Histopathological and biochemical alterations of kidneys in albino mice infected by Visceral leishmaniasis and the role of Aloe vera crude

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Abstract:
Leishmaniasis is a disease caused by Protozoan parasite Leishmania. It is transmitted to humans by the bite of blood sucking insects called sand flies. Visceral Leishmaniasis (VL) has widely distributed in the world including Yemen. The aim study to the histopathological and biochemical alteration of the albino mice kidneys infected with visceral leishmaniasis. 56 mice were classified into three groups: (A) 20 BALB/c mice as control, (B) 20 mice infected by ($2x10^7$) parasites for each animal through tail vein and (C) 16 mice were infected by ($2x10^7$) parasites and treated with Aloe vera extract (15mg/kg/5days). These animals were sacrificed at 20, 40, 60, 80 and100 day post infection (P.i.). The results showed mortality rate in mice reached 11.11% and recognized significant decrease in body weight from controls; Various histopathological changes including: damage of glomerulus where the it epithelia were hypertrophied and possessed pyknotik nuclei .necrotic changes in proximal and distal convoluted tubules, degeneration and infiltration of glomerular tufts by chronic inflammatory cells and red blood cells, swelling of tubular epithelium and vacuolation. Also showed a significant increase in the values of urea and Creatinine of infected mice, The giving Aloe Vera plant crude (15 mg / kg / 5 days) to mice infected with visceral Leishmania shows stimulated tissues healing and repair gradually while some of the pathological changes are stilllimited.
Key word: Visceral Leishmaniasis (VL), BALB/c mice, Aloe vera, and histopathological and biochemical alteration and Taiz and Yemen.

الملخص :

داء اللشمانيا هو مرض يسببة طفيل اولي يسمى الليشمانيا. وينتقل المرض من الانسان عن طريق لدغة الحشرات الماصة الدم يسمى ذباب الرمل. وهو منتشر على نطاق واسع في العالم بما فيها اليمن، وتهدف هذه الدراسة إلى معرفة التغيرات الهيستوباثولوجية والبيوكيمائية لكلى الفئران. وقد تم تصنيف ٥٦ فأرًا الى ثلاث مجموعات (أ) ٢٠ فار كنترول، (ب) ٢٠ فأرًا أصيبت ب $10x2^7$ طفيل الليشمانيا عن طريق وريد الذيل (ج) ١٦ فأرًا أصيبت ب $10x2^7$ طفيل الليشمانيا وعولجت بمستخلص نبات الصبار (١٥ مع/كغ/٥ أيام). الحيونات ذبحت يوم (١٠٠,٨٠,٦٠,٤٠,٢٠) بعد الإصابة وأظهرت النتائج في معدل وفيات الفئران بلغ ١١,١١ % وبينت إنخفاض ملحوظ في وزن الجسم وتورم وظهور فجوات. وأظهرت أيضًا زيادة كبيرة في قيم اليوريا والكراتينين في الفئران المصابة، وإعطاء مستخلص الصبار للفئران المصابة بالليشمانيا الحشوية أظهر وحفز شفاء الأنسجة وإصلاحها تدريجيًا مع أن بعض التغيرات الباثولوجية ظلت محدودة .

INTRODUCTION:

Visceral leishmaniasis is a disease fatal parasite and infects animals and human it is transformed through sand fly (Sharafet, 1999) .it is preference in tropical and subtropical regions and occurs in Yemen in some governorate such Taiz, Lahg, Appien and Hagga (Haidar, *et al.*,2001).

The parasite infect epithelial system and mononuclear phagocytes especial the liver, spleen, bone marrow and

kidney. It's produced some histopathological alterations as hypertrophy and hyperplasia (Raina, *et al*., 2010), also, some distribution in kidney functions where urea and createnin are increased and the bilrubin value is increased(Ciaramella**,***et al*., 1997).In addition, increasing total protein (Slappendel&Ferrer,1998)and decreasing albumin value (Denerolle,1996) visceral leishmaniasis is make acute decreased in weight body (Celik, et al.,2007).

WHO reported that 80 % from people treated with natural therapy (Newman, *et al.*,2000), *Aloe vera* Is used as common in treatment major diseases, in India recent study was showed the effect of *Aloe vera* plant on amastigote and promastigotes of *Leishmania* in white mice (Dutta, *et al.*, 2008). As well as found Durrani, *et al.* (2010) that garlic, harmal and onion have effective anti leishmaniasis. Other study reported that Curcumin and Methanolic extract of Green tea showed antileishmanial activity against promastigotes in vitro, and was effective against amastigotes (Askar, et al., 2011). Bafghi *et al,. (2008)* reported that *Rubia Tinctorium* extract has effects against cutaneous leishmaniasis in BALB/c mice.

Thus this study aimed to Study the pathological effects of visceral leishmaniasis on kidneys in experimental albino mice at differentperiods and Study the healing effect of Aloe vera leaf crude extract on mice with visceral leishmaniasis.

MATERIALS AND MOTHODES:

1- visceral *Leishmania* parasites :*Leishmania spp* parasite isolated from young patients from Taiz city (The Yemeni Swedish hospital) and cultured on NNN medium and generated on nutrient broth medium supplied with10% fotal calve serum, the culture incubated at 26°c.The parasite was collected, responded with normal saline and counted (1-2× 10^7/ 250µl) (Mullen, *et al.*, 1998)

2- Experiment animals: albino mice which used in current study provided us from Sana'a university 60 mice were divided as following:

- control group (20mice)
- infected group (36 mice) it injected with 1-2× 10^7/mice, it divided to Positive control group (20 mice) and treated group (16 mice) where at 30 day post infection treated with crude of *Aloe vera* plant (15 mg /kg/ day)

is make acute decreased in weight body (Celik, *e*

3)- design experiment :post infection 4 mice at 20, 40, 60, 80, and 100 day were weighted, decapitate and collected the blood for biochemichel tests also, dissected and was obtained the liver, installed in 10 % buffered formalin for histopathological analysis

4) - crude of *Aloe vera* plant :*Aloe vera* leaves collected from Taiz city and identified by Dr. Abdu Alwally Al-Khulaidi (Centre of Agriculture Researches Taiz), the leaves crushed in blannder and filtred and the dos was preapred as (15 mg /kg/ 5day)

RESULTS AND DISCUSSION:

The mortality rate in albino mice due to visceral leishmaniasis reached to 11.11% and occurred in the first periods demonstrated in the table. Leishmaniasis is a deadly vector-borne disease that causes significant mortality in Africa, Asia, Latin America and Mediterranean regions (Sharma & Singh, 2008) visceral leishmaniasis has a high mortality rate if not recognized (Collin, et al., 2006); mortality is very high in untreated cases (90% and 100%) (Melby, 2000 and Joshi, et al., 2006), mortality in other study was 7.3% (Rahim & Ashkan, 2007). Mortality rate of 4% was recorded in the study by Abdeen, et al. (2002). Our investigations showed the illness, cutaneous lesions (alopecia, dandruff production, hair loss, and ulcers), Ocular lesions, and, diarrhoea,

onychogryphosis. These results agree with other studies on animals (Slappendel and Ferrer, 1998; Ferrer, et al., 1991 and Ciaramella, et al., 1997).

The table demonstrates the body weights average, standard division and percent of change of control and experimental groups, where was showed decreasing in the body weight of infected mice reached maximum value 25 % at 100 day P.I. comparing with control. In the treated mice with Aloe vera extract (15mg/kg/5day) were showed gradual increasing amounted 8 % at 100 day P.I. comparing with infected mice. Also illustrated that there were significant differences in the values of weight between the control and infected mice group, it illustrated that there were significant differences in the values of weight between the infected and treated mice group except at 100 day was no significant (Fig.7), our result similar to other authors on animals and human. (Mueller, et al., 2006; and Sciberras,2007).

Table shows the concentrations, mean and percent of change in the values of urea between the three mice groups. The values of urea were high of infected animals than treatment and control groups.

Also, illustrated highest values in the infected group followed by treatment group and finally control group; presented significant variation between control and infected group also between infected and treated group. In our study, it was shown significant differences in the values of urea between the three mice groups, the difference was not significant between infected and control group at day 20 post infection (P.I.), but was only significant difference at 80 day in infected and treated groups. Generally, , it was noticed an increased concentration Azotemia (high serum values of urea and creatinine) of infected mice, this result agrees with several authors (Ferrer, et al., 1992; Kontos & Koutinas, 1993; Denerolle, 1996 ; Ciaramella, et al., 1997; Slappendel & Ferrer, 1998; Solano-Gallega, et al., 2001; Langoni, et al., 2005 and Joshi, et al., 2006). However, some studies disagree with our data on the serum urea and creatinine levels that are usually normal (Pagliano, et al., 2005; Bhattacharya, et al., 2006 and López-Peña, et al.,2009).

In the present study, in kidneys of infected mice with VL was observed hemorrhages, destruction in some renal tubules, lymphocyte infiltrations glomerular inflammation، glomerular tuft was increased, intravascular accumulations of neutrophils and monocytes, destructions of Bowman's capsule, diffuse chronic interstitial inflammation. It has presented hypertrophy and hyperplasia of the arterioles inters tubulars and hyaline degeneration, agreed with several literatures that show the above observations. There is a frequent in the kidney of animals and human resulting from visceral leishmaniasis (Mancianti, et al., 1988 ; Poli, et al., 1991 ; Costa, 2003; Zatelli, et al., 2003 and Zini, et al., 2004).

According to the data provided by some workers, our study presented diffuse membrano-proliferative glomerulonephritis in the most part of the glomerular tuft. There has increased by proliferation of endothelial, epithelial, or mesangial cells. Intravascular accumulations of neutrophils and monocytes were accompanied the cellular proliferation, Glomerular capillary walls were thickened due to the thickening of the basement membrane and the parietal and visceral endothelial and epithelial swelling. Thickening of Bowman's capsule have occurred due to the hyperplasia of parietal epithelial cells,

thickening of the basement membrane and peri glomerular fibroses (Tafuri, et al., 1989; Nieto, et al., 1992; and Font, et al., 1993).

In the present study, the infected mice with VL, were treated orally with Aloe vera extract (15mg/kg body weight×5days), which reduced the main histopathological alteration and parasitemia in the kidney with advance of renal functions. There was an increased body weight,. In addition, Aloe vera decreased urea and Creatinine values in infected and treated mice compared with infected mice without treatment, all results weresignificant.

Non significant possibly was due to short duration initial treatment or probable due to highly parasitism load and there was no response immune system in early stage of treatment. As well as, non significant probable are due to finished activity of treatment due to low dose administration or possible due to reinfection parasite as finished life of macrophage which destructed and releasedparasite.

According to the current study, Aloe vera is effective in wound healing especially at 80, and 100 day post infection. Treatment with Aloe vera, agreed with Joseph & Raj (2010) they reported that a 62.5% reduction in wound diameter was noted in mice receiving 100 mg/kg/day oral Aloe vera these data suggests that Aloe vera is effective in wound healing.

Aloe vera has the ability to repair and heals tissues may due to several effects such as immune modulatory, anti-inflammatory, antimicrobial, antioxidant, and skin burns cure materials. In addition, it has numerous mineral salt, amino acid, vitamins, and enzymes (Boudreau & Beland, 2006; Habeeb, et al., 2007; Ramachandra & Srinivasa, 2008 and Joseph & Raj, 2010). On the other hand, a recent study was demonstrated the effect of AVL on Leishmania parasite, in BALB/c mice, there was an affection against amastigote, reduced parasitemia by >90% in the liver, spleen, and bone marrow without impairment of hepatic and renal functions (Dutta, et al., 2008) which agrees with our results**.**

Table: comparing the values of Weights and kidney functions test in three experimental groups control (C), infected (I) and treatment (T)

Days (P.I)	20		40			60			80			100		
group	C	I	C	I	T	C	I	T	C	I	T	C	I	T
Weights ±S.D. %	28.9^{a} ±0.8	25.8^{a} ±1.1	31.0^{b} ± 1.1	27^{a}± 1 .01	28.4^{b} ±0.5	33.6^{c} ±1.7	26.6^{a} ±1.1	30.1^{b}±1 .4	35.6^{c} ±1.4	27.4^{a} ±0.7	31.3^{b} ±1.6	36.8^{d} ±0.8	27.6^{a} ±2.0	29.9 ±1.7
		-11		-13	-8.4		-21	-11		-23	-12	-	-25	-19
urea ±S.D. %	17.3 a ±0.7	39.5b ±17.7	17.7 a ±0.9	33.2 b ±4.2	22.0 a ±4.6	17.1 a ±1.2	32.0 b ±6.0	21.1 a ±4.5	17.0 a ±1.3	33.6 b ±5.8	21.2 a ±3.7	16.9 a ±1.0	36.8 b ±2.3	21.8 ±0.5
	-	128	-	87.3	24.1	-	87.3	23.6	-	97.1	24.5	-	118	28.9
Creatinin ±S.D. %	0.8 a ±0.02	1.1 b±0.5 0	0.8 a±0.10	1.0 b±0.10	0.9a ±0.1	0.8 a±0.05	0.9 b±0.10	0.9a ±0.1	0.8a ±0.04	1.0b ±0.10	0.9a ±0.1	0.8 a±0.10	1.0b ±0.03	0.9 a ±0.1
	-	40.2	-	17.9	14.8	-	15	13.5	-	14.6	3.28	-	23.8	12.1
Mortality	11.11% (4 dead mice from total infected mice before 20 day)													

*= significant (P < 0.05), N.S=Non significant (P > 0.05)

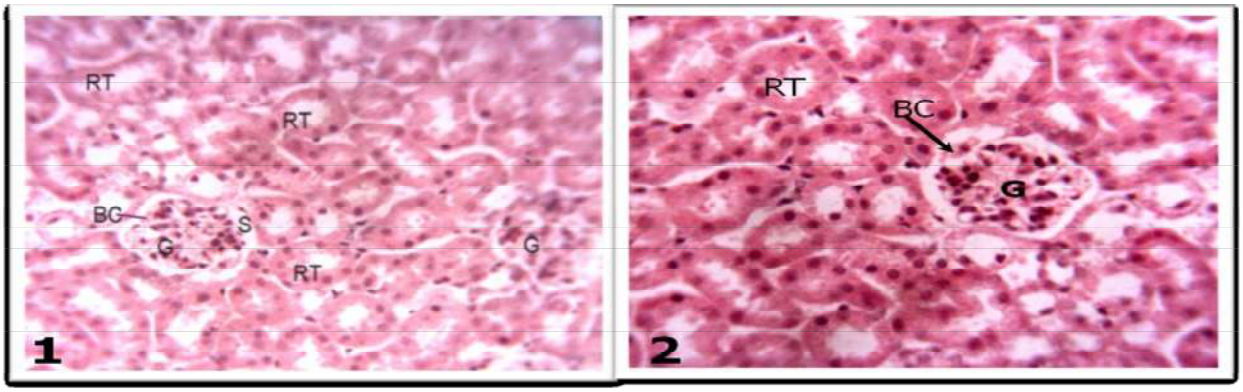


Fig. (1&2): Photomicrographs of transverse section in the kidney of control mice (none infected), indicating normal structure Bowman's capsule (BC), glomerulus (G), renal tubules (RT) and Bowman's space (S) (H&E, X400).

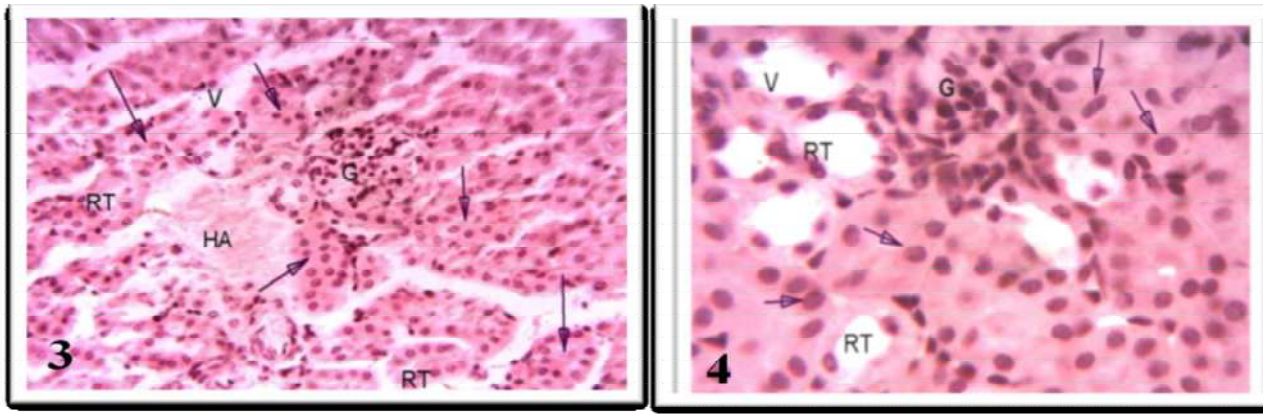


Fig. (3&4): Photomicrographs of transverse section in the kidney of infected mice, displaying damage and loss of inner brush border lining proximal convoluted tubules (RT), destruction and wide glomerulus (G), hemorrhages (HA), dissemination abnormal of mononuclear cells in each microscopic fields (arrows) and vacuolation (V) (H&E, X400 &X1000).

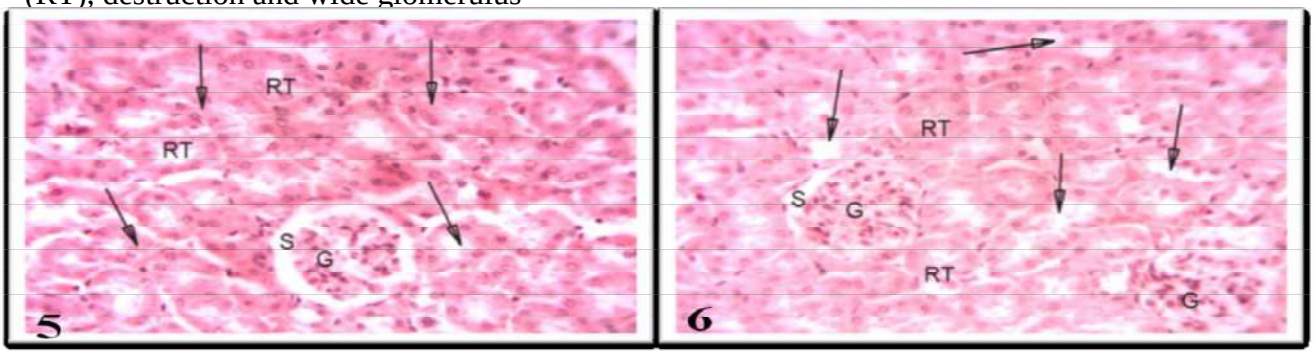


Fig. (5&6): Photomicrographs of transverse section in the kidney of infected mouse and treated with *Aloe vera* extract (40 day P.I.), displaying semi normal glomerulus (G), few vacuoles (arrows) and semi normal cubical cells in renal tubules (RT). (H&E, X400).

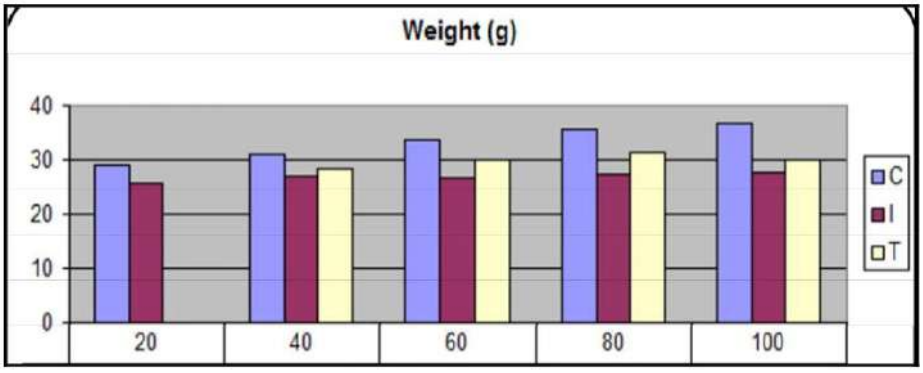
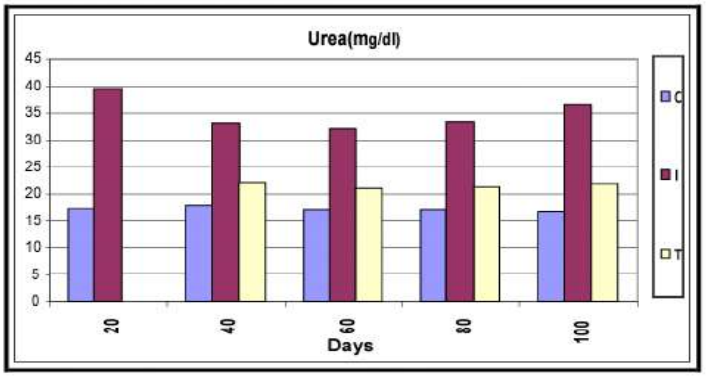
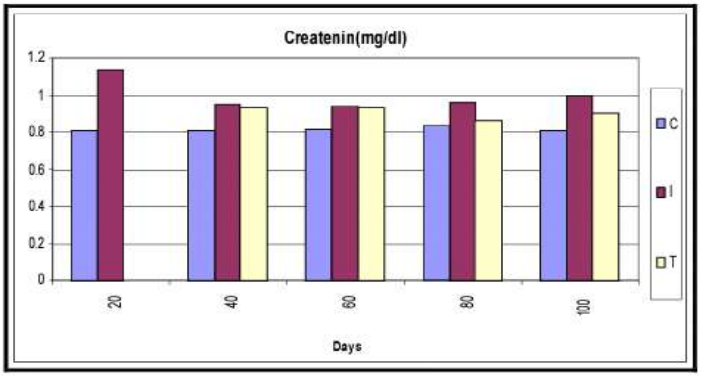


Figure (7)



Figure(8)



Figure(9)

CONCLUSIONS:

Visceral leishmaniasis is occurs in Taiz Governorate particularly in children and the pathological pictures in infected mice with VL are weight loss, hair loss, ulcers, diarrhea, increasing urea and creatinine. *Aloe vera* plant illustrated a positive anti leishmanial activity where it has repaired some the lesion, damage and decreased some destruction in liver tissue.

REFERENCES

Abdeen, Z.; Sawalha, S.; Bader, K.; Yousef, O.; Azmi, K.; Khanfar, H.; Shtayeh, M.; Warburg, A.; Greenblatt, C. ; Schnur, L. ; Jaffe, C. ; and Baneth, G. (2002): Epidemiology of visceral leishmaniasis in the Jenin District, West Bank, Palestine, 1989-1998. Am. J. Trop. Med. Hyg., 66:329-333.

Bhattacharya, S.; Dipika, S. and Karbwang, J. (2006): Childhood visceral leishmaniasis Indian.J. Med. Res., 123: 353-356.

Boudreau, M.D. and Beland, F.A.(2006): An evaluation of the biological and toxicological properties of Aloe barbadensis (miller), Aloe vera. J. Environ. Sci. Health.,24(1):103-54.

Ciaramella, P.; Oliva, G. and Luna, R. (1997): A retrospective clinical study of canine leishmaniasis in 150 dogs naturally infected by *Leishmania* infantum. Vet. Rec., 22: 539- 543.

Collin, S.; Coleman, P.; Ritmeijer, K. and Davidson, R. (2006): Unseen kala-azar deaths in South Sudan (1999–2002) Trop. Med. Int. Heal., 11:509–512.

Costa, F.; H. Goto, L.; Saldanha, S.; Silva, I.; Sinhorini, T.; Silva, and Guerra, L. (2003): Histopathologic patterns of nephropathy in naturally acquired canine visceral leishmaniasis. Vete. Patho., 40:677–684.

Denerolle, P. (1996): Leishmaniose anine: difficulté du diagnostic du traitement. Pra. Méd.Chir. Anim. Com., 31: 137–145.

Dutta, A.; Sarkar, D.; Gurib-Fakim, A.; Mandal, C. and Chatterjee, M. (2008): In vitro and in vivo activity of *Aloe vera* leaf exudates in experimental visceral leishmaniasis. Parasitol. Res. Doi.,10:1-8

Ferrer, L.; Juanola, B.; Ramos, J. and Ramis, A. (1991): Chronic colitis due to *Leishmania*infection in two dogs. Vet. Path., 28 : 342-343.

Font, A.; Closa, J.; Molina, A. and Mascort, J. (1993): Thrombosis and nephrotic syndrome in a dog with visceral leishmaniasis. J. Sma. Ani. Pract., 34: 466–470.

Habeeb, F.; Shakir, E.; Bradbury, F.; Cameron, P.; Taravati, M.; Drummond, A.; Gray, A. and Ferro, V. (2007): Screening methods used to determine the anti-microbial properties of *Aloe vera* inner gel. Methods., 42:315-20.

Joshi, A.; Bhatt, L.; Regmi, S. and Ashford, R. (2006): An assessment of the effectiveness of insecticide spray in the control of visceral leishmaniasis in Nepal. J. Nep. Hea. Res. Council., 1: 1–6.

Kontos, V. and Koutinas, A. (1993): Old world canine leishmaniasis, Compend. Contin. Educ.Prac. Vete., 15: 949-960

Langoni, H.; Lucheis, S.; DaSilva, R.; Castro, A. and Paes, A. (2005): american visceral leishmaniasis: a case report. J. Venom. Anim. Toxins incl. Trop. Dis., 11(3): 372-84.

López-Peña, M.; Alemañ, N; Muñoz, F.; Fondevila, D. and Suárez, M. (2009): Visceral leishmaniasis with cardiac involvement in

a dog: A case report Acta. Vet. Scan.,51:20-22.

Mancianti, F.; Falcone, M.; Giannelli, C. and Poli, A. (1995): Comparison between and enzyme-linked immunosorbent assay using a detergent-soluble Leishmania infantum antigen and indirect immunofluorescence for the diagnosis of canine leishmaniosis. Vet. Parasitol., 59: 13–21.

Melby,P.(2000): Leishmania. In: Behrman RE, Kliegman RM, Jenson HB eds. Nelson Textbook of Pediatrics, 16th edn. Philadelphia, WB Saunders, pp:1041-1045.

Mueller, M.; Balasegaram, I.; Koummuki, Y.; Ritmeijer, K.; Santana, R. and Davidson R. (2006): A comparison of liposomal amphotericin B with sodium stibogluconate for the treatment of visceral leishmaniasis in pregnancy in Sudan. J. Anti. Chem., 58: 811–815.

Nieto, C.; I. Navarrete; M.; Habela; F.; Serrano, S. and Redondo, E. (1992): Pathological changes in kidneys of dogs with natural Leishmania infection. Vet. Paras., 45 (1-2):33-47.

Pagliano, P.; Carannante, N. and Rossi, M. (2005): Visceral leishmaniasis in pregnancy: a case series and a systematic review of the literature. J. Anti. Chem., 55:229–233.

Poli, A.; Abramo, F.; Mancianti, F.; Nigro, M.; Pieri, S. and Bionda, A. (1991): Renal involvement in canine leishmaniasis: a light microscopic, immunohistochemical and electron-microscopic study. Nephr., 57:444–452.

Rahim, M. and Ashkan M. (2007): Epidemiological, Clinical And Therapeutic Features Of Pediatric Kala-Azar Southeast Asian. J. trop. Med. Publ. heal., 38: 626-630.

Ramachandra, C. and Srinivasa, P. (2008): Processing of Aloe vera Leaf Gel: A Review. Amer J. Agri. Bio. Sci., 3 (2): 502-510.

Sciberras, R. (2007): The use of K39 test in the diagnosis of visceral leishmaniasis, Malta. Med.J., 19: 42-45.

Slappendel, R. and Ferrer, L. (1998): Leishmaniasis. In: Greene, C. Clinical microbiology and infectious diseases of the dog and cat. Philade. W.B. Saunders., 450-458.

Solano-Gallega, L.; Morell, P.; Arboix, M.; Alberola, J. and Ferrer, L. (2001): Prevalence of Leishmania infantum infection in dogs living in an area of canine leishmaniasis endemicity using PCR on several tissues and serology. J. Clin. Microbiol.,39:560–3.

Tafuri, W.; Michalick, M. and Dias, M. (1989): Optical and electron microscopic study of the kidney of dogs naturally and experimentally infected with Leishmania (Leishmania) chagasi. Rev. Inst. Med. Trop. São Paulo., 3:139-145.

Zatelli, A.; Borgarelli, M.; Santilli, R and

Bonfanti, U. (2003): Glomerular lesions in dogs infected with Leishmania organisms. Am. J. Vet. Res.,64:558-561.

Zini, E.; Bonfanti, U. and Zatelli, A. (2004): Diagnostic relevance of qualitative proteinuria evaluated by use of sodium dodecyl sulfate-agarose gel electrophoreis and comparison with renal histologic findings in dogs. Am. J. Vet. Res.,65:964-971.