Prophylactic interferon-γ and interleukin-17 facilitate parasite clearance in experimental visceral leishmaniasis

Prabin Kumar1,2, Pragya Misra1, Narendra Kumar Yadav3, Sumit Joshi3, Amogh A. Sahasrabuddhe3, Anuradha Dube3, Narayan Rishi2, Dipendra Kumar Mitra1

1Department of Transplant Immunology and Immunogenetics, All India Institute of Medical Sciences, New Delhi, 2Amity Institute of Virology and Immunology, Amity University, Noida, 3Division of Parasitology and Molecular and Structural Biology, CSIR-CDRI, Jankipuram Extension, Lucknow, Uttar Pradesh, India

INTRODUCTION

Visceral leishmaniasis (VL) caused by protozoan parasite: Leishmania donovani is a disease of the reticuloendothelial system. If left untreated, in both humans and mice, the infection can spread from the skin to lymph nodes, liver, spleen, and bone marrow (BM), further leading to hepatomegaly, splenomegaly, and ultimately leads to death. Effective immunity against parasite in VL is characterized by the emergence of a strong, parasite-specific Th1 response. Interferon (IFN)-γ-inducible nitric oxide synthase (iNOS) and interleukin (IL)-12-STAT-4-mediated immunological responses play a crucial role in the resistance against Leishmania infection. However, the prophylactic role of IL-17 in visceral leishmaniasis has not been validated.

Background and Objective: The synergy of interleukin (IL)-17 along with other pro-inflammatory cytokines is well known in various autoimmune and infectious diseases. A longitudinal study in the Sudanese population showed an association of IL-17 with the protection of kala-azar outbreak. The protective role of IL-17 is also known in terms of expansion of IL-17-producing cells in vaccine-induced immunity. However, the prophylactic role of IL-17 in visceral leishmaniasis has still not been validated. In the present study, we evaluated the prophylactic efficacy of IL-17A and interferon (IFN)-γ in Leishmania donovani-challenged Balb/c mice.

Materials and Methods: Two doses of recombinant IL (rIL)-17A and/or IFN-γ were administered intraperitoneally after/at 1 week interval and then the mice were challenged with amastigote form of L. donovani. At 45 days of postchallenge, mice were sacrificed and evaluated for change in the body and organ weight, parasitic load in visceral organs, and fold change in gene expression of cytokines.

Results: We observed that the prophylactic use of rIL-17A and IFN-γ alone or in combination significantly inhibited the parasitic load in visceral organs. Furthermore, pro-inflammatory cytokine gene expression increased up to 2–4-folds in mice treated with recombinant cytokines.

Conclusion: Our results suggest that prophylactic use of recombinant IFN-γ and IL-17A inhibits parasitic growth in visceral organs of L. donovani-challenged experimental mice model, especially through upregulation of pro-inflammatory cytokines’ gene expression.

Keywords: Balb/c mice, interleukin-17, prophylactic, visceral leishmaniasis
pathways are important for effective parasite clearance on activation and induction of microbicidal functions of macrophage in VL.[9]

Recent studies have changed the Th1/Th2 cell dichotomy to Th1/Th2/Th3 after induction of Th17 (Th3) cell, a new T-cell subset in effector immunity.[9] The protective role of Th17 against extracellular bacterial and fungal infection is well established.[9] A recent study also demonstrated that Th17-IL23 pathway may play a major role in the induction of protective immunity against intracellular pathogens by regulating both innate and adaptive immune responses.[10] Both detrimental and protective role of IL-17 is known in different forms of clinical leishmaniasis. In cutaneous leishmaniasis, IL-17 is known for progression of disease in susceptible mice, wherein it induces neutrophil recruitment at disease site that persuades in disease progression. In VL also both protective and detrimental role of IL-17 is documented.[3,7,8] IL-17A (-/-) mice have been shown to have high resistance to VL infection.[9] Conversely, a human-based study in Sudanese population showed an increased level of IL-17 and IL-22 for protection against reexposure to VL.[9] Few recent studies have also reported the role of IL-17A as an important mediator of resistance against *Leishmania* infection, demonstrating that it acts synergistically with IFN-γ to promote parasite killing.[10] We have also observed a strong inverse correlation between the levels of IL-17A and IFN-γ with parasitic load at the local site (BM) in VL patients.[11] Hence, the overall findings pave the way to ponder the involvement of IL-17 in vaccine-induced protective immunity. Since there is no vaccine against any clinical forms of leishmaniasis, recombinant IL (rIL)-17 could be used along with vaccine candidate(s) to boost the protective immunity.[12] Moreover, a limited number of drugs used for the treatment of leishmaniasis are associated with high toxicity and unresponsiveness. Thus, to overcome these limitations and open a new avenue for protection and treatment from leishmaniasis, we have used recombinant IFN (rIFN)-γ and IL-17A cytokines for both prophylactic and adjunct therapy (along with suboptimal dose of amphotericin-B) in *Leishmania* mice model. Although the use of recombinant cytokine(s) as a prophylactic agent may not be a better alternative for protection from leishmaniasis, we performed in mice model to validate our earlier human-based findings. In the present study, we have used rIL-17A and IFN-γ alone or in combination as a prophylactic agent in a mice challenged model. The present study thus highlights the application of these recombinant cytokines as a prophylactic agent in *Leishmania* mice model. We show here that application of rIFN-γ and IL-17 resulted in parasitic clearance accompanied with increased Th1 types of cytokines gene expression, thus advocating that the use of recombinant cytokine(s) as a prophylactic agent may be beneficial for controlling of VL. Furthermore, this approach may also help in boosting the vaccine-induced immunity.

**MATERIALS AND METHODS**

**Animal and parasites**

Laboratory-bred Balb/c mice from the Animal House Facility of CDRI were used as the experimental host. The usage of mice was approved by the Institute’s Animal Ethical Committee (protocol number IAEC/2016/T-7[12/16] dated November 04, 2016). The mice were used as per the National Institutes of Health guidelines. Clinical strains of *L. donovani* were obtained from the patient who was admitted at Balaji Utthan Sansthan, Patna, and cultured in our laboratory under the standard *in vitro* conditions as described elsewhere.[13] This strain was also established and maintained in mice model through the serial passage, that is, from amastigote to amastigote.

**Prophylactic cytokine administration**

Five groups containing six mice each were used for the study. These were treated prophylactically with recombinant cytokines (IL-17A and IFN-γ) alone or in combination [Table 1]. The mice in Group I (M1) were given phosphate-buffered saline (PBS) only (negative control) and the Group II (M2) mice were infected with amastigote parasite (positive control). Intraperitoneally, 10 µg/0.01 ml/animal of recombinant mouse cytokine(s) was administered in the study groups of mice: rIL-17A ([Group III; M3] [R & D System, Cat. No. 421-ML-025]), rIFN-γ ([Group IV; M4] [R & D System, Cat. No. 485-MI-100]), and both rIL-17A as well as rIFN-γ (Group V; M5). A booster dose of the same amount of recombinant cytokines was given intraperitoneally to all the mice of respective experimental groups (i.e., Groups M3–M5) at an interval of 7 days.

**Infection**

Except naïve group (M1), all the groups of mice were challenged intravenously with 10⁷ amastigotes of *L. donovani* after 7 days

**Table 1: Experimental conditions of mice**

| Group of mice | Conditions |
|---------------|------------|
| M1 (negative control) | Without infection: Injected with only PBS |
| M2 (positive control) | Infected with amastigote parasite |
| M3 | Injected with recombinant IL-17 |
| M4 | Day 0: 10 µg/0.01 ml |
| Day 7: First booster dose (10 µg/0.01 ml) |
| M5 | Injected with recombinant IFN-γ |
| Day 0: 10 µg/0.01 ml |
| Day 7: First booster dose (10 µg/0.01 ml) |

IL: Interleukin, IFN: Interferon, PBS: Phosphate-buffered saline
of the booster dose of prophylactic cytokine(s). Necropsies of all the mice were done at day 45 postchallenge (P.C.). Prophylactic efficacy was assessed in terms of parasitic load in the liver, spleen, and BM. Calculations were done as the number of amastigotes/1000 cell nuclei in each organ as compared to untreated controls (M2). Percentage inhibition was assessed in comparison to the infected control by following formula:

$$\text{Number of parasite counted from infected control} - \frac{\text{Number of parasite from study groups}}{\text{Number of parasite counted from infected control}} \times 100$$

Primary culture of liver and splenic tissues
Liver and splenic extracts of different groups of mice were cultured in RPMI media to assess the parasitic load. Mice were dissected to isolate the liver and spleen. Isolated organs (liver/spleen) were washed 2–3 times with 1 × PBS-containing gentamycin antibiotic (2 mg/100 ml). Small pieces of tissue from the isolated organs were then transferred into flasks containing 10–15 ml of incomplete RPMI with gentamycin. Flasks were examined daily for up to 10–20 days for any moving promastigotes.

Immunological assay
Cytokine gene expression by real-time polymerase chain reaction
To quantitatively assess the mRNA expression of cytokines in experimental groups of mice, real-time polymerase chain reaction (PCR) was performed. Three individual mice were chosen randomly from each group and their spleen tissues were taken for RNA isolation. RNA isolation was carried out using Tri-reagent (Sigma-Aldrich: Cat No. 93289-100ML) at day 45 and quantified using GeneQuant (Bio-Rad). cDNA was synthesized from 1 μg of total isolated RNA using a first-strand cDNA synthesis kit (Fermentas: Cat No. K1622).

Real-time quantitative PCR (RT-PCR) was conducted as per the protocol described earlier. Housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase, was used for normalization in all quantifications. To avoid contamination or nonspecific reactions, a no-template cDNA was included. Cycle threshold (CT) values obtained indicated the number of PCR cycles which are required for the fluorescence signal to exceed the detection threshold value (background noise). Comparative CT method was used to calculate the differences in gene expression. The fold change in the expression of genes in recombinant cytokine-treated groups as compared to that of the infected group was determined by the formula fold change in expression $2^{ΔΔCT}$. [15,16]

Statistical analysis
Statistical significance was determined using the unpaired Student's t-test using GraphPad Prism® for Windows, Version 7. Results were expressed as mean ± standard deviation (SD) and percentage inhibition (mean value of analyzing group/mean value of infected control group) ×100.

RESULTS
Change in body weight over time in mice treated with recombinant cytokine(s) was similar to that of control
The mice in study groups (M1 to M5) were used for evaluation of change in body weight and organ weight after prophylactic treatment with recombinant cytokine(s) on day 45 P.C. We observed a significant decrease in body weight of mice which were not treated with cytokine(s) before infection (M2; mean ± SD; 20 ± 1.93 g) as compared with uninfected control group of mice (M1; mean ± SD; 31.22 ± 3.73 g). Furthermore, we observed almost similar weight gain in mice, which was treated with cytokine(s) before infection (M3; mean ± SD; 27 ± 1.22 g), (M4; mean ± SD; 27 ± 2.26 g), and (M5; mean ± SD; 28 ± 2.18 g) as compared with uninfected control [Figure 1a]. In addition, on comparing the change in body weight, we observed significant gain in body weight in cytokine(s)-treated group

![Figure 1](image_url)

**Figure 1:** In cytokines-treated group of mice change in body weight over time was similar to that of control: M1: The group of mice without any treatment uninfected. M2: Infected control group of mice. M3: Injected recombinant interleukin-17A. M4: Injected recombinant interferon-γ. M5: Both recombinant interleukin-17A and recombinant interferon-γ were injected. The recombinant cytokine(s) was given intraperitoneally. (a) Body weight in gram; (b) Liver weight in gram; and (c) weight of spleen in gram. Each bar represents the pooled data (mean ± standard deviation value) of five replicates
of mice as compared to the untreated infected group of mice (M2) [Figure 1a]. Further, we also evaluated the groups of mice for change in weight of visceral organs (liver and spleen) but did not observe any significant change in weight of liver (approximately 1.2 ± 0.02 g; in all the study groups of mice) [Figure 1b]. However, on infection, a significant gain in spleen weight was observed in mice which were not treated with cytokine (M2; mean ± SD; 0.8 ± 0.14 g) [Figure 1c] compared with uninfected control (M1; mean ± SD; 0.24 ± 0.03 g). Similar spleen weight was also observed in cytokine-treated groups of mice as that of uninfected control.

**Significantly decreased parasitic load in visceral organs of mice treated with cytokine(s)**

Parasitic load in visceral organs (liver, spleen, and BM) was evaluated to perceive the prophylactic, protective role of recombinant cytokine(s) in the study groups of mice. We observed significant percentage inhibition of parasitic load in visceral organs in recombinant cytokine-treated group of mice compared with infected control (M2) on day 45 P.C. in liver (M3 = 49.0%), (M4 = 56.6%), and (M5 = 55.6%) [Figure 2a]; spleen (M3 = 65.6%), (M4 = 69.7%), and (M5 = 71.2%) [Figure 2b]; and BM (M3 = 71.1%), (M4 = 73.7%), and (M5 = 73.3%) [Figure 2c]. Furthermore, we did not observe any parasitic growth in primary culture of liver and spleen tissues after 10 days of postculture, indicating that both the cytokines were sufficient to inhibit the parasitic growth in visceral organs when given alone or in combination.

**Th1 gene expression was upregulated in mice treated with recombinant cytokine(s)**

The mRNA expression of Th1 (iNOS, tumor necrosis factor (TNF)-α, IL-2, and IL-12) cytokines was evaluated by real-time PCR on day 45 P.C. A maximum of 3–4-fold increase in expression level of iNOS gene was observed in the group of mice which were treated with a combination of recombinant cytokines (M5), whereas other recombinant cytokine-treated groups (M3 and M4) also showed marginal increase in iNOS expression (2–3 folds) when compared with infected controls [Figure 3a]. We observed a 4–5-fold increase in TNF-α level in combined recombinant cytokine-treated group (M5); however, in other groups of mice treated with either IFN-γ or IL-17 (M3 and M4, respectively), the expression level varied between 3 and 4 folds when compared with infected controls [Figure 3b].

A 2–3-fold increase in expression of early pro-inflammatory cytokines IL-2 and IL-12 was also observed when compared with an infected group on day 45 P.C. in all the treated groups (M3, M4, and M5)[Figure 3c and d].

**Figure 2:** Significantly decreased parasitic load in visceral organs of cytokine(s)-treated group of mice: Parasitic load in visceral organs: (a) Liver, (b) spleen, and (c) bone marrow; Parasite burden (number of amastigotes/1000 cell nuclei) in the groups of mice treated with recombinant cytokines (M3, M4, and M5) at day 45 postchallenge (M2: mean of infected was used to evaluate percentage inhibition of parasitic load in visceral organs, due to lack of parasitic infection in M1: negative controls group: infection was not observed). Each bar represents the pooled data (mean and standard deviation value) of five replicates.

**Figure 3:** Th1 gene expression was upregulated in mice treated with recombinant cytokine(s): The fold change in pro-inflammatory cytokine gene expression in treated groups (M3, M4, and M5) was compared with infected control group (M2). a) Inducible nitric oxide synthase (iNOS) gene expression was upregulated 3-4. b) Tumor necrosis factor-α (TNF-α) gene expression was upregulated 4-6 folds c) Interleukin-2 (IL-2) gene expression was upregulated 2-6 folds. d) Interleukin-12 (IL-12) gene expression was upregulated 2-3 folds in treated groups compared with untreated control.
**DISCUSSION**

Leishmaniasis is known to cause an immunocompromised state of immunity with an imbalance of pro-inflammatory and anti-inflammatory cytokine milieu. Lack of effective CD4\(^+\)/CD8\(^+\) T-cell responses underlies the disease progression that ultimately limits the therapeutic efficacy of antileishmanial drugs.\(^{[17]}\) There is a persistent lack of vaccine against all clinical form of human leishmaniasis\(^{[18]}\) has resulted in only chemotherapy-based treatment. Hence, there is an urgent need for prophylactic/or therapeutic vaccine alone or in combination with immune-chemotherapy agents.

Cytokines mediate a wide variety of biological activities, including inflammation induced by an immune response as well as tissue repair and remodeling of immune system. Excessive production of one arm of cytokine(s) (Th1/Th2) or the lack of another arm contributes to the immunopathogenesis. Restoring the optimal cytokine balance may have a therapeutic value which could be achieved either by blocking of excessive cytokine(s) or by induction of recombinant cytokine(s). Recombinant cytokines are being successfully used in different diseases such as IL-2 in cancer,\(^{[19]}\) IFN-\(\alpha\) in viral infection and cancer,\(^{[20]}\) IFN-\(\beta\) in multiple sclerosis,\(^{[21]}\) IFN-\(\gamma\) in cancer and osteoporosis,\(^{[22]}\) and IL-11 in postchemotherapy-induced thrombocytopenia.\(^{[23]}\) However, till date, there is no documented proof of the clinical use of rIL-17 in any disease. Thus, we wanted to explore the possibility of prophylactic protective role of rIL-17 in VL. In this study, we observed that the group of mice treated with rIL-17A showed an almost similar gain in body weight as that of IFN-\(\gamma\)-treated group. Moreover, the marginal gain in body weight was seen when we used both cytokines for treatment, which was comparable to normal control group of mice. The gain in body weight was most likely associated with prophylactic treatment of mice with recombinant cytokine(s). Furthermore, as with earlier findings, we also observed a significant decrease in body weight in infected groups of mice (M2; without treatment), indicating that active infection possibly perturbs the normal metabolic activity that restrains in normal weight gain over time.\(^{[24]}\)

Infection, however, did not influence any change in weight of liver [Figure 1b]. This is probably due to early resolution of disease in the liver associated with granuloma formation.\(^{[2,25]}\) Furthermore, we observed that the group of mice treated with IL-17 alone showed decrease in parasitic burden by nearly 49%–71.1% (liver, spleen, and BM), which was marginally increased to 56.6%–73.7% when given along with IFN-\(\gamma\), which further improved when we used both cytokines [Figure 2]. Moreover, we did not observe parasitic growth in primary culture of tissue taken from the spleen suggesting that the recombinant cytokine alone or in combination may be sufficient to completely control parasitic growth in host visceral organs. We hypothesize that prophylactic cytokine(s) may be able to boost the parasitic clearance. Furthermore, recombinant cytokines may also be used with immune modulator for effective control of parasite. In our other study of adjunct therapy, recombinant cytokine treatment showed more than 90% inhibition of parasitic growth in visceral organs; moreover, primary culture of tissues from visceral organs again did not show any parasitic growth. In addition, the dominance of Th1 cytokines gene expression was also observed (unpublished data).

IL-12 has been shown to induce IFN-\(\gamma\) production in IL-10-deficient mice and acts in synergism to have an additional paramount effect on parasitic clearance in tissue.\(^{[26]}\) We observed that its level in treated groups of mice was raised; which is an indicative of skewing of Th2 cytokines toward Th1. The dominant effect of IFN-\(\gamma\) and TNF-\(\alpha\) on macrophage parasitical response and other effector killing mechanisms is well documented.\(^{[27,28]}\) These cytokines were observed to be moderately expressed in infected mice and showed ≈ 2–3-fold increase in all the recombinant cytokine-treated groups of mice. Interestingly, we did not observe any significant increase of IL-4, IL-10, and transforming growth factor-\(\beta\) in prophylactic and in adjunct therapy group of mice (data not shown).

Based on the above findings, we propose that IFN-\(\gamma\), as well as IL-17A, may contain the parasitic load in VL. This is possibly due to macrophage activation by IFN-\(\gamma\) which is further facilitated by IL-17A. Therefore, we suggest that prophylactic use of IFN-\(\gamma\) and IL-17A (alone or in combination) may synergize and can be used as prophylactic and adjunct therapy with low doses of conventional toxic drugs.

**CONCLUSION**

Prophylactic use of recombinant IFN-\(\gamma\) and IL-17A inhibits parasitic growth in visceral organs of L. donovani challenged experimental mice model, especially through the upregulation of pro-inflammatory cytokines’ gene expression. Thus the application of these recombinant cytokine(s) as a prophylactic agent may help in controlling/protection from VL. Moreover, this approach may also help in boosting vaccine induced immunity.

**Financial support and sponsorship**

Nil.
Kumar, et al.: Prophylactic use of recombinant cytokine(s) help in parasitic clearance in VL

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Gupta G, Oghumu S, Satoskar AR. Mechanisms of immune evasion in leishmaniasis. Adv Appl Microbiol 2013;82:155-84.
2. Locquem C, Bahuls AL, Hide M. Study of Leishmania pathogenesis in mice: Experimental considerations. Parasit Vectors 2016;9:144.
3. Terrazas C, Vanucci S, Kimble J, Moretti E, Boyaka PN, Satoskar AR, et al. IL-17A promotes susceptibility during experimental visceral leishmaniasis caused by Leishmania donovani. FASEB J 2016;30:1135-43.
4. Ravindran B. Are inflammation and immunological hyperactivity needed for filarial parasite development? Trends Parasitol 2001;17:70-3.
5. Curtis MM, Way SS. Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. Immunology 2009;126:177-85.
6. Khader SA, Gopal R. IL-17 in protective immunity to intracellular pathogens. Virulence 2010;1:423-7.
7. Banerjee A, Bhattacharya P, Joshi AB, Ismail N, Dey R, Nakhasi HL, et al. Role of pro-inflammatory cytokine IL-17 in Leishmania pathogenesis and in protective immunity by Leishmania vaccines. Cell Immunol 2016;309:37-41.
8. Quirino GF, Nascimento MS, Davoli-Ferreira M, Sacramento LA, Lima MH, Almeida RP, et al. Interleukin-27 (IL-27) mediates susceptibility to visceral leishmaniasis by suppressing the IL-17-neutrophil response. Infect Immun 2016;84:2898-99.
9. Pitta MG, Romano A, Cabantous S, Henri S, Hammad A, Kouriba B, et al. IL-17 and IL-22 are associated with protection against human kala azar caused by Leishmania donovani. J Clin Invest 2009;119:2379-87.
10. Nascimento MS, Carregaro V, Lima-Júnior DS, Costa DL, Ryffel B, Duthie MS, et al. Interleukin 17A acts synergistically with interferon γ to promote protection against Leishmania infantum infection. J Infect Dis 2015;211:1015-26.
11. Kumar P, Misra P, Thakur CP, Saurabh A, Rishi N, Mitra DK, et al. T cell suppression in the bone marrow of visceral leishmaniasis patients: Impact of parasitic load. Clin Exp Immunol 2018;191:318-27.
12. Das A, Ali N. Vaccine development against Leishmania donovani. Front Immunol 2012;3:59.
13. Rai AK, Thakur CP, Kumar P, Mitra DK. Impaired expression of CD26 compromises T-cell recruitment in human visceral leishmaniasis. Eur J Immunol 2012;42:2782-91.
14. Kushwaha PK, Gupta R, Tripathi CD, Sundar S, Dube A. Evaluation of Leishmania donovani protein disulfide isomerase as a potential immunogenic protein/vaccine candidate against visceral leishmaniasis. PLoS One 2012;7:e35670.
15. Schmitten TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008;3:1101-8.
16. Livak KJ, Schmitten TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods 2001;25:402-8.
17. Osman M, Mistry A, Keding A, Gabe R, Cook E, Forrester S, et al. A third generation vaccine for human visceral leishmaniasis and post kala azar dermal leishmaniasis: First-in-human trial of chAd63-KH. PLoS Negl Trop Dis 2017;11:e005527.
18. Gilespie PM, Baumier CM, Strych U, Hayward T, Hotze PJ, Bottazzi ME, et al. Status of vaccine research and development of vaccines for leishmaniasis. Vaccine 2016;34:2992-5.
19. Choudhry H, Helmi N, Abdulah WH, Zeyadi M, Zamzami MA, Wu W, et al. Prospects of IL-2 in cancer immunotherapy. Biomed Res Int 2018;2018:9056173.
20. Roberts NJ, Zhou S, Diaz LA Jr., Holdhoff M. Systemic use of tumor necrosis factor alpha as an anticancer agent. Oncotarget 2011;2:739-51.
21. Lis K, Kuzawitska O, Balikowiec-Iskra E. Tumor necrosis factor inhibitors – State of knowledge. Arch Med Sci 2014;10:1175-85.
22. Xu Z, Hurehla MA, Deng H, Uluçkan O, Bu F, Berdy A, et al. Interferon-gamma targets cancer cells and osteoclasts to prevent tumor-associated bone loss and bone metastases. J Biol Chem 2009;284:4658-66.
23. Xiao Y, Liu J, Huang XF, Guo JX, Fu PC, Huang XH, et al. A clinical study on juheli (Recombinant human interleukin – 11) in the second prevention of chemotherapy induced thrombocytopenia. Asian Pac J Cancer Prev 2016;17:485-9.
24. Hoffmann JL, Machado JG, Dias-Melicio LA, Langoni H. Experimental infection with Leishmania chagasi in immunosuppressed Balb/c mice: Cytokines and parasite burdens. J Vetom Anim Toxins Incl Trop Dis 2009;15:391-410.
25. Bankoti R, Stäger S. Differential regulation of the immune response in the spleen and liver of mice infected with Leishmania donovani. J Trop Med 2012;2012:639304.
26. Singh OP, Sundar S. Immunotherapy and targeted therapies in treatment of visceral leishmaniasis: Current status and future prospects. Front Immunol 2014;5:296.
27. Liu D, Uzonma JE. The early interaction of Leishmania with macrophages and dendritic cells and its influence on the host immune response. Front Cell Infect Microbiol 2012;2:83.
28. de Souza Carmo EV, Katz S, Barbieri CL. Neutrophils reduce the parasite burden in Leishmania (Leishmania) amazonensis-infected macrophages. PLoS One 2010;5:e13815.