IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 5 Ver. V (Sep. – Oct. 2017), PP 07-15
www.iosrjournals.org

Histological Study on the effect of Miltefosine in treatingVisceral Leishmaniasis

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Abstract:*Leishmaniasis a vector- borne disease caused by obligate intra -macrophage protozoa, is characterized by diversity and complexity. Leishmania are one of different genera within the family Trypanosomatidae. Visceral leishmaniasis occurs universally, but >90% of the cases are in five countries: north-eastern India, Bangladesh, and Nepal in the Indian subcontinent, Sudan in Africa and north-eastern Brazil in South America. Sodium stibogluconate (Sb) has become ineffective in the 1990's in most of the high-burden areas and must be replaced. However, none of the traditional alternatives was satisfactory. Oral drugs are very suitable as the need for hospitalization and related costs are eliminated, home handling is possible, coverage and access is better. Miltefosine (hexadecylphosphocholine, HePC), was the first oral drug that has proved to be highly effectual against VL, including antimony-resistant cases. This study tried to show the susceptibility of HePC on the treatment of liver tissues infected with VL in comparison to Sb. The results showed that HePC had a significant effect in treating VL infected liver tissues compared with those treated with Sb, which didn't revealed any pronounced improvement in all used concentrations. On the contrary, it led to deterioration of infected liver tissues in when used in high concentrations.*
Keywords:*Leishmaniasis, Visceral leishmaniasis. Miltefosine.*

Date of Submission: 22-08-2017 Date of acceptance: 06-10-2017

I. Introduction

Visceral leishmaniasis(VL), also known as kala-azar, is a diffused protozoan infection caused by the *Leishmania donovani* complex and transmitted byphlebotomine sandflies (1). Transmission occurs in 88 tropical and subtropical countries where the sandfly vector is present (2). The zoonotic form, for which dogs are the chief reservoir, is present in the Mediterranean basin, China, Middle East, and South America, and is caused via*Leishmaniainfantum*or*Leishmania chagasi*. The anthroponotic form is caused by *L. donovani* and is widespread in east Africa and the Indian subcontinent (1, 3).*Leishmania* parasites exist a dual-form life cycle, as either a promastigoteflagellar or an amastigoteshape. The promastigotes are found in the insect vector and are entered into the mammalian host during the vector's blood meal. And then, they are phagocytised by macrophages, dendritic cells and/or neutrophils attracted to the biting site in the skin. Once within the phagosome, promastigotes differentiate into amastigotes, multiply by simple division until disrupt the host cell. In the mammalian host, these protozoa are compel intracellular parasites of macrophagedendritic cell lineages (4). The parasites can have various host cells and organs tropism, infecting either external cells or visceral cells, which developments in hepatosplenomegaly and bone marrow infiltration (5). The numbers of leishmaniasis cases are rising worldwide. Some reasons are the scarcity of vaccines, difficulties in controlling vectors and the escalating number of parasites resistance to chemotherapy (6). Pentavalentantimonials has been the recommended drugs for VL and CL. Meglumineantimoniate (Glucantime) and sodium stibogluconate (Pentostan)(Sb) – have variable efficacy against VL and cutaneous leishmaniasis (CL), and require injectable administration, that can be intravenous (IV), intramuscular (IM) or intralymphatic (IL)(7). Due to side effects such as heightcardiotoxicity (8), pancreatitis (9) and nephrotoxicity (10), patients should be hospitalized and monitored, as treatment may require to be suspended. These drugs are costly and difficult to obtain, and they are becoming increasingly less efficient because of the appearance of drug-resistant parasites especially in certain countries such as India (11).

Miltefosine, known as hexadecylphosphocholine (HePC) was simultaneously discovered as an anticancer and antileishmanial drug (12, 13). It is the most current antileishmanial drug on the market and the first effectual oral treatment against VL, being approved as first line drug for childhood VL (14). It is an attractive agent in areas, such as India, that have drug resistance against traditional chemotherapy (11).

The study aimed to valuation the efficacy of HePC on the liver tissues which infected with Iraqi *L. donovani* strain in comparison with the standard drug (Sb).

II. Materials and methods

Parasite culture

Leishmania donovani strain (DUAA/IQ/2005/MRU15) was grown in Novy-MacNeal-Nicolle medium (NNN medium) supplemented with 100 IU/ml gentamycin at 25-26°C (15).

Preparation of drugs :

Pentostam (Sb)

Pentostam present as ampoules prepared for injection (100 mg/ml of Sb) (Glaxo Operations UK Limited Castle).

Miltefosine (HePC)

Miltefosine present as powder form (10 g) with molecular weight 407.57 g/mol and purity 99%. It was manufactured by (Xian Wango Biopharm Co.,Ltd. China). 80 mg of HePC was dissolved in 20 ml of distilled water in order to prepare the stock solution of HePC (4mg/ml) or (104 µg/ml), then the used concentrations prepared from it .

Study desine :

One hundred and twenty female BALB/c mice (6-8 weeks old) were obtained from the Monitoring and Pharmaceutical Research Center in Baghdad. Mice were randomly divided into 12 groups (10 mice / group), 11 of them were infected intrapretonealy with 2×10^7 of *L. donovani* promastigotes.

After one month of infection, five groups of infected mice were treated intra-pretonealy with Sb (4, 8, 12, 16, 20 mg/kg) twice daily. Other five groups of infected mice were treated orally with HePC (3.2, 4.8, 8, 9.6, 12.8 mg/kg) twice daily also. The remain infected group left without treatment as a positive control, while the twelfths (12th) group left without infection and treatment as a negative control.

After 2 and 4 weeks of treatment, the weights of liver of all groups were measured. Liver's impression smears were prepared, methanol fixed, and stained with Giemsa stain, and histological sections from this organ were prepared and stained with hematoxyllin -eosin stain.

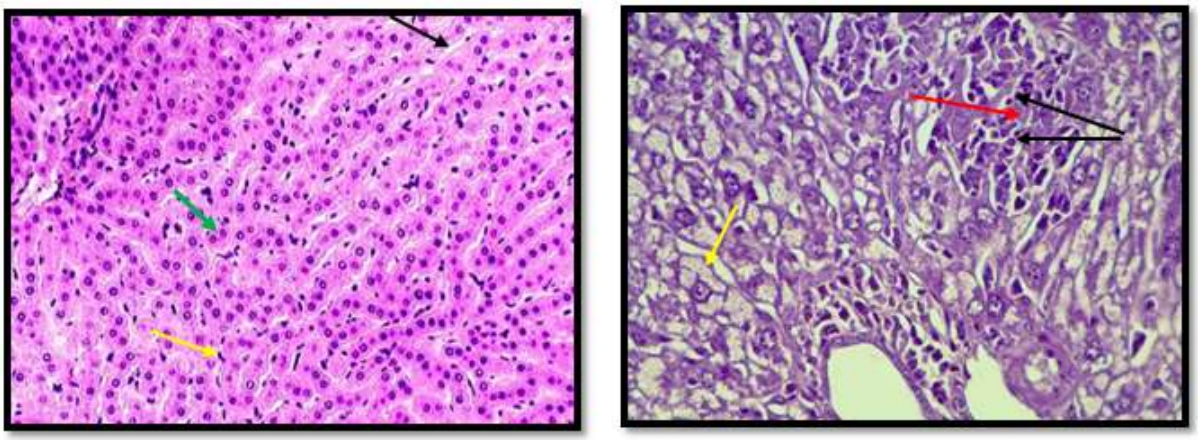
III. Results

Normal structure of liver (control group – uninfected mice)

The parenchymal cells of the liver are hepatocytes. These polygonal cells are connected to one another in anastomosing plates, with borders that face either the sinusoids or neighboring hepatocytes. Hepatocytes make touch with blood in sinusoids, which are extensible vascular channels lined with greatlyfenestrated endothelial cells and populated with phagocytic Kupffer cells (Figure 1).

Histological structure of liver infected with *L. donovani*

The section in mice liver infected with *L. donovani* and non-treated, exhibited the presence of hepatocyte necrosis and infiltration of inflammatory cells (mononuclear cells having unilateral nucleus). The rest of hepatocytes showed the accumulation of cholesterol materielwhich look like plant cells (Figure 2).



Figure(1): Section in normalstructureofliver /hepatocyteFigure(2): Section in infected liver with *L. donovani*/necrosis(green arrow)/ sinusoids(blackarrow)/kupffer cell(H&E 40X).(red arrow)/ inflammtory cell (blackarrows)/(yellow arrows) accumulation of cholesterol material (yellow arrow)(H&E 40X).

Histological structure of liver infected with *L. donovani* and treated with different concentrations of pentostam(Sb)

The effect of 4 mg/kg of Sb :

After the 2nd week, liver sections showed the accumulation of cholesterol materiel and thickening in the cell wall look like plant cells, with wide infiltration of inflammatory cells as a result of hepatocytes necrosis (Figure 3). After the 4th week the beginning of decrease glycoprotein granules with simple infiltration of inflammatory cells was observed (Figure 4).

The effect of 8mg/kg of Sb :

After the 2nd week, liver sections showed the accumulation of glycoprotein granules with simple infiltration of inflammatory cells, in addition to the existence of a focus of necrotic hepatocytes (Figure 5). After the 4th week, the existence of apoptotic cells with simple increment of kupffer cells was clear (Figure 6).

The effect of 12mg/kg of Sb :

After the 2nd week, liver sections revealed irregular expansion of sinusoids; also, there is a degeneration and necrosis in some hepatocytes with infiltration of inflammatory cells inside sinusoids (Figure 7). After the 4th week, the accumulation of cholesterol materiel inside hepatocytes was look like plant cells. There was very little infiltration of inflammatory cells near pylori region (Figure 8).

The effect of 16mg/kg of Sb :

After the 2nd week, liver sections revealed the accumulation of cholesterol material inside hepatocytes look like plant cells. There was a necrosis in some hepatocytes with simple infiltration of inflammatory cells (Figure 9). After the 4th week, large accumulation of glycoprotein granules inside hepatocytes was presented, while the infiltration of inflammatory cells didn't notice. Another section showed the existence of apoptotic cells, there was very simple expansion in sinusoids with increment of kupffer cells (Figure 10 a,b).

The effect of 20mg/kg of Sb :

After the 2nd week, liver sections exhibited the irregular expansion of sinusoids with simple infiltration of inflammatory cells inside sinusoids. Also the beginning of accumulation of cholesterol materials and thickening of cell wall was clear (Figure 11). After the 4th week, the natural shape of hepatocytes was lost with necrosis and infiltration of inflammatory cells with possibility of deposition the amyloid substance (Figure 12).

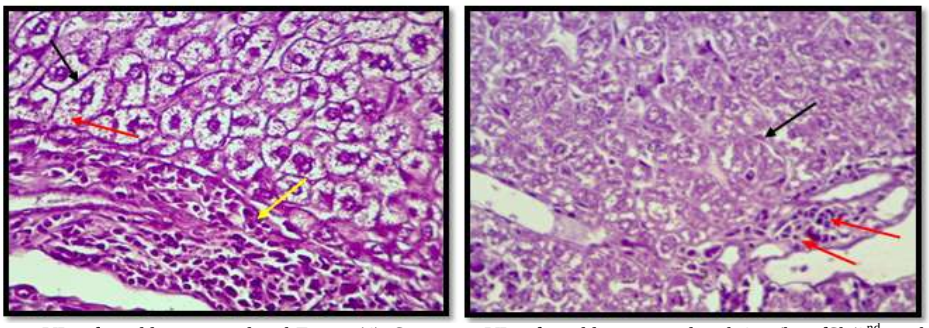


Figure (3): Section inVL infected liver treated withFigure (4): Section in VL infected liver treated with4mg/kg ofSb/2nd week / inflammatory cell(yellow4 mg/kg of Sb/4th week / kupffer cell (black arrow) / arrow) /cholesterol material (red arrow) /thickeninginflammatorycells (red arrows) (H&E 40X).of cell membrane(black arrow)(H&E 40X).

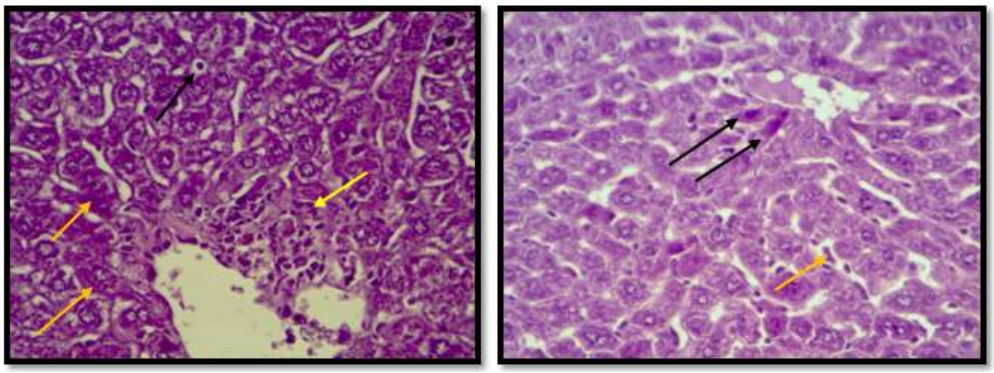
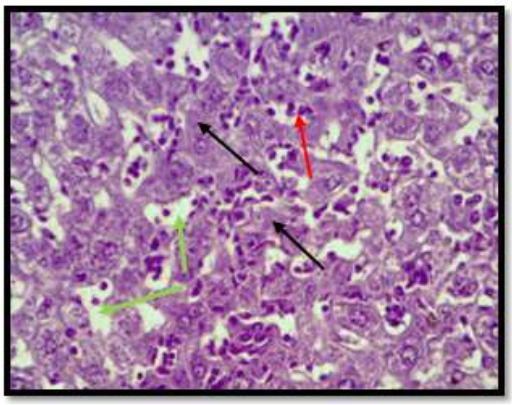


Figure (5): Section in VL infected liver treated with Figure (6): Section in VL infected livertreated with 8mg/kg of Sb /2nd week / accumulation of glycoprotein8mg/kg of Sb /4th week / apoptotic cell (black arrows) / granules (orange arrows) / inflammtory cell(yellowkupffercell(orange arrow) (H&E 40X).arrow)/apoptotic cell(black arrow) (H&E 40X)



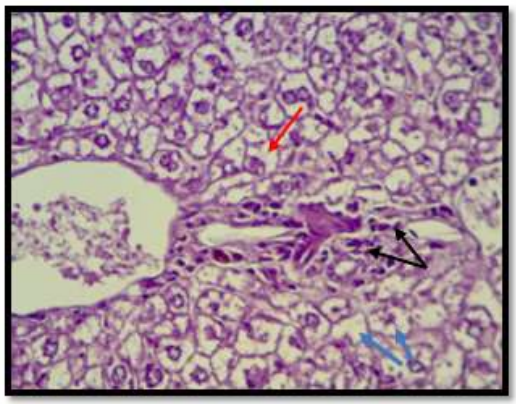
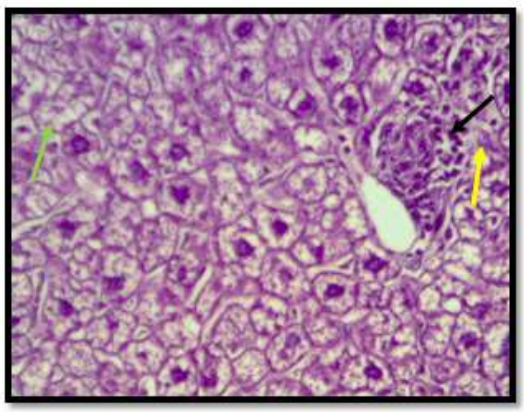


Figure (7): Section inVL infected liver treated with12mgFigure (8): Section inVL infected liver treatedwith12mg /kg of Sb/2nd week/ necrosed cell (black arrows)/irregular/kgof Sb /4th week / inflammtory cell (black arrows)sinusoids (green arrows) / inflammtory cell(redarrow)necrosedcell (blue arrows) / accumulationofcholesterol(H&E 40X)(redarrow)(H&E 40X)



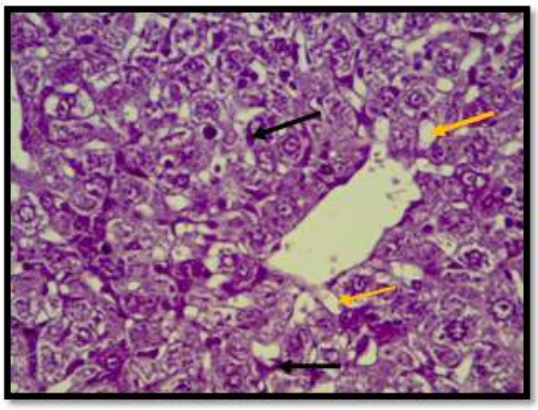


Figure (9):Section inVL infected liver treated with 16mgFigure (10 a):Section inVL infected liver treated with16mg/kg of Sb /2nd week / cholesterol accumulation(greenarrow)/kg of Sb /4th week / sinusoids expansion(orangearrows)/ necrosed cell (yellow arrow) / inflammatorycell(black/ kupffer cell (black arrows) (H&E 40X). arrow) (H&E 40X).

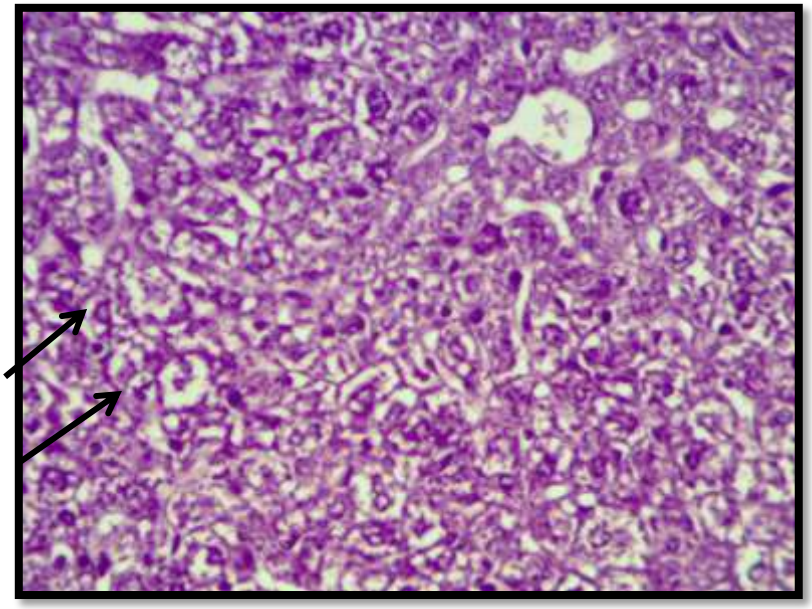
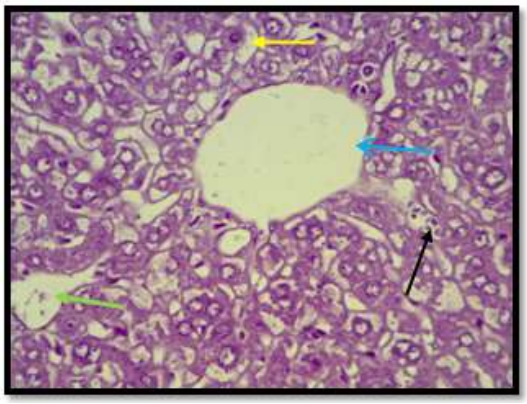


Figure (10b) : Section in VL infected liver treated with 16mg/kg of Sb/four weeks / apoptotic cell (black arrows) (H&E 40X).



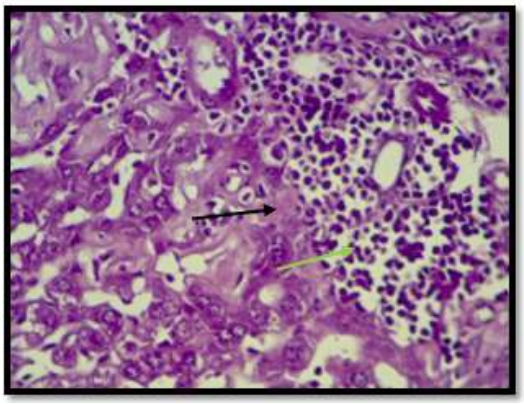


Figure (11): Section inVL infected liver treated with 20mgFigure (12):Section inVL infected liver treated with20mg/kg of Sb /2nd week / cholesterol accumulation (yellowarrow)/kg of Sb /4th week / look like amyloid substance(black/sinusoids expansion (green arrow) /inflammatorycell (blackarrow) / necrosis with inflammtory cell(green arrow) arrow) /central vien (blue arrow)(H&E 40X).(H&E 40X).

Histlogical structure of liver infected with *L. donovani* and treated with different concentrations of miltefosine(HePC)

The effect of 3.2mg/kg of HePC :

After the 2nd week, liver sections revealed simple dilatation of sinusoids. The hepatocytes looked like the normal shape, and there was simple increment of kupffer cells (Figure 13). After the 4th week, simple dilatation of sinusoids with slight depletion of glycoprotein granules was exhibited with simple degeneration in some hepatocytes (Figure 14).

The effect of 4.8mg/kg of HePC :

After the 2nd week, liver sections showed the hepatocytes looked like normal hepatocytes except slight depletion of glycoprotein granules (Figure 15). After the 4th week the beginning of depletion of glycoprotein granules and the appearance of simple degeneration and apoptosis in some hepatocytes was presented (Figure 16).

The effect of 8 mg/kg of HePC :

After the 2nd week, liver sections revealed that the hepatocytes arranged in ribbons around the central vein (looked like normal) and it was obtained glycoprotein granules (Figure 17). After the 4th week the hepatocytes looked like normal cells with simple dilatation of sinusoids (Figure 18).

The effect of 9.6 mg/kg of HePC :

After the 2nd week, liver sections showed that the hepatocytes looked like normal cells with simple dilatation of sinusoids (Figure 19). After the 4th week, also the hepatocytes still looked like normal cells with slight depletion of glycoprotein granules (Figure 20).

The effect of 12.8 mg/kg of HePC :

After the 2nd week, liver sections revealed the normal shape of hepatocytes with slight depletion of glycoprotein granules (Figure 21). After the 4th week, also the hepatocytes were still the same (Figure 22).

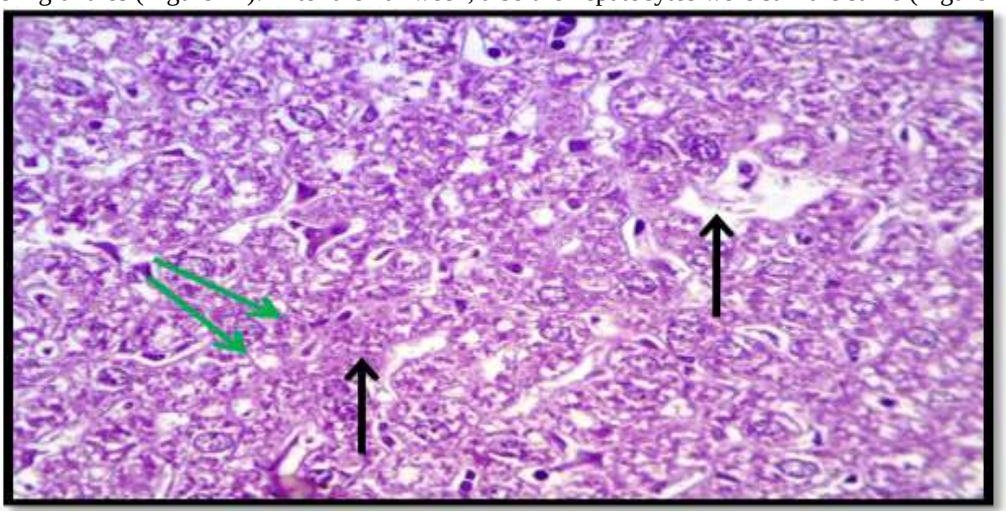


Figure (13) : Section in VL infected liver treated with 3.2mg/kg of HePC /two weeks /kupffer cell (green arrows)/ sinusoids expansion (black arrows) (H&E 40X).

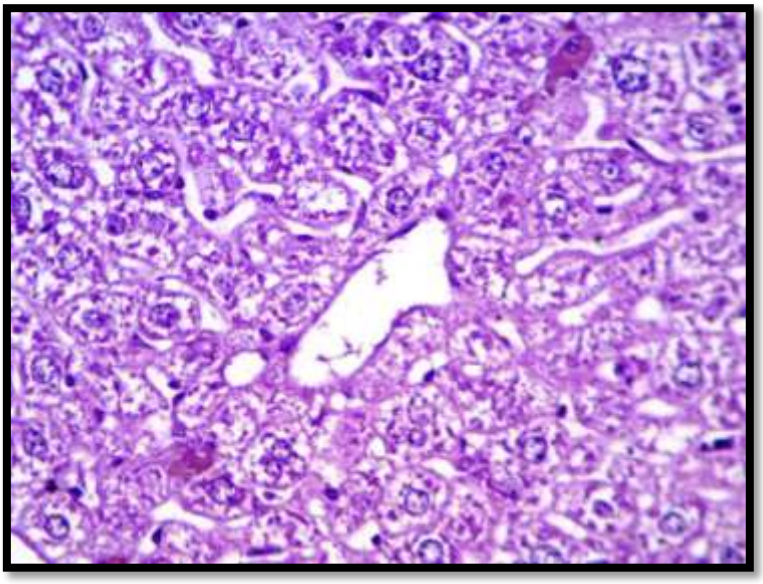


Figure (14) : Section in VL infected liver treated with 3.2mg/kg of HePC/four weeks / degeneration but not resemble plant cell (red arrow)/ simple sinusoids expansion (green arrow)/slight depletion of glycoprotein (black arrow)/ central vein (blue arrow) (H&E 40X).

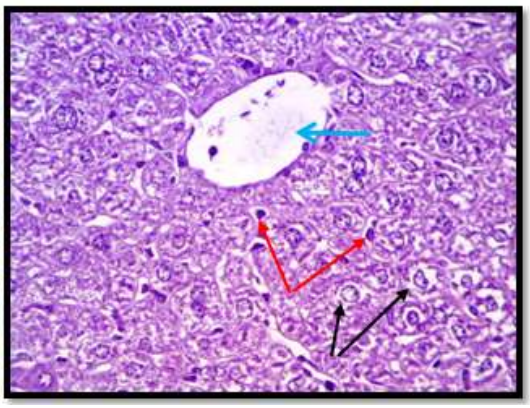
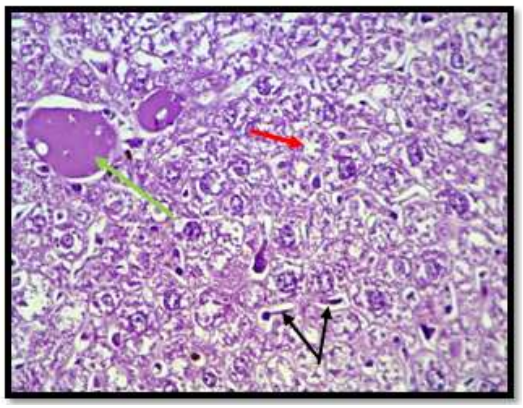


Figure (15): Section inVL infected liver treated with 4.8mg/kg of HePC /2nd week / kupffer cell (red arrows)/slitemg /kg of HePC /4th week/kupffer cell (black arrows) / deplesion ofglycoprotein(black arrows)/centralarterioldegeneration of cell (red arrow) / congestion /(green (blue arrow) (H&E 40X).arrow)(H&E 40X).



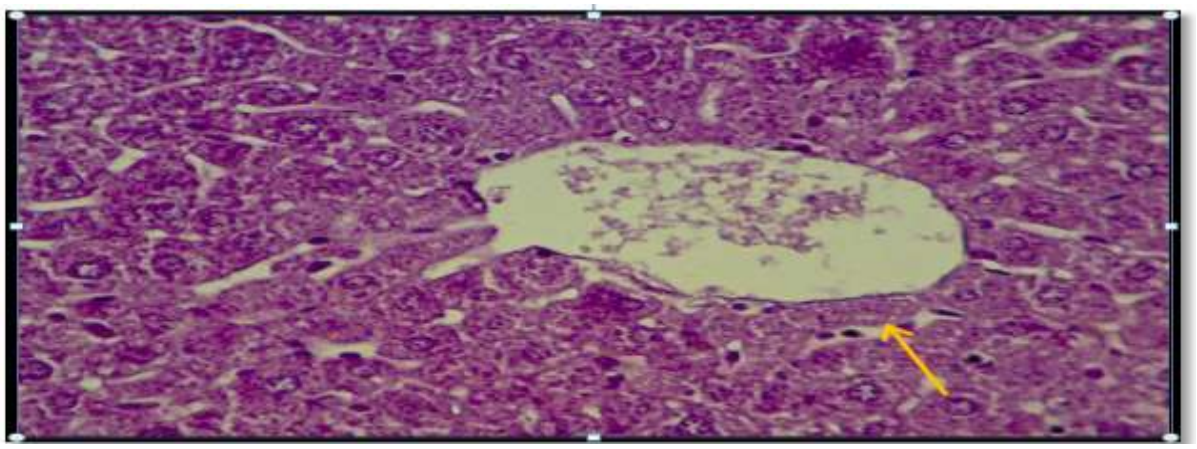


Figure (17) : Section in VL infected liver treated with 8 mg/kg of HePC/two weeks / kupffer cell (orange arrow)(H&E 40X).

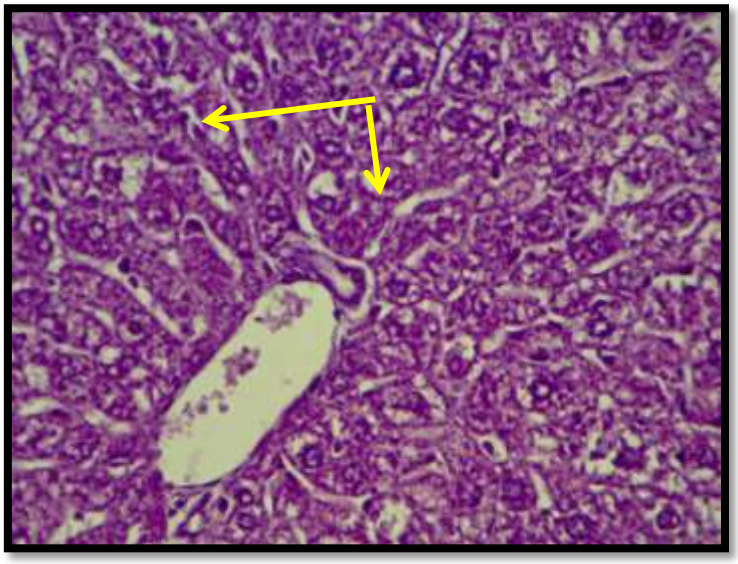


Figure (18) : Section in VL infected liver treated with 8 mg/kg of HePC/four weeks / dilatation of sinusoids (yellow arrows)(H&E 40X).

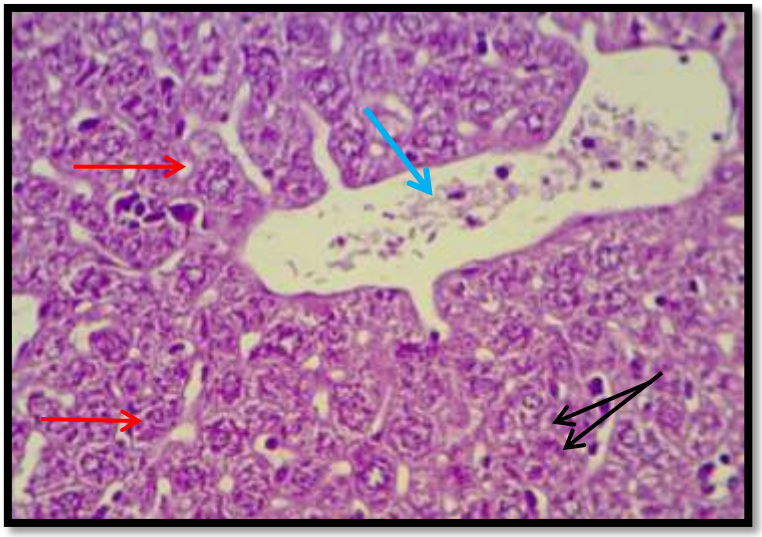


Figure (19) : Section in VL infected liver treated with 9.6 mg/kg of HePC/two weeks / slite dilatation of sinusoids (red arrows)/ kupffer cell (black arrows)/ central vein (blue arrow) (H&E 40X).

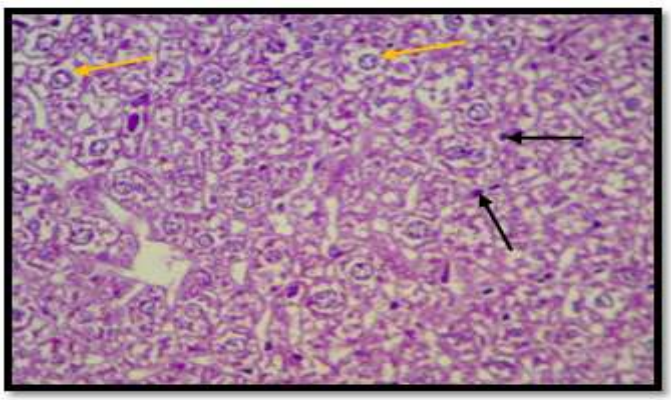


Figure (20): Section inVL infected livertreated with 9.6mg/kgof HePC /2nd week / slite dilatation of sinusoids(redkg of HePC /4th week /slitedeplesionof glycoprotein(orange arrows)/kupffer cell (blackarrows)central vein(blue arrow)arrows)/kupffercell(black arrows)(H&E 40X).(H&E40X).

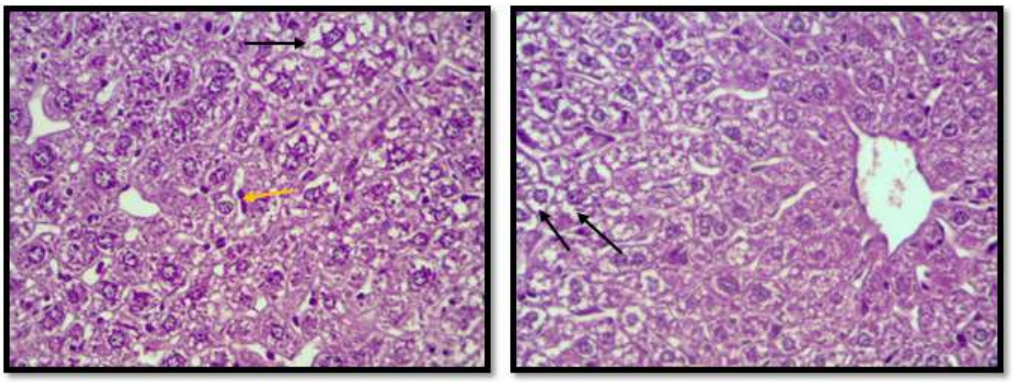


Figure (21): Section inVL infected liver treated with 12.8 mg/kg of HePC/2nd week/slitedeplesionof12.8mg/kg of HePC /4th week/slitedeplesion of glycoprotein(black arrow) / kupffer cell (orange arrow) glycoprotein(black arrows)(H&E 40X).(H&E 40X).

IV. Discussion

Infected mice with visceral leishmaniasis revealed necrosis in liver tissues, this manifest the gradual death of liver cells. Also, the VL infected hepatocytes showed accumulation of cholesterol in some liver cells which looked like plant cells. Liver has a variety of functions in lipid metabolism: 1) uptake, oxidation and transformation of free fatty acids, 2) synthesis of plasma lipoproteins, 3) transformation of lipoproteins, 4) catabolization of LDL, VLDL and 5) secretion of enzymes for lipoprotein metabolism (16). So, accumulation of cholesterol in liver cells was an evidence of impairment of liver function as a result of leishmaniasis infection.

The VL infected mice which treated with the least concentration of Sb (4 mg/kg), exhibited no improvement in liver tissue over a whole month of treatment. As the concentration of Sb increased (12 mg/kg), a slight improvement was observed in the liver tissues. Also, groups treated with high concentrations of Sb (16 and 20 mg/kg), did not notice a good improvement in the infected liver tissues, on the contrary, there was incidence of deterioration in some infected liver tissues. Morphological and functional integrity of liver is vital to the health of the human organism (17), therefore, accumulation of cholesterol in the liver cells, sinusoids expansion, increase infiltration of inflammatory cells and increase observation of kupffer cells (central to both hepatic and systemic response to pathogens), all of these were clear evidence of significant liver dysfunction due to the infection and is not showing pronounced improvement as a result of Sb treatment.

In the case of treatment with HePC, the results showed that the lower concentration of HePC (3.2mg/kg) start to show a slight improvement in the infected liver tissues. Subsequently, with the increased concentrations of the dose given to the infected mice, pronounced improvements were observed in the liver tissues, which became almost similar to the normal tissues compared with cases treated with Sb drug and untreated once.

Although there were a lot of studies done on HePC, this study was the first one clarified the histological effect of HePC on liver tissue. Many previous studies showed the importance of HePC in the treatment of many diseases, and its impact on different types of *Leishmania*, and other parasites. HePC showed extensive anti-protozoal activity (18) it is the solely effective oral treatment for leishmaniasis (19). Mahmood*et al.* (20) showed the potential effect of HePC against experimental cryptosporidiosis in immunocompromised mice, this study showed that administration of HePC for twenty days revealed significant reduction in the number of oocysts in infected groups. In another study, the activity of HePC against *C. parvum* was demonstrated *in vivo* showing 78-98% inhibition of parasite at 45hour post infection (21). Eissa and Amer (22) studied the effect of HePC on murine giardiasis, HePC achieved a pronounced improvement in the pathological mucosal changes and a lesser degree of inflammatory infiltrates. According to Abdou*et al.* (23), only one experimental immune-suppressed mouse was demonstrating large cell dysplasia in the liver. Treated groups with HePC showed the improvement of liver cells architecture, also, Eissa*et al.* (24) showed that the efficacy of HePC in the treatment of mice infected with either invasive, juvenile or adult stages of *Schistosomamansoni* resulted in significant diminution of hepatic granulomata size and amelioration of hepatic pathology. The amelioration in liver pathology could be elucidated by the uptake of HePC by liver tissue and it may probably play a role in healing liver tissues. Mar'ia*et al.* (25) were used different concentrations of HePC (8,16 and 25 mg/kg/day) to treat lesions caused by *L. (L.) amazonensis*, the results showed that low concentrations (8 or 16 mg/kg/day) srevealed adequate clinical effectiveness with no symptoms of distress or morbidity suggesting low toxicity of the drug at these doses, while the high dose (25 mg/kg/day), although it was not allowed to develop the lesions, but there was high mortality rate during treatment.

Esmaeili*et al.* (26) used HePC against *Leishmania major* (MRHO/IR/75/ER): *in vitro* and *in vivo* studies. Their results showed that HePC has a good suppression effects on the promastigotes, suggest that oral

HePC might be a promising approach for developing new anti-Leishmanial drugs. Mohebali*et al*. (27), in a randomized clinical trial, showed that 2 weeks, 3 months, and 6 months after the end of treatment by HePC, the clinical outcome is apparently at least as good as using meglumineantimoniate for CL caused by *L. major*. Induction of programmed cell death is one of the advantages of HePC against the other presently used drugs, including antimonials (28).

References

[1]. Chappuis, F.; Sundar, S.; Hailu, A.; Ghalib, H.; Rijal, S.; Peeling, R.W.; Alvar, J.; and Boelaert, M. (2007). Visceral leishmaniasis: what are the needs for diagnosis, treatment and control?. Nat Rev Microbiol. 5: 873–82.

[2]. Piscopo, T.V.; and MalliaAzzopardi, C. (2007). Leishmaniasis. Postgrad. Med. J. 83: 649– 657.

[3]. Herwaldt, B.L. (1999). Leishmaniasis. Lancet. 354: 1191–99.

[4]. Hammarton, T.C.; Mottram, J.C.; and Doerig, C. (2003). The cell cycle of parasitic protozoa: potential for chemotherapeutic exploitation. Prog. Cell Cycle Res. 5: 91–101.

[5]. Tripathi, P.; Singh, V.; and Naik, S. (2007). Immune response to Leishmania: paradox rather than paradigm. FEMS Immunol. Med. Microbiol. 51: 229–242.

[6]. Lucio, H.; Freitas-Junior ; Eric Chatelain ; Helena Andrade Kim; Jair, L.; and Siqueira-Neto. (2012).Visceral leishmaniasis treatment: What do we have, what do we need and how to deliver it?. International Journal for Parasitology: Drugs and Drug Resistance. 2: 11–19.

[7]. Sundar, S.; and Chakravarty, J. (2008). Paromomycin in the treatment of leishmaniasis. Expert OpinInvestig Drugs. 17:787–94.

[8]. Matoussi, N.; Ameur, H.B.; Amor, S.B.; Fitouri, Z.; and Becher, S.B. (2007). Cardiotoxicity of n-methyl-glucamineantimoniate (Glucantime). A case report. Med. Mal. Infect. 37 (Suppl 3): S257–S259.

[9]. Shahian, M.; and Alborzi, A. (2009). Effect of meglumineantimoniate on the pancreas during treatment of visceral leishmaniasis in children. Med. Sci. Monit. 15: CR290–293.

[10]. Zaghloul, I.Y.; and Al-Jasser, M. (2004). Effect of renal impairment on the pharmacokinetics of antimony in hamsters. Ann. Trop. Med. Parasitol. 98: 793–800.

[11]. Craig, G.; and Stark, M.D. (2017). Leishmaniasis Medication. Medscape.

[12]. Croft, S.L.; Neal, R.A.; Pendergast, W.; and Chan, J.H. (1987). The activity of alkyl phosphorylcholines and related derivatives against *Leishmania donovani*. Biochem. Pharmacol. 36: 2633–2636.

[13]. Scherf, H.R.; Schuler, B.; Berger, M.R.; and Schmahl, D. (1987). Therapeutic activity of ET-18-OCH3 and hexadecylphosphocholine against mammary tumors in BD-VI rats. Lipids. 22: 927–929.

[14]. Bhattacharya, S.K., Jha, T.K., Sundar, S., Thakur, C.P., Engel, J., Sindermann, H.; Junge, K.; Karbwang, J.; Bryceson, A.D.M.; and Berman, J.D. (2004). Efficacy and tolerability of miltefosine for childhood visceral leishmaniasis in India. Clin. Infect. Dis. 38: 217–221.

[15]. Hossein, M.; Darabi, H.; Kariminia, A.; Rivier, D.; Bovay, P.; Mauel, J.; Ajdary, S.; and Kharazmi, A. (2002). Adjuvant Effect of *Leishmania major* promastigotes on the immune response of mice to ovalbumin. Iranian Biomed J. 6: 123-28.

[16]. Calvert, G.D.; and Abbey, M. 1985. Plasma lipoproteins apoproteins and proproteins concerned with lipid metabolism. Adv. Clin. Chem. 24: 218-298.

[17]. Holldrof, A. W.; Krahl-Mateblowski, U.; Mateblowski, M.; and Wutherich, S.(1992). Pathwayes of metabolism in liver. 9th Ed. .

[18]. Dorlo, T.P.; Balasegaram, M.; Beijnen, J.H.; and de Vries, P.J. (2012). Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. J AntimicrobChemother. 67(11):2576–2597.

[19]. Sundar, S.; and Olliaro, P.L. (2007). Miltefosine in the treatment of leishmaniasis: Clinical evidence for informed clinical risk management. TherClin Risk Manag. 3(5):733–40.

[20]. Mahmood, M.N.; Ramadan, F.N.; Hassan, M.S.; Sabry, H.Y. and Magdy, M.M. (2016). Introducing Miltefosine as an Anti-cryptosporidial Agent in Immunocompromised Mice. J Plant PatholMicrobiol . 7: 354.

[21]. Shahiduzzaman, M.; Dyachenko, V.; Obwaller, A.; Unglaube, S.; and Daugschies, A. (2009). Combination of cell culture and quantitative PCR for screening of drugs against *Cryptosporidium parvum*. Vet Parasitol 162: 271-277.

[22]. Eissa, M.M.; and Amer, E.I. (2012). *Giardia lamblia*: a new target for miltefosine. Int J Parasitol 42: 443-452.

[23]. 23 – Abdou, A.G.; Harba, N.M.; Afifi, A.F.; and Elnaidany, N.F. (2013). Assessment of *Cryptosporidium parvum*infection in immunocompetent and immuno- compromised mice and its role in triggering intestinal dysplasia. Int J Infect Dis. 17: e593-600.

[24]. Eissa, M.M.; El-Azzouni, M.Z.; Amer, E.I.; and Baddour, N.M. (2011). Miltefosine, a promising novel agent for *Schistosomiasismansoni*. Int J Parasitol. 41: 235-242.

[25]. Mar´ıa, F.; Garc´ıaBustos; Alejandra Barrio; Gabriela, G.; Prieto Emma, M.; de Raspi, Rub ´en O.; Cimino, Rub´en M.; Cardozo,Luis A.; Parada, Matthew Yeo; Jaime Soto, Delfor A.; Uncos, Cecilia Parodi; and Miguel A. Basombr´ıo. (2014). *In Vivo* Antileishmanial Efficacy of Miltefosine Against *Leishmania* (*Leishmania*) *amazonensis*. J. Parasitol., 100(6): 840-847.

[26]. Esmaeili, J.; Mohebali, M.; Edrissian, G. H.; Rezayat, S. M.; Ghazi-Khansari, M.; and Charehdar, S. (2008). Evalution Of Miltefosin Against *Leishmania major* (MRHO/IR/75/ER*): In Vitro* and *In Vivo* Studies. ActaMedicaIranica, 46(3): 191-196.

[27]. Mohebali, M.; Fotouhi, A.; Hooshmand, B.; Zarei, Z.; Akhoundi, B.; Rahnema, A.; Razaghian, A.R.; Kabir, M.J.; and Nadim, A. (2007). Comparison of miltefosime and meglumineantimoniate for the treatment of zoonotic cutaneous leishmaniasis (ZCL) by a randomized clinical trial in Iran. Acta Trop. 103: 33–40.

[28]. Arnoult, D.; Akarid, K.; Grodet, A.; Petit, P.X.; Estaquier, J.; and Ameisen, J.C. (2002). On the evolution of programmed cell death: apoptosis of the unicellular eukaryote *Leishmania major* involves cysteine proteinase activation and mitochondrion permeabilization. Cell Death Differ. 9:65–81.