.

:

Biochemical Alterations of Liver Enzymes and Microelements During *Leishmania Major* Infection in Balb/C Mice after Treatment with Paromomycin

Sharareh Davachi[1], Hossein Nahrevanian[2*], Eskandar Omidinia[3], Reza Hajihosseini[4], Marzyeh Amini[5], Mahin Farahmand[6], Fatemeh Mirkhani[7], Seifoddin Javadian[8]

[1] MSc student, University of Payame Noor, Tehran Center, Tehran, Iran; [2] PhD, Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran; [3] PhD, Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran; [4] PhD, University of Payame Noor, Tehran Center, Tehran, Iran; [6, 5] MSc, Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran; [7,8] BSc, Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran

Summary

Cutaneous leishmaniasis (CL) is a zoonotic disease caused by *Leishmania* parasites. CL is still one of the health problems in tropical areas and in Iran. In this study, the Paromomycin was used for treatment of CL in Balb/c mice infected with *Leishmania major*. It is purposed that Paromomycin can be used as an appropriate achievement for control and therapy of CL. Paromomycin was used to inhibit leishmaniasis in susceptible Balb/c mice infected with *Leishmania major* MRHO/IR/75/ER; as a prevalent strain of CL in Iran. Serum concentrations of essential trace elements including copper (Cu) and Zinc (Zn) were determined by Flame Atomic Absorption Spectrophotometer (FAAS). Moreover, liver enzymes including Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Alkaline Phosphatase (ALP) were also studied by Auto Analyzer Technical RA1000. Paromomycin therapy caused significant changes in the values of liver enzymes, Zn, Cu and Cu/Zn ratio as observed in healthy and infected Balb/c mice. Although, SGOT, SGPT and Cu were decreased in test groups, Zn and ALP increased in same groups in comparison with controls in healthy and infected groups of mice. The biochemical alteration of trace elements and liver enzymes was indicated during this study, which may attributable to anti-leishmanial effects of Paromomycin injection during *L. major* infection. This result clarifies Paromomycin as an effective therapy for the treatment of CL in rodent model of leishmaniasis.

Keywords: Balb/c, *Leishmania major*, Paromomycin, liver enzymes, microelement

*** Corresponding author:** Dr. H. Nahrevanian, Department of Parasitology, Pasteur Institute of Iran, Pasteur Ave., Tehran 13164, Iran; Tel / Fax: +98-21-66968855,
E-mail: mobcghn@gmail.com, mobcghn@pasteur.ac.ir

.

Introduction

Leishmaniasis is a geographically widespread severe disease, with an increasing incidence of two million cases per year and 350 million people from 88 countries at risk. The causative agents are species of *Leishmania*, a protozoan flagellate [1], which is transmitted by species of *Phlebotomine* sandflies [2]. Leishmaniasis has a worldwide distribution with important foci of infection in Central and South America, southern Europe, North and East Africa, the Middle East, and the Indian subcontinent. Currently the main foci of visceral leishmaniasis (VL) are in Sudan and India and those of cutaneous leishmaniasis (CL) are in Afghanistan, Syria, and Brazil. In addition to the major clinical forms of the disease, VL and CL, there are other cutaneous manifestations, including mucocutaneous leishmaniasis (MCL), diffuse cutaneous leishmaniasis (DCL), recidivans leishmaniasis (LR), and post-kala-azar dermal leishmaniasis (PKDL) that are often linked to host immune status [3]. Several species of *Leishmania* are involved, including *L. major*, *L. tropica* and *L. aethiopica* in the Old World, and several species of the *L. braziliensis* and *L. mexicana* in the New World [4].

In recent years, a protective immune response against intracellular *Leishmania*, has been defined as Th1, whereas protection against extracellular pathogens, such as helminths, requires a Th2 response. The murine model of experimental leishmaniasis has been instrumental for the elaboration of the Th1/Th2 paradigm; however the preferential action of Th1 or Th2 cytokines results in cure or progression of the disease, respectively [5-7]. Mice that spontaneously resolve their infections develop Th1-type responses, while non-healing Balb/c mice develop Th2-type responses [8, 9]. In addition, activated macrophages (MQ) participate in the inflammatory response by releasing chemokines and factors that recruit additional cells to site of infection. In addition, MQ activate the expression of genes responsible for the high-output synthesis of intermediates, which contribute to the regulation of the inflammatory response [10].

Trace metals, including Zn and Cu, are directly involved in metabolic processes critical to cell differentiation and replication. Zn is an essential trace element with cofactor functions in a large number of proteins of intermediary metabolism, hormone secretion pathways and immune defense mechanisms [11-13]. The essentiality of Zn for humans was first documented by Prasad in the 1960s. One of the main clinical manifestations associated with Zn deficiency is increased susceptibility to infectious diseases [14, 15]. Thus, there was an increased interest of researchers in studying the role of Cu and Zn in animal and human models of immunity [16-19]. Although many immunologic functions depend on these processes, Cu and Zn are believed to be essential to functioning of immunocompetent cells residing in circulating blood, and their deficiency may result in impairment of immune responsiveness [19, 20].

Moreover, several enzymes which contribute to immune system responses require Zn and Cu for their activities [21]. The changes in the levels of trace elements are part of immunity of organism and are induced by IL-1, TNF-α and IL-6 [22-24]. These substances are immunocytokines liberated dose-dependent mode, mostly by activated macrophages, in response to several stimuli, including exercise, trauma, stress, or infection [25]. The most known changes in inflammation and infections are alterations in Fe, Zn and Cu levels in association with elevated levels of acute phase proteins, such as ceruloplasmin [26]. The process of elimination of *Leishmania*, requires a Th1 type immune response, and Zn deficiency decreases Th1 but not Th2 immune response [27, 28].

The human liver contains thousands of enzymes, which help necessary chemical reactions to take place. Enzymes trigger activity in the body's cells, speeding up and facilitating naturally occurring biochemical reactions, and maintaining various metabolic processes within the liver [29].

Serum Glutamic Pyruvic Transaminase (SGPT) is an enzyme that helps metabolize protein, Serum Glutamic Oxaloacetic Transaminase (SGOT) plays a role in the metabolism of the amino acid alanine and Alkaline Phosphatase (ALP) is an enzyme needed in small amounts to trigger specific chemical reactions [29] and to diagnose liver function and its integrity [30-32].

Paromomycin sulfate is an aminoglycoside antibiotic, first isolated from *Streptomyces krestomuceticus* in 1950s with the chemical formula of $C_{23}H_{45}N_5O_{14} \times H_2SO_4$. Paromomycin is also effective against some protozoa and cestodes and it is the only aminoglycoside with clinically important anti-leishmanial activity [33, 34]. Injectable Paromomycin was extensively used as an antibiotic until 1980s, before cephalosporins and quinolones became popular antibiotics. Paromomycin is poorly absorbed after oral application, however it is still marketed as treatment for Amoebiasis and Giardiasis. Paromomycin in methylbenzethonium chloride ointment is used as a topical treatment for *L. major* CL in Israel [34] and its anti-leishmanial properties were well recognized [35-41]. Paromomycin, with its excellent efficacy, low cost, shorter duration of administration and good safety profile, has the potential to be used as a first-line drug [42].

To our knowledge so far, the injectable form of Paromomycin has not been applied for the treatment of CL yet. In the current study, for the first time, the anti-leishmanial effects of Paromomycin were clarified in Balb/c mice infected with *L. major*. This study aimed to investigate liver enzymes; Zn and Cu levels alter in murine model of CL, and whether these variations might be involved in the immune responses against the parasite. This study will complete the previous studies on the topical treatment of leishmaniasis with Paromomycin ointment and lotion, which is able to find an appropriate solution for control and therapy.

Methods

Animals

Female inbred Balb/c mice (supplied by the Karaj Laboratory Animal Unit, Pasteur Institute of Iran) were used in this study. The initial body weight was 19.2 ± 1.3 g (mean ± standard error of mean, SEM) and mice were housed at room temperature (20–23 °C) on a 12-h light and 12-h dark cycle, with unlimited access to food and tap water. Experiments with animals were done according to the ethical standards formulated in the Declaration of Helsinki, and measures taken to protect animals from pain or discomfort. It has been approved by Ethical Committee of the Pasteur Institute of Iran, in which the work was done.

In vitro cultivation of *L. major*

The *L. major* MRHO/IR/75/ER used in this study was the standard strain isolated from Iran. The infectivity of the parasite was maintained by regular passage in susceptible Balb/c mice and parasite were cultured in the RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 292 mg/ml L-glutamine and 4.5 mg/ml glucose (all supplied by Sigma). Under these conditions, the stationary phase of parasite growth was obtained in 10 days [43, 44].

Infection of Balb/c mice with *L. major*

Promastigotes of *L. major* were harvested from culture media, counted and used to infect Balb/c mice. The base of the tail was injected intradermally with inoculums of 2×10^6 promastigotes.

Experiments and groups

The experiments were performed once in six groups considering time, budget and long-period monitoring of animals according to the ethical issues for sample size and replication in order to protect animals from further pain or discomfort.

.

It has been approved by institutional ethical review board in which the work was done. Balb/c mice were divided into six test and control groups (n =8 mice/group) including Group 1(Control: Uninfected mice + D.W.), Group 2 (Test: Uninfected mice + 0.4 mg/kg Paromomycin), Group 3 (Test: Uninfected mice + 0.6 mg/kg Paromomycin), Group 4 (Control: *L. major* + D.W.), Group 5 (Test: *L. major* + 0.4 mg/kg Paromomycin) and Group 6 (Test: *L. major* + 0.6 mg/kg Paromomycin).

Drug: Paromomycin and drug vehicles

Paromomycin (Sigma) was developed as a therapeutic against leishmaniasis. 300 mg Paromomycin was dissolved in 30 ml D.W. and injected for 3 weeks after infection in experimental groups. Control groups were injected only with D.W. and test groups were injected with 0.4 and 0.6 mg/kg of Paromomycin.

Preparation of Serum

Serum was prepared from blood taken by cardiac puncture, from mice terminally anaesthetized. Serum was prepared by microfuging blood at 6000 g for 5 min, and supernatants were transferred to an eppendorf and stored at -70ºC [45].

Measurements of blood trace metals and liver enzymes

Serum was removed by centrifugation and stored in metal-free tubes at -70° C. Zn and Cu were determined by direct aspiration of 1:10 dilution of serum in deionized water into the Flame Atomic Absorption Spectrophotometer (FAAS, Thermo Jarrel Ash, Germany) [46]. Serum SGOT, SGPT and ALP were determined by Auto Analyzer Technical RA1000 [30].

Statistical analysis

Values for SGOT, SGPT, ALP, Cu and Zn are presented as the mean ± SEM for groups of *n* samples. The significance of differences was determined by Analysis of Variances (ANOVA) using Graph Pad Prism Software (Graph Pad, San Diego, California, USA).

Results

Statistically significant differences in serum Cu were observed among groups. Serum Cu decreased in test group compared to control group in uninfected Balb/c mice [1.29 ± 0.024 µg/mL (Group 1, Control); 1.22 ± 0.027 µg/mL (Group 3, Test), with $P<0.05$] and also serum Cu decreased in test groups compared to control group in infected Balb/c mice [1.33 ± 0.016 µg/mL (Group 4, Control); 1.23 ± 0.02 µg/mL (Group 5, Test) and 1.25 ± 0.021 µg/mL (Group 6, Test), with $P<0.01$] (Figure 1).

Serum Zn increased in test groups as compared with control group in uninfected Balb/c mice groups [1.25 ± 0.068 µg/mL (Group 1, Control); 1.27 ± 0.056 µg/mL (Group 2, Test) and 1.43 ± 0.01 µg/mL (Group 3, Test), not significant differences (NSD)] and also serum Zn increase in test groups observed as compared with control group in infected Balb/c mice groups [1.20 ± 0.041 µg/mL (Group 4, Control); 1.24 ± 0.026 µg/mL (Group 5, Test) and 1.29 ± 0.020 µg/mL (Group 6, Test), NSD] (Figure 2).

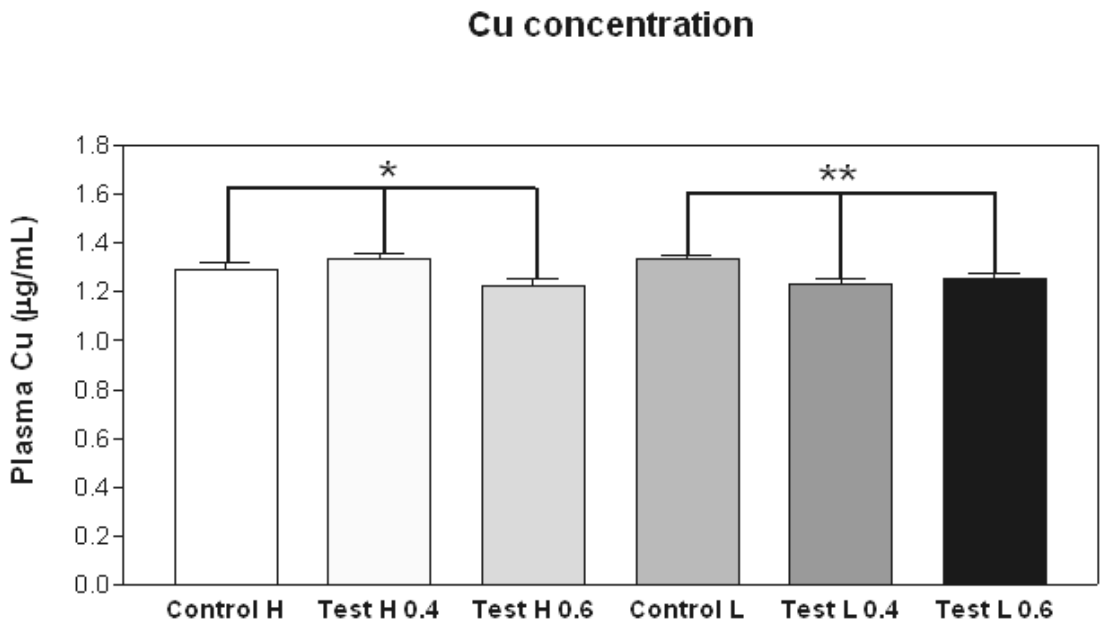

Figure 1: Serum Cu levels in control and test groups
Serum Cu was determined by FAAS in control and *L major* infected Balb/c mice with and without treatment with Paromomycin. Significance of differences (**P*<0.05, ***P*<0.01) was determined by One-Way ANOVA test using Graph Pad Prism Software (*n*=8 mice/group). [H, Healthy mice; L, Leishmania infected mice; 0.4, 0.4mg/kg Paromomycin; 0.6, 0.6mg/kg Paromomycin].

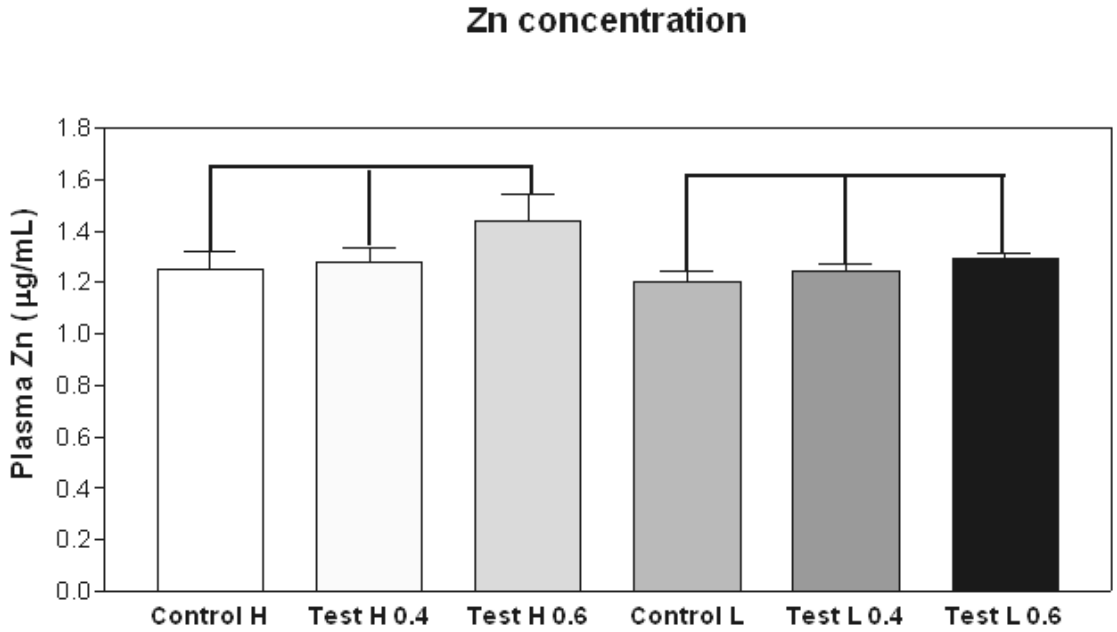

Figure 2: Serum Zn levels in control and test groups
Serum Zn was determined by FAAS in control and *L major* infected Balb/c mice with and without treatment with Paromomycin. No significance of differences was determined by One-Way ANOVA test using Graph Pad Prism Software (*n*=8 mice/group).

The ratio of Cu / Zn was decreased, but this decline was NSD in test and control groups. The ratio of Cu / Zn decreased in test group compared to control group in uninfected Balb/c mice group [1.06 ± 0.118 (Group 1, Control) and 0.86 ± 0.127 (Group 3, Test)] ;and also the ratio of Cu / Zn decreased in test groups compared to control group in infected Balb/c mice [1.15 ± 0.065 (Group 4, Control); 1 ± 0.039 (Group 5, Test) and 1.01 ± 0.026 (Group 6, Test), NSD] (Figure 3).

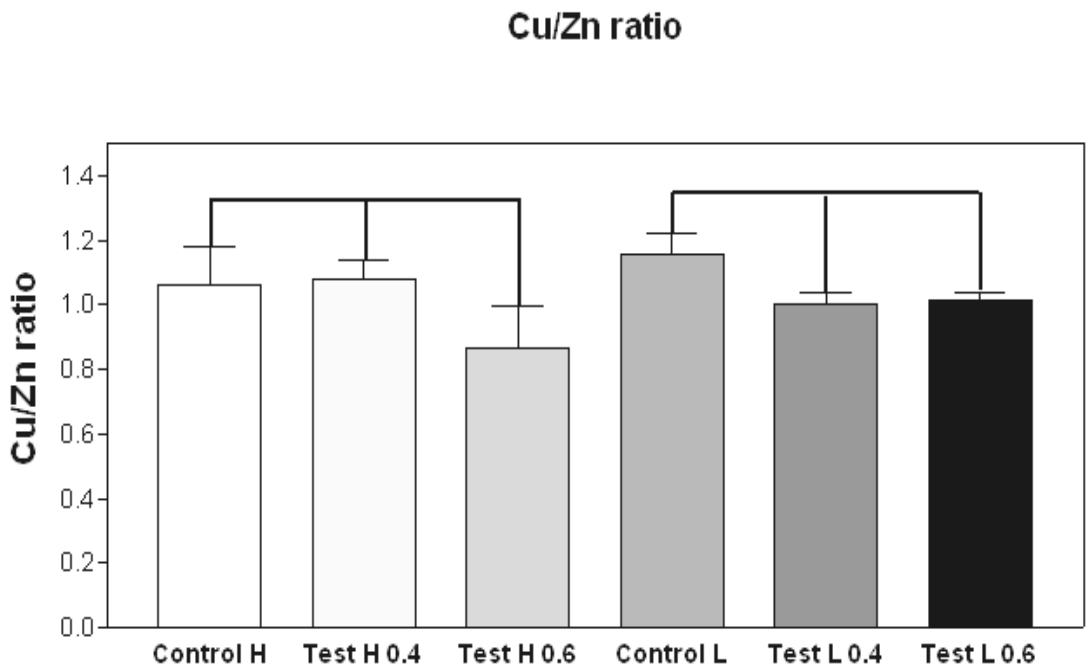

Figure 3: Serum Cu / Zn ratios in control and test groups
Cu / Zn ratios were calculated by subtracting Cu to Zn values. No significance of differences was determined by One-Way ANOVA test using Graph Pad Prism Software (n=8 mice/group).

Statistically significant differences in serum SGOT and SGPT were observed among groups. SGOT decreased in test groups compared to control group in uninfected Balb/c mice [233.60 ± 17.58 U/L (Group 1, Control); 210 ± 33.50 U/L (Group 2, Test) and 141.16 ± 14.41 U/L (Group 3, Test), with P<0.05] and also SGOT decreased in test groups compared to control group in infected Balb/c mice [243.66 ± 19.7 U/L (Group 4, Control); 179.16 ± 15.28 U/L (Group 5, Test) and 155.53 ± 19.42 U/L (Group 6, Test), with P<0.05] (Figure 4).

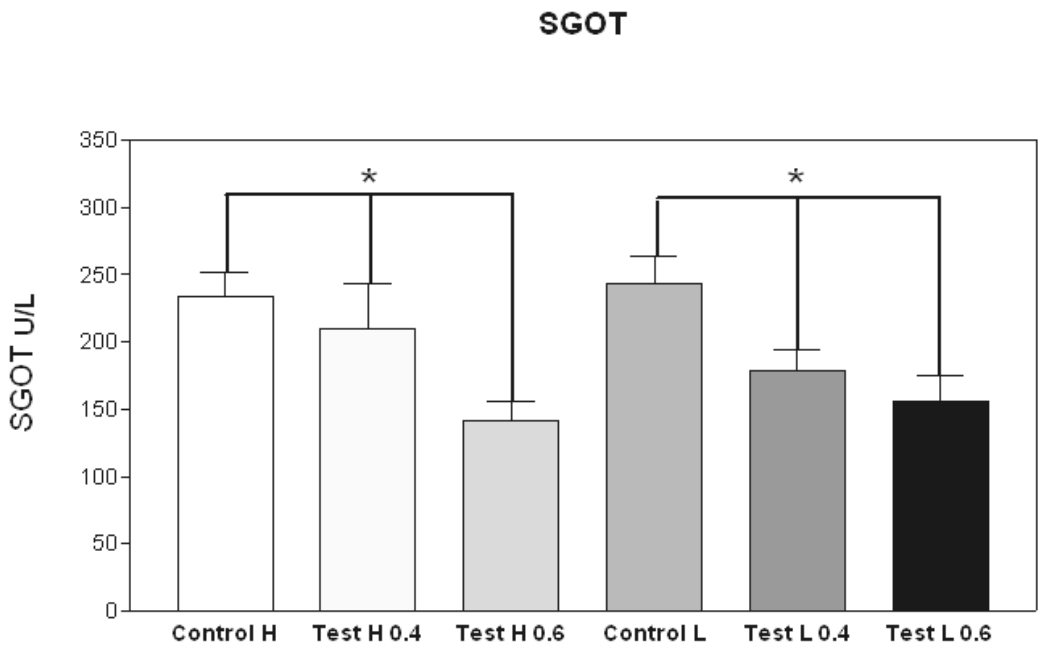

Figure 4: SGOT concentration in control and test groups
SGOT was determined by Auto Analyzer Technical RA1000 in control and *L major* infected Balb/c mice with and without treatment with Paromomycin. Significance of differences (**P*<0.05) was determined by One-Way ANOVA test using Graph Pad Prism Software (*n*=8 mice/group).

SGPT decreased in test groups compared to control group in uninfected Balb/c mice [90.40 ± 5.71 U/L (Group 1, Control); 82.83 ± 3.89 U/L (Group 2, Test) and 53.16 ± 7.51 U/L (Group 3, Test), with *P*<0.01] and also serum SGPT decreased in test groups compared to control group in infected Balb/c mice [81.5 ± 3.73 U/L (Group 4, Control); 66.5 ± 8.35 U/L (Group 5, Test) and 49.66 ± 6.48 U/L (Group 6, Test), with *P*<0.05] (Figure 5).

Serum ALP increase in test group observed as compared with control group in uninfected mice [411.4 ± 17.74 U/L (Group 1, Control) and 432.16 ± 35.79 U/L (Group 3, Test), NSD]. Serum ALP increased in test groups observed as compared with control group in infected mice [208.83 ± 19.77 U/L (Group 4, Control); 313 ± 26.94 U/L (Group 5, Test) and 369.83 ± 40.36 U/L (Group 6, Test), with *P*<0.01] (Figure 6).

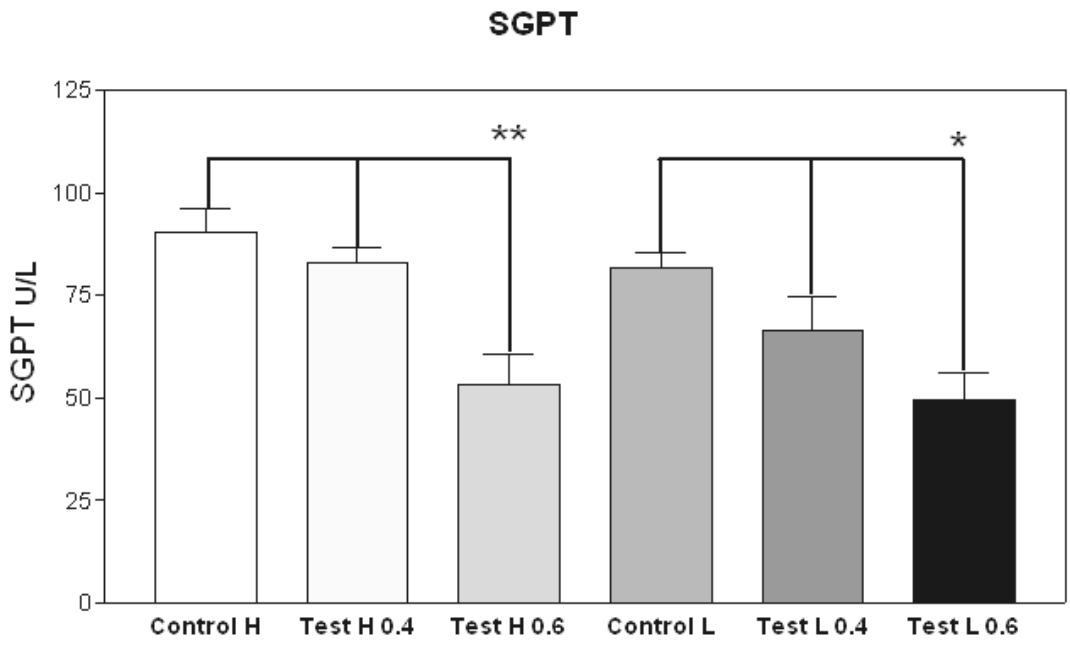

Figure 5: SGPT concentration in control and test groups
SGPT was determined by Auto Analyzer Technical RA1000 in control and *L major* infected Balb/c mice with and without treatment with Paromomycin. Significance of differences (*P<0.05, **P<0.01) was determined by One-Way ANOVA test using Graph Pad Prism Software (n=8 mice/group).

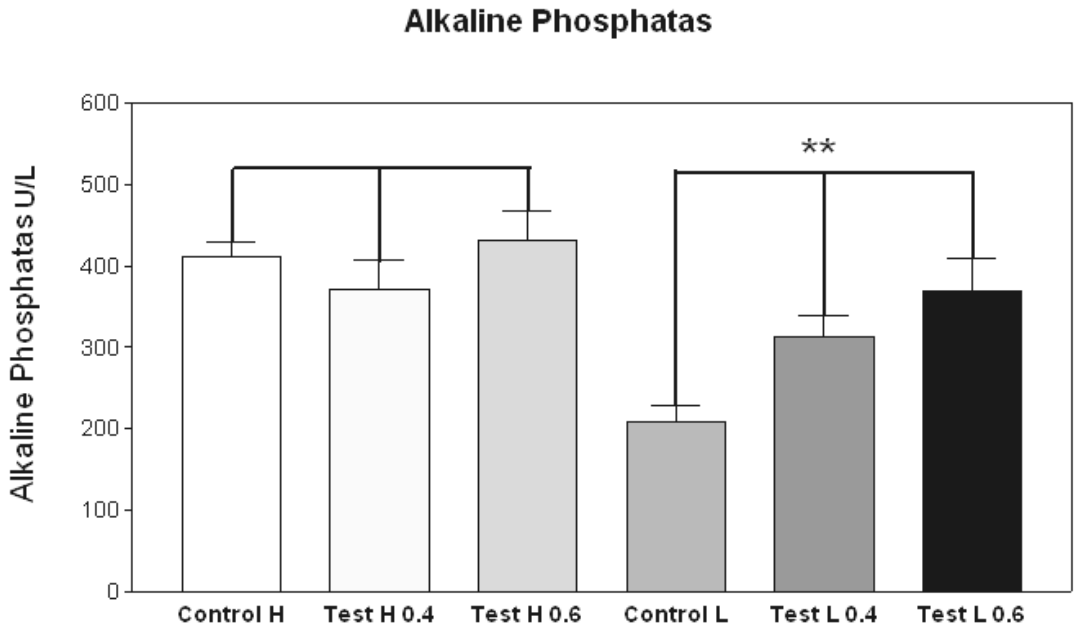

Figure 6: ALP concentration in control and test groups
ALP was determined by Auto Analyzer Technical RA1000 in control and *L major* infected Balb/c mice with and without treatment with Paromomycin. Significance of differences (**P<0.01) was determined by One-Way ANOVA test using Graph Pad Prism Software (n=8 mice/group).

.

Discusssion

Micro- elements and liver enzymes play a major role in leishmania disease and their increasing rates could affect disease progress and its pathologies. It is concluded that the concentrations of essential trace elements including Zn and Cu were probably altered by the *leishmania* parasite during CL infection, which should be considered as therapy or vaccine strategies for CL. In addition, due to the importance of Zn and possibly deleterious effect of Cu in protective Th1 response, it is proposed that trace Zn and Cu levels should be taken into account for CL treatment and vaccine strategies [47].

Zn was reported to increase the immune response [48] and association between Zn concentration and disease status may result to Zn redistribution in response to infection [49]. High levels of Zn and low values of Cu might be correlated to anti-parasitic pattern of host immunity [27]. Results of this study revealed an increase in Zn and a decrease in Cu levels in test groups under treatment with Paromomycin, which might be a cause, rather than a consequence of CL in this model. This is in agreement with previous publication on leishmaniasis as a non-protective Th2 / humoral immune response [50]. High Zn and low Cu levels in serum of infected host under treatment with Paromomycin might be responsible for the ability of the host to clear the parasite. This could be associated with disease inflammation and production of various cytokines during treatment with Paromomycin. In addition, Cu levels in this study were observed to be higher in infected control group without treatment than test group, which is reported to be attributable to the disease induction [26]. On the other hand, characteristic changes in metabolism of trace elements are integral parts of the acute-phase response. The alternations of Zn and Cu may probably depend on cytokines specially IL-1 and TNF-α. It is concluded that serum trace elements were probably altered by the some immunocytokines as a host defense strategy during CL infection [51, 22].

In this study the ratio of Cu/Zn was also lower in test groups than control groups. Cu/Zn ratio can be a useful marker for immune dysfunction and its implication in both humoral and cellular anti-leishmania immune response, which should inspire future strategies for therapy and immunoprophylaxis of human leishmaniasis [27].

The rise of ALP and the fall of SGOT and SGPT levels as key liver enzymes, might be correlated to treatment with Paromomycin, indicating an anti-parasitic immune response. Liver plays a key role in the metabolic conversion and elimination of many enzymes [52], therefore SGOT, SGPT and ALP, could be used to determine whether the liver is functioning normally or if it has an injury or disease [53].

In this research for the first time, the injectable form of Paromomycin rather than orally inoculation was used, resulting an inhibitory effect of Paromomycin against parasite replication during treatment of CL in *L. major* infected Balb/c mice. Paromomycin clarified its anti-leishmanial effects with presenting the least toxicity on host phsiopathological markers including body weight, survival rate, hepato / splenomegaly, nephrotoxicity and blood leukocyte / reticolocyte ratio. Considering biochemical, parasitological, pharmacological and physiopathological points of view, it is suggested that Paromomycin can be used as an alternative therapy for classic leishmania treatment, however supplementary studies are required.

Acknowledgments and Declaration:

This study was supported financially by Pasteur Institute of Iran. We are grateful the heads of Departments of Parasitology and Biochemistry, Pasteur Institute of Iran, and Payame Nour University, Tehran Center for the kind collaboration during this study. There is no conflict of interest of any of the authors with the results of this study.

.

References

1. Lukes J, Mauricio IL, Schonian G. Evolutionary and geographical history of the Leishmania donovani complex with a revision of current taxonomy. Nat Acad Sci USA, 2007;22:9375–80.

2. Dey A and Singh S. Transfusion Transmitted Leishmaniasis: a case report and review of literature. Ind J Med Microbiol, 2006;24:165-70.

3. Croft SL, Sundar Sh, Fairlamb A. Drug resistance in leishmaniasis. Clin Microbiol Rev, 2006;1:111–26.

4. El-On J, Bazasky E, Sneir R. Leishmania major: In vitro and In vivo anti-leishmanial activity of paromomycin ointment (Leshcutan) combined with the immunomodulator Imiquimod. Exp Parasitol, 2007;116:156-62.

5. Coffman RL, Chatelain R, Leal LM, Varkila K. Leishmania major infection in mice: a model system for the study of CD4+ Tcell subset differentiation. Res Immunol, 1991;142:36-40.

6. Reiner SL, Locksley RM. The regulation of immunity to Leishmania major. Ann Rev Immunol, 1995;13:151-77.

7. Convit J, Ulrich M, Fernandez CT ,Tapia, FJ, Cáceres-Dittmar G, Castés M, Rondón AJ. The clinical and immunological spectrum of American cutaneous leishmaniasis. Trans Royal Soc Trop Med Hyg, 1993;87:444–8.

8. Holzmuller Ph, Sereno D, Cavaleyra M, Mangot I, Daulouede S,Vincendeau Ph, Lemesre JL.Nitric oxide-mediated proteasome-dependent oligonucleosomal DNA fragmentation in Leishmania amazonensis amastigotes. Infect Immunit, 2002;70:3727-35.

9. Louis J, Gumy A, Voigt H, Rocken M, Launois P. Experimental cutaneous leishmaniasis: a powerful model to study in vivo the mechanisms underlying genetic differences in Th subset differentiation. Europ J Dermatol, 2002;12:316–8.

10. Bosca L, Zeini M, Traves PG, Hortelano S. Nitric oxide and cell viability in inflammatory cells: a role for NO in macrophage function and fate. Toxicol, 2005;208:249-58.

11. Hambidge KM, Krebs NF. Zinc deficiency: a special challenge. J Nutriton, 2007;137:1101–5.

12. Vallee BL, Auld DS. Zinc coordination, function, and structure of zinc enzymes and other proteins. Biochemistry, 1990;29:5647–59.

13. Kindermann B, Doring F, Pfaffl M, Daniel H. Identification of genes responsive to intracellular zinc depletion in the human colon adenocarcinoma cell line HT-29. J Nutrition, 2004;134:57–62.

14. Prasad AS. Zinc deficiency. British Med J, 2003;326:409–10.

15. Rink L, Gabriel P. Zinc and the immune system. Proc Nutrition Soc, 2000;59:541-52.

16. Salgueiro MJ, Zubillaga M, Lysionek A, Cremaschi G, Goldman CG, Caro R, De Paoli T, Hager A, Weill R, Boccio J. Zinc status and immune system relationship: a review. Biolog Trace Elem Res, 2000;76:193-205.

17. Svenson KLG, Hallgren R, Johansson E, Lindh U. Reduced zinc in peripheral blood cells from patients with inflammatory connective tissue disease. Inflamm, 1985;9:189-99.

18. Scuderi P. Differential effects of copper and zinc on human peripheral blood cytokine secretion. Cell Immunol, 1990;126:391-405.

19. Bonomini M, Palmieri P, Evangelista M, Manfrini V, Albertazzi A. Zinc–mediated lymphocyte energy charge modification in dialysis patients. Am Soc Artific Intern Organs, 1991;37:387-99.

20. Bonomini M, Manfrini V, Capelli P, Albertazzi A. Zinc and cell-mediated immunity in chronic uremia. Nephron, 1993;65:1-4.

21. Panemangalore M, Bebe FN. Effect of high dietary zinc of plasma ceruloplasmin and erythrocyte superoxide dismutase activities in copper-depleted and repleted rats. Biolog Trace Elem Res, 1996;55:111-26.

22. Faryadi M, Mohebali M. Alterations of serum zinc, copper and Iron concentrations in patients with acute and chronic cutaneous leishmaniasis. Irn J Pub Health, 2003;32:53-8.

23. Barber EF, Cousins RJ. Interleukin-1-stimulated induction of ceruloplasmin synthesis in normal and copper-deficient rats. J Nutrition, 1988;118:375-81

24. Klassing KC. Nutritional aspects of leukocytic cytokines. J Nutrition, 1988;118:1435-43.

25. Mastousek AJ, Burguera JL, Burguera M, Anez N. Changes in total content of iron, copper and zinc in serum, heart, liver, spleen and skeletal muscle tissues of rats infected with Trypanosoma cruzi. Biolog Trace Elem Res, 1993;37:51-69.

26. Kolak M, Bingol NK, Ayhan O, Avci S, Bulut V. Serum copper, zinc and selenium levels in rheumatoid arthritis. Romatizma, 2001;2:66-71.

27. Weyenbergh JV, Santana G, D'Oliveira A, Santos AF, Costa CH, Carvalho EM, Barral A, Barral-Netto M. Zinc/copper imbalance reflects immune dysfunction in human leishmaniasis: an ex vivo and in vitro study. BMC Infec Dis, 2004;4:50-7.

28. Cunningham-Rundles S, Bockman RS, Lin A, Giardina PV, Hilgartner MW, Caldwell-Brown D, Carter DM. Physiological and pharmacological effects of zinc on immune response. Ann New York Acad Sci, 1990;587:113-22.

29. http://www.puristat.com/livercleansing/liverenzymes.aspx, 2007.

30. Green RM, Flamm S. AGA technical review on the evaluation of liver chemistry tests. Gastroenterol, 2002;123:1367-84.

31. Berk PD, Korenblat KM. Approach to the patient with jaundice or abnormal liver test results. Cecil Medicine. 23rd ed. Philadelphia, Pa: Saunders Elsevier, 2009;150.

32. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol, 1957;28:56-9.

33. Neal RA, Murphy AG, Olliaro P, Croft S. Aminosidine ointments for the treatment of experimental cutaneous leishmaniasis. Trans Royal Soc Trop Med Hyg, 1994;88:223–5.

34. Davidson RN, den Boer M, Ritmeijer K. Paromomycin. Trans Royal Soc Trop Med Hyg, 2008;1010:16-24.

35. Kellina OI. A study of experimental cutaneous leishmaniasis in white mice [in Russian]. Meditsinskaia Parazitologiia i Parazitarnye Bolezni, 1961;30:684-91.

36. Neal RA, Allen S, McCoy N, Olliaro P, Croft SL. The sensitivity of Leishmania species to aminosidine. J Antimicrob Chemoth, 1995;35:577-84.

37. Neal RA. The effect of antibiotics of the neomycin group on experimental cutaneous leishmaniasis. Ann Trop Med Parasitol, 1968;62:54-62.

38. Kanyok TP, Reddy MV, Chinnaswamy J, Danziger LH, Gangadharam PR. In vivo activity of Paromomycin against susceptible and multidrug-resistant Mycobacterium tuberculosis and M. avium complex strains. Antimicrob Agents Chemoth, 1994;38:170-3.

39. Vicens Q, Westhof E. Crystal structure of Paromomycin docked into the eubacterial ribosomal decoding A site. Structure, 2001;9:647-58.

40. Asilian A, Davami M. Comparison between the efficacies of photodynamic therapy and topical paromomycin in the treatment of old world cutaneous Leishmaniasis: a placebo-controlled, randomized clinical trial. Clin Exp Dermatol, 2006;31:634-7.

41. Mussi SV, Fernandes AP, Ferreira LA. Comparative study of the efficacy of formulations containing fluconazole or paromomycin for topical treatment of infections by Leishmania major and Leishmania amazonesis. Parasitol Res, 2007;100:1221-6.

42. Sundar S, Chakravarty J. Paromomycin in the treatment of leishmaniasis. Expert Opin Invest Drugs, 2008;17:787-94.

43. Farahmand M, Nahrevanian H, Assmar M, Mohebali M, Zarei Z. Expression of A2 proteins in amastigotes of Leishmania infantum produced from canine isolates collected in the district of Meshkinshahr, in north–western Iran. Ann Trop Med Parasitol, 2008;102:81-4.

44. Nahrevanian H, Farahmand M, Aghighi Z, Assmar A, Amirkhani A. Pharmacological evaluation of anti-leishmanial activity by *in vivo* nitric oxide modulation in Balb/c mice infected with Leishmania major MRHO/IR/75/ER: An Iranian strain of cutaneous leishmaniasis. Exp Parasitol, 2007;116:191-8.

45. Nahrevanian H, Dascombe MJ. Nitric oxide and reactive nitrogen intermediates in lethal and nonlethal strains of murine malaria. Parasit Immunol, 2001;23:491-501.

.

46. Nourmohammadi I, Nazem N, Ehsani-Zenuz A, Moaveni A. Serum levels of Zn, Cu, Cr and Ni in Iranian subjects with atherosclerosis. Arch Iranian Med, 2001;4:21-4.

47. Beck FW, Prasad AS, Kaplan J, Fitzgerald T, Brewer GJ. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. Am J Physiol, 1997;272:1002-7.

48. Wallwork JC, Botnen JH, Sandstead HH. Influence of dietary zinc on rat brain catecholamines. J Nutrition, 1982;112:514-9.

49. Bennett PM, Jepson PD, Law R J, Jones BR, Kuiken T, Baker JR, Rogan E, Kirkwood JK.. Exposure to heavy metals and infectious disease mortality in harbour porpoises from England and Wales. Environm Pollution, 2001;112:33-40.

50. Ribeiro-de-Jesus A, Almeida RP, Lessa H, Bacellar O, Carvalho EM. Cytokine profile and pathology in human leishmaniasis. Braz J Med Biol Res, 1998;31:143-8.

51. Kocyigit A, Erel Ö, Seyrek A, Gürel MS, Aktepe N, Avcý S, Vural H. Effects of antimonial therapy on serum zinc, copper and iron concentrations in patients with cutaneous leishmaniasis. East J Med, 1998;3:58-61.

52. Jonker JW, Stedman CA, Liddle C, Downes M. Hepatobiliary ABC transporters: physiology, regulation and implications for disease. Front Biosci, 2009;14:4904-20.

53. Jennifer Heisler. Liver enzyme and liver function tests and results. Med Rev Board, 2009;4:1-8.

Coauthor contributions

S. Davachi involved in all experiments. H. Nahrevanian designed and conducted parasitological and pharmacological parts of project. E. Omidinia and R. Hajihosseini designed and developed biochemical assays. M. Amini and M. Farahmand carried out parasitological methods on *Leishmania major* in vitro and in vivo. F. Mirkhani and S. Javadian performed and quantified trace elements by Flame Atomic Absorption Spectrophotometer and liver enzymes by Auto Analyzer Technical RA1000.