate of efficacy between 75% and 100%, and development of resistance is of increasing concern [9], other modalities of treatment of cutaneous leishmaniasis had been used with variable rate of success, like Systemic use of antifungal drugs [10] dapsone [11] azithromycin and rifampicin [12, 13] oral zinc sulphate and interferon [8, 14, 15]. Many local physical modalities of treatment also use with variable cure rate like, heat therapy [16] laser [17] cryotherapy [18] and medical treatment like hypertonic sodium chloride [19] chloroquine [20] topical application of paromomycin [21] and azole agents [22]. D-Penicillamine (D-PA): firstly, isolated by Abraham, et al. as an amine from degradation of penicillin in 1942 [23]. D-PA, unlike its precursor, does not have such antibiotic efficacy and interesting of compound arose from his situation during degradation process of parent penicillin [23]. Later, D-PA had been using in medicine extensively; firstly, by Walshe in 1956 as revolution therapy for Wilson disease [24], also used in treatment of rheumatoid arthritis (RA), juvenile RA, palindromic rheumatism,

cystinuria, alcohol detoxification, primary biliary cirrhosis, chronic active hepatitis, heavy metal removal, scleroderma, morphoea and keloid. Moreover, D-PA has been used as a ligand in the radiopharmaceutical's preparation for imaging kidney and liver [25]. It is worth to be mentioned, the L-isomer is toxic so only D-isomer is medically use [26]. D-PA is an effective chelator of lead, mercury, copper and zinc, and many other heavy metals forming stable, soluble complexes which are excreted in the urine, readily [27, 28]. Pharmacokinetically, D-PA is well absorbed (40-70%) from the gastrointestinal tract, peak concentrations are obtained in blood (1-3 hours) after intake, more than 80% of blood-circulation D-PA is plasma-protein bounded, that eliminated primarily via the urinary tract [27]. Immune enhancement efficacy of D-PA had been established by its stimulatory effect on reticuloendothelial function via an interaction with macrophage plasma membrane [29], through binding to the macrophages surface aldehydes, D-PA leads to interaction between macrophages and T cells, that subsequently leads to induction of an immune response [30]. D-PA increase the output of the following cytokines (interleukin-6, interleukin-23, tumour necrosis factor) which give considerable clue that D-PA enhance activity of macrophage cell [30]. It's mentioned that T-cells and macrophages collectively, are crucial immune cells in D-PA penicillamine-induced autoimmunity [31].

PATIENTS AND METHODS

study is unicentric open inpatient control therapeutic trial. Conducted in outpatient ward of department of dermatology and venerology, Al-Yarmouk Teaching Hospital, Baghdad, Iraq. During time from January 2023 to August 2023. For each patient, informative history and clinical exam were achieved which including age, gender, occupation, residence, allergy to penicillin, duration of lesion(s), type whether ulcerative or not, lesion site(s). After confirming the diagnosis clinically and parasitological (Leishman bodies in Giemsa-stained of direct smear from the lesion scrapings), all patients enrolled in essential investigations that include complete blood count, liver

function before initiation of treatment and every week thereafter. Patients with following criteria were excluded from trial therapy, these criteria are more than 6 months duration of disease, their size more than 5 cm, lesions close to eye or that occur in nose or ear, receiving anti-leishmaniasis therapy in last 6 weeks, patients with hepatic or haematological and other immunocompromised disease, pregnant and lactating female and patient who is had penicillin allergy. After giving enough information to the patients, written informed consent was obtained from all participants, Gross photographs, by using SONY® Cyber-shot DSC-W12.1MP Digital camera with 5x Optical zoom were taken of lesions and after receiving the guardian's consent pre and post treatment.

Ethical Approval

The protocol of this study was approved by scientific committee of our institution. For each patient, informed consent was taken, and confidential record was kept through all study stages. All patients enrolled in study should have two or more lesions and study sample was divided in two groups as below:

Category A (Penicillamine Group)

lesions were treated with intralesional (5%) penicillamine which prepared by dissolving (500mg) of Penicillamine (Penicilin)® purchased from SAMARTH LIFE SCIENCES PVT LTD. INDIA, in (10ml) distilled water in sterilized container to obtained (5%) concentration of D-PA and autoclaved at 121 Celsius degrees for 20 min before use. Lesions sponged off with a mild antiseptic solution then fresh prepared D-penicillamine was administered through multiple superficial puncture technique without anaesthesia by using 26-gauge insulin needle. After completed of first session, the patient is advised to return in after one week, at which a second injection is done, with totally six sessions with one week interval between subsequent sessions.

Category B (Control Group)

No therapy was getting for patients in this group (as all tested patients have two or more lesions, one lesion used as control).

Assessment the Responding to Treatment

Each lesion was assessed before started therapy and then weekly (before giving corresponding dose) through detecting the colour,

erythema, induration, ulcer, and crust. Lesion diameters were obtained by marking the induration (after palpated), then calculate its diameter by tape measure with millimetre unite in case of round regular lesions, and two maximum diameters for irregular lesions. To evaluation the response to therapy, study adopted Sharquie modified leishmania score (Table1). Assessment of patients carry on weekly and through 6 weeks to evaluate the response and scoring it and notice any drug side effects whether locally or systematically. For documentary purpose, take a picture for each lesion in same place and distance by using SONY® cyber-shot DSC W12.1 MP digital camera with 5X optical zoom.

Statistical Analysis

This study applied the program of IMB SSPS (V. 24) for both descriptive statistics that includes mean $\pm$ SD; and for analytic statistics that includes chi-square (χ^2) and *P* value, the last considered significant if was less than (0.005).

Result

Totally 49 patients with 144 lesions of acute cutaneous leishmaniasis have been enrolled in study. 38(77.55%) were males and 11(22.45%) were females. Range of patients ages from (6-66) years with mean $\pm$ SD of 33.94 ± 15.33 years. From 144 total lesions there was 80 (55.56%) ulcerative type and 64 (44.44%) non-ulcerative type. Lesions duration ranged from (5-21) weeks with mean $\pm$ SD of 11.97 ± 4.43 weeks. The most affected site was upper limbs then lower limbs then head and neck, and finally trunk with mean $\pm$ SD $65(45.14\%)$ 43(29.86%) 27(18.75%) 9(6.25%) respectively. as shown demographic features in (table2). Category A: totally 95 lesions were treated, 87(91.15%) of them showed cure, of which 69(72.6%) lesions have complete response and 18(18.9%) lesions have marked response after three to six sessions. The remaining 8(8.4%) patients showed moderate response after 6 sessions. Regarding the types of response through weekly assessment is showing in (table3) and (figure1) and total scores for each patient per week shown in (table 4). Statistically, the *P* value considered significant because its counting < 0.05 . Category B: controlled 49 lesions showed no sign of healing, apart from 5(10.2%) lesions became ulcerated by the end of study.

Table 1: Sharquie's Modified Leishmania Score.

Score	Colour Changes in the Lesion	Reduction Rate in Lesion Colour	Reduction Rate in The Induration of Lesion	Reduction Rate of Ulcer/Crust
4	Bright red	-	-	-
3	red	0% - 25%	0% - 25%	0% - 25%
2	Dusky red	25% -50%	24% -50%	25% - 50%
1	Dark brown	50% -75%	50% -75%	50% -75%
0	Light brown	>75% - Clearance	>75% - Clearance	>75% - Clearance

Score 13-16: Mild response. Score 9-12: Moderate response. Score 5-8: Marked response. Score 0-4: Complete response & clearance. Both marked and complete response had been considered as a cure.

Table 2(a): Demographical Data

Parameter	Values
Gender	
Males	38(77.55%)
Females	11(22.45%)
Age in years	
Mean	33.94
SD	15.33
Residence	
Urban	29(59.18%)
Rural	20(40.82%)
Duration in weeks	
Mean	11.97
SD	4.43

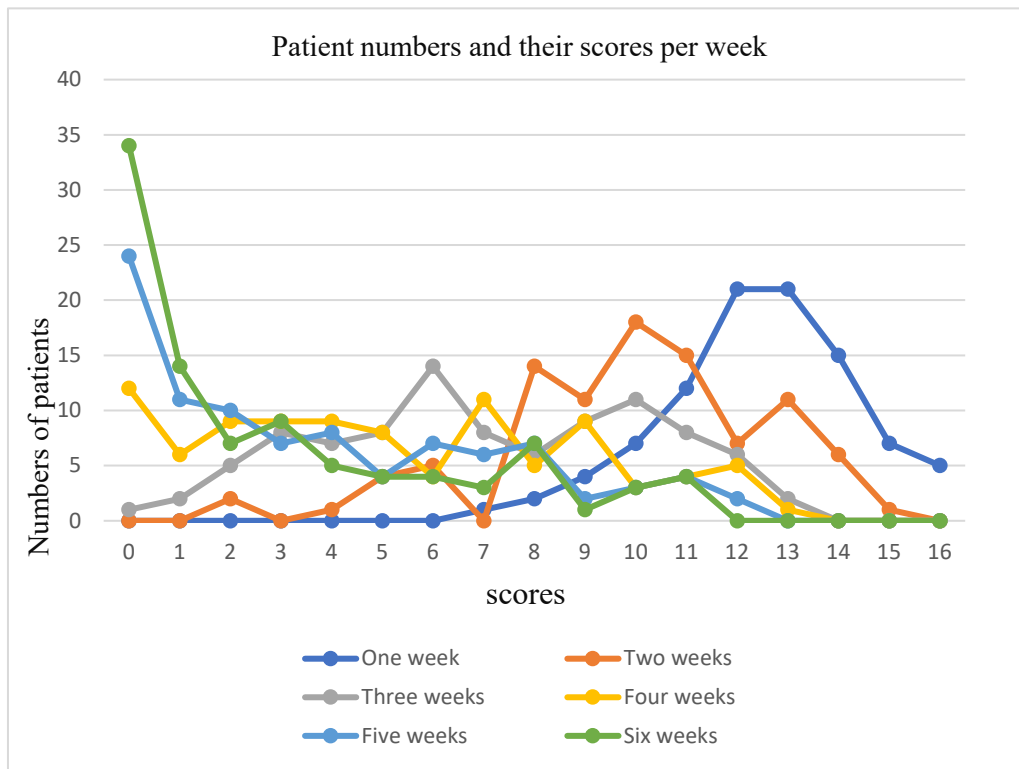
Table 2(b): Demographical Data

Parameter	Values
Site of lesion(s)	
Head +Neck	27(18.75%)
Upper limbs	65(45.14%)
Lower limbs	43(29.86%)
Trunk	9(6.25%)
Morphology	
Ulcerative	80 (55.56%)
Non ulcerative	64 (44.44%)

Table 3: Types of Response Per Week

Visit	Mild Response (%)	Moderate Response (%)	Marked Response (%)	Complete Response (%)	P Value*
One Week	48 (50.5%)	44 (46.3%)	3 (3.2%)	0	0,000
Two Weeks	18 (18.9%)	51 (53.7%)	23 (24.2%)	3 (3.2%)	0,000
Three Weeks	2 (2.1%)	34 (35.8%)	36 (37.9%)	23 (24.2%)	0,000
Four Weeks	1 (1.1%)	21 (22.1%)	28 (29.5%)	45 (47.3%)	0,000
Five Weeks	0	11 (11.6%)	24 (25.3%)	60 (63.1%)	0,000
Six Weeks	0	8 (8.4%)	18 (18.9%)	69 (72.6%)	0,000

P value was calculated by total score of specific weeks with that of control group.

**Figure 1:** Patient Numbers and their Scores Per Week**Table 4:** Total Scores for Each Patient Per Week

Score	One Week	Two Weeks	Three Weeks	Four Weeks	Five Weeks	Six Weeks
0	0	0	1	12	24	34
1	0	0	2	6	11	14
2	0	2	5	9	10	7
3	0	0	8	9	7	9
4	0	1	7	9	8	5
5	0	4	8	8	4	4
6	0	5	14	4	7	4
7	1	0	8	11	6	3
8	2	14	6	5	7	7
9	4	11	9	9	2	1
10	7	18	11	3	3	3
11	12	15	8	4	4	4
12	21	7	6	5	2	0
13	21	11	2	1	0	0
14	15	6	0	0	0	0
15	7	1	0	0	0	0
16	5	0	0	0	0	0
Total	95	95	95	95	95	95



FIGURE 2: 17 Years Old Male with 3 Months Duration CL on Dorsum of Foot that cured after 4 Sessions.



FIGURE 3: 10 Years Old Boy with Facial CL Show Marked Healing after 5 Sessions.

Discussion

Although CL is self-healing skin disease, treatment is recommended because prolong time for cure and ugly scarring formation [7]. Regarding demographics features, male: female ratio was (3.4: 1) that parallel to many studies [20, 32]. Mean age group was 33.94 ± 15.33 , which goes parallel others studies [7, 20]. Regarding to site of lesions, upper and lower limbs account major area of involvement 108 (75%); trunk occupy lowest number 9 (6.25%), which can explain as CL sand fly-born disease which mainly occur in exposed area of the body and to lest degree in hidden area. This result show similarity with other study regarding to predominance area of patients [20]. Head and neck account only 27(18.75%) from total 144 evaluated lesions, which may be related to exclusion criteria for lesions nears the ears, closed to eyes, and on the nose. Intraleisional injection method for therapy of CL is traditionally used and it's recommended by WHO in endemic regions, so that different kinds of therapies were evaluated by intraleisional technique [7, 19, 20]. It is reasonable to treat acute CL individually due to the acute CL is lesions locally persist and once parasite infected skin tissue becomes unable to migrate, additionally just little dose of therapy was utilised while the benefit locally and directly with absorbed slowly. This study produced cure rate 87(91.15%) among controlled treated patients. Which hassling with results of different studies that utilise different intraleisional therapies like; Sodium stibogluconate antimony (94.6%) [7], Intraleisional 8.33% rifamycin infiltration (92.7%) [33]. Hypertonic sodium chloride solution (96%) [19], and Ciprofloxacin 0.2% (81.5%) [34].

Most side effects were mild and transient like minimal pain during injection, localised oedema, hyperpigmentation. Regarding to immune response to acute cutaneous leishmaniasis, its well documented that impedance to disease is mainly related to Th1 cell development also to output of certain cytokines with pro-inflammatory effects such as interleukine-12, interleukine-1, interferon gamma, tumour necrosis factor alfa and interleukin-2, which produce direct stimulatory action upon macrophage and subsequently killing of parasite [35]. Intraleisional D-PA for treatment of acute cutaneous leishmaniasis, which has not been tried before, does not have any antibiotic activity [23]. It seems to be produced its effect by stimulatory response upon lesional macrophage through Th1 immune response via an interaction with aldehydes on the surface of macrophage plasma membrane [29], which lead to interaction between macrophages and T cells, that subsequently leads to induction of Th1 immune response [30], that manifest by output of IL-23, TNF-alpha and IL-6 that give extra proof that D-penicillamine enhance macrophage cell activity [30]. It's mentioned that both of Th-1 cell also macrophages have crucial role in D-penicillamine induce autoimmunity [31].

Conclusion and Recommendations

Intraleisional D- penicillamine is new, safe, affordable, and considerably effective modality for treatment of acute cutaneous leishmaniasis. Study recommended different D-penicillamine concentrations and farther clinical comparative studies.

References

- [1] A. J. Altamirano-Enciso, M. C. Marzochi, J. S. Moreira, A. O. Schubach, and K. B. Marzochi, "On the origin and spread of cutaneous and mucosal leishmaniasis, based on pre- and post-Colombian historical sources," *História, Ciências, Saúde-Manguinhos*, vol. 10, pp. 853-882, 2003.
- [2] N. C. Hepburn, "Cutaneous leishmaniasis: an overview," *Journal of postgraduate medicine*, vol. 49, no. 1, pp. 50-54, 2003.
- [3] W. H. Markle and K. Makhoul, "Cutaneous leishmaniasis: recognition and treatment," *American family physician*, vol. 69, no. 6, pp. 1455-1460, 2004.
- [4] W. H. Organization, "Manual for case management of cutaneous leishmaniasis in the WHO Eastern Mediterranean Region," 2014.
- [5] J. R. Al-Rawi, M. J. Ahmed, and A. S. Salloom, "Evaluation of skin biopsies in Al-Yarmouk teaching hospital," *International Journal of Health Sciences*, vol. 6, no. S6, pp. 289-299, 2022.
- [6] W. H. Organization, "Communicable Disease Working Group on Emergencies, HQ Division of Communicable Disease Control, EMRO, WHO OFFICE, Baghdad," *WHO Office, Baghdad. Communicable Disease Toolkit, IRAQ CRISIS. WHO*, pp. 39-44, 2003.
- [7] K. Sharquie, K. Al-Talib, and A. Chu, "Intralesional therapy of cutaneous leishmaniasis with sodium stibogluconate antimony," *British Journal of Dermatology*, vol. 119, no. 1, pp. 53-57, 1988.
- [8] K. Sharquie, R. Najim, I. Farjou, and D. Al-Timimi, "Oral zinc sulphate in the treatment of acute cutaneous leishmaniasis," *Clinical and experimental dermatology*, vol. 26, no. 1, pp. 21-26, 2001.
- [9] N. Hepburn, "Cutaneous leishmaniasis," *Clinical and experimental dermatology*, vol. 25, no. 5, pp. 363-370, 2000.
- [10] F. H. Salmanpour, MK Noughpish, R, "Comparative study of the efficacy of oral ketoconazole with intra-lesional meglumine antimoniate (Glucantime) for the treatment of cutaneous leishmaniasis," *Journal of dermatological treatment*, vol. 12, no. 3, pp. 159-162, 2001.
- [11] N. Al-Mutairi *et al.*, "Treatment of Old World cutaneous leishmaniasis with dapsone, itraconazole, cryotherapy, and imiquimod, alone and in combination," *International journal of dermatology*, vol. 48, no. 8, pp. 862-869, 2009.
- [12] M. L. Silva-Vergara, L. d. A. Silva, F. R. Z. Maneira, A. G. d. Silva, and A. Prata, "Azithromycin in the treatment of mucosal leishmaniasis," *Revista do Instituto de Medicina Tropical de Sao Paulo*, vol. 46, pp. 175-177, 2004.
- [13] H. Jaffar, "Rifampicin in cutaneous leishmaniasis—a therapeutic trial in Saudi Arabia," *Journal of Pakistan Association of Dermatologists*, vol. 16, no. 1, pp. 4-9, 2006.
- [14] N. Kırkcıoğlu and R. Tandoğdu, "Interferon gamma therapy for cutaneous leishmaniasis," *Archives of dermatology*, vol. 126, no. 6, pp. 831-832, 1990.
- [15] A. Khatami, A. Firooz, F. Gorouhi, and Y. Dowlati, "Treatment of acute Old World cutaneous leishmaniasis: a systematic review of the randomized controlled trials," *Journal of the American Academy of Dermatology*, vol. 57, no. 2, pp. 335. e1-335. e29, 2007.
- [16] M. Selim, Z. Vlasin, and L. Jaroskova, "Leishmaniasis: currently recommended treatment," 1990.
- [17] S. Shamsi Meymandi, S. Zandi, H. Aghaie, and A. Heshmatkhah, "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*, vol. 25, no. 5, pp. 587-591, 2011.
- [18] M. Gurei *et al.*, "Efficacy of cryotherapy and intralesional pentostam in treatment of cutaneous leishmaniasis," *Journal of the Egyptian Society of Parasitology*, vol. 30, no. 1, pp. 169-176, 2000.
- [19] H. AK, "Intralesional therapy of cutaneous leishmaniasis with hypertonic sodium chloride solution," *Postgraduate thesis, University of Baghdad*, 1993.
- [20] R. Yasmin, I. Khan, and S. A. Ahmad, "Response to treatment of cutaneous leishmaniasis with intralesional chloroquine vs intralesional meglumine antimoniate," *Journal of Pakistan Association of Dermatologists*, vol. 21, no. 4, pp. 270-275, 2011.
- [21] A. Asilian *et al.*, "Treatment of cutaneous leishmaniasis with aminosidine (paromomycin) ointment: double-blind, randomized trial in the Islamic Republic of Iran," *Bulletin of the World Health Organization*, vol. 81, no. 5, pp. 353-359, 2003.
- [22] E. B. Larbi, A. Al-Khawajah, Y. Al-Gindan, S. Jain, A. Abahusain, and A. Al-Zayer, "A randomized, double-blind, clinical trial of topical clotrimazole versus miconazole for treatment of cutaneous leishmaniasis in the eastern province of Saudi Arabia," *The American journal of tropical medicine and hygiene*, vol. 52, no. 2, pp. 166-168, 1995.
- [23] E. Abraham, E. Chain, W. Baker, and R. Robinson, "Penicillamine, a characteristic degradation product of penicillin," *Nature*, vol. 151, no. 3821, pp. 107-107, 1943.
- [24] J. Walshe, "Penicillamine, a new oral therapy for Wilson's disease," *The American journal of medicine*, vol. 21, no. 4, pp. 487-495, 1956.
- [25] H. E. Howard-Lock, C. J. Lock, A. Mewa, and W. F. Kean, "D-penicillamine: chemistry and clinical use in rheumatic disease," in *Seminars in arthritis and rheumatism*, 1986, vol. 15, no. 4: Elsevier, pp. 261-281.
- [26] J. E. Wilson and V. Du Vigneaud, "Inhibition of the growth of the rat by L-penicillamine and its prevention by aminoethanol and related compounds," *Journal of Biological Chemistry*, vol. 184, no. 1, pp. 63-70, 1950.
- [27] L. S. Goodman, *Goodman and Gilman's the pharmacological basis of therapeutics*. McGraw-Hill New York, 1996.
- [28] U. S. P. Convention, *Drug information for the health care professional*. Micromedex, 2002.
- [29] L. Binderup, E. Bramm, and E. Arrigoni-Martelli, "Effect of D-penicillamine in vitro and in vivo on macrophage phagocytosis," *Biochemical Pharmacology*, vol. 29, no. 17, pp. 2273-2278, 1980.
- [30] J. Li and J. P. Uetrecht, "D-penicillamine-induced autoimmunity: relationship to macrophage activation," *Chemical research in toxicology*, vol. 22, no. 9, pp. 1526-1533, 2009.
- [31] M. J. Masson, M. Teranishi, J. M. Shenton, and J. P. Uetrecht, "Investigation of the involvement of macrophages and T cells in D-penicillamine-induced autoimmunity in the Brown Norway rat," *Journal of immunotoxicology*, vol. 1, no. 2, pp. 79-93, 2004.
- [32] R. Armijos *et al.*, "The epidemiology of cutaneous leishmaniasis in subtropical Ecuador," *Tropical Medicine & International Health*, vol. 2, no. 2, pp. 140-152, 1997.
- [33] N. K. Al-Sudany and Y. J. Ali, "Intralesional 8.33% Rifamycin infiltration; New treatment for cutaneous leishmaniasis," *Journal of Dermatology & Dermatologic Surgery*, vol. 20, no. 1, pp. 39-45, 2016.
- [34] K. Al Hamdi, A. Awad, and H. Moker, "Evaluation of intralesional 0.2% ciprofloxacin as a treatment for cutaneous leishmaniasis," *EMHJ-Eastern Mediterranean Health Journal*, 16 (1), 89-93, 2010, 2010.
- [35] N. Maspi, A. Abdoli, and F. Ghaffarifar, "Pro-and anti-inflammatory cytokines in cutaneous leishmaniasis: a review," *Pathogens and global health*, vol. 110, no. 6, pp. 247-260, 2016.



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