

Preparation of *leishmania donovani* Antigens and Study The Immunological Response in Infected Mice

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Abstract

This study includes determine the effectiveness of *leishmania* Ags, by stimulate cellular and humoral immunity against infection with *L.donovani* in mice and study the pathological changes in the internal organs , and the antigen which was given the best results separated by column chromatography and study molecular weight . In this study used one handed white swiss BALB/C mice from both sexes,were randomly divided in to five groups,The first group was immunized with (Lipophosphoglycan Ag) LPG Ag I/P.The second group was immunized with (Autoclaved *Leishmania* Ag) ALAg I/P .The third group was immunized with (Whole *Leishmania* Ag) WLAG I/P. The immunized group give booster dose 14 days after the first dose. The fourth group as control +ve group. The fifth group was inoculated with sterile PBS as control negative. At 21 days ,post immunization ,cell mediated immunity was estimated by skin test used Soluble Sonicated *Leishmania* Antigens (SSLAgS) and at 28 days post immunization , mice from all groups were sacrificed and blood was collected for indirect passive haemagglutination test.The remained mice of all groups were challenged I/p with viable virulent *L.donovani* which obtain from medical Research Center/College of Medicine Al- Nahrain University., then all mice from each group were sacrificed at 30, 60 days post challenge . The results showed that the mean skin thickness at 24,48 hr. in immunized mice induced by LPG Ag were not significantly ($p>0.05$)higher than their values induced by ALAg and WL Ag at 24 and 48 hours respectively post test. In 72 hours results showed that the mean skin thickness in immunized mice induced by LPG Ag were significantly ($p< 0.05$) higer than their valus induced by ALAg and WLAG. In control animal there was no change in thickness of skin.The levels of Abs titer against LPG Ag was higer than their values against AL Ag and WLAG.In immunized and infected animals express slight enlargement in liver and spleen weight were not significant differences between these groups at 30 days $p>0.05$ in liver weight and weight of spleen at 30,60 days but there is statistical significant differences between these groups at 60 days $p< 0.05$ compared with control groups non infected .The histopathological examination of immunized animals showed that animals immunized with LPG Ag during the course of the experiment observed mild pathological lesions and give the highest immunological response, LPG Ag was fractionated by using column chromatography and three peaks of molecular weight 42561 , 30600 ,18320 dalton respectively was seen.

keywords: visceral *leishmania* ,vaccination, LPG Antigen,pathology,column chromatography

Introduction

Leishmaniasis is a zoonotic disease, major protozoan health problem worldwide, present a significant impact on immunosuppressed patients,with important clinical epidemiological diversity depending on the virulence factors of the parasite and the immune response by the host, there are three major of leishmaniasis in human, Cutaneous (CL) Muco Cutaneous (MCL) and Visceral Leishmaniasis (VL) (^{2, 46}) Humans are occasional hosts and

animals,mainly dogs, are the reservoir of the parasite (¹⁰) Human infection characterized by prolonged fever, enlargement of spleen and liver, anaemia and weight loss (²⁹). Affects upto a half million children and adults in both the third and first world settings (¹) Iraq has been reported to be one of the endemic area of Kala azar (^{3,4}), And it is usually detected in infant and children than other due to environmental risk which include

presence of domestic animal ,and rodent^(7,19).

In animals, it is maintained in dogs, wild rodents, and other animals in endemic areas⁽⁴⁷⁾ In dogs the disease symptoms include fever, hypergamma-globulinemia, hepatosplenomegaly and anemia. From the analysis of the clinical and pathological symptoms accompanying canine VL disease⁽¹⁵⁾ in Iraq there is very little attempts to use vaccination against *L.donovani* infection to find effective protection and control against it therefore, the present study aim to :

Materials and methods

Preparation of *leishmania* antigens were prepared from virulence *L.donovani* strain was obtained from the Medical Research center / College of Medicine Al-Nahrain University.

a- Lipophosphoglycan LPG according to⁽⁴⁴⁾.
b-Autoclaved Antigen (AL Ag) according to⁽⁴⁵⁾.

c-Whole Antigen(WLAg).according to⁽²⁾.

1-First group : (20mice) inoculated intrapretonially with 0.5 ml of LPG Antigen which contained 0.15 mg of protein ,in to two dose with 14 days intervals .

2-second group: (20) inoculated intrapretonially with 0.05 ml of AL Ag. which contained 0.15 mg of protein ,in to two dose with 14 days intervals .

3- third group:(20 mice) immunized intrapretonially with 0.05 ml of WLAg which contained 0.15mg of protein , and the second dose from the same antigen after 14 days by I/P administration .

4-fourth group :(20 mice) inoculated intrapretonially with virulent *L. donovani* 2×10^7 as control positive.

5-fifth group:(20 mice) inoculated intrapretonially with 0.5ml of sterail PBS

1- Extraction of three antigens from the *L.donovani* Lipophosphoglycan Antigen (LPG Ag) ,Autoclaved *Leishmania* Antigen (AL Ag) Whole *Leishmania* Antigen (WL Ag) .

2- Study immunological response and pathological changes in laboratory mice.

3- Fractionation the best results antigen by column chromatography technique depending on molecular weight of its contents.

7.2 PH I/P as acontrol negative group.At the day 21 post-immunization,skin test was done to five mice from the 1st, 2nd and 3rd groups to measure cell mediated immunity(CMI).At the day 28 post-immunization , blood was collected from another five immunized mice from 1st, 2nd, 3rd, 4th and 5th to measure Abs titers by passive haemagglutination test (PHA). Another 10 immunized mice 1st, 2nd and 3rd group were inoculated I/P with 0.5 ml of *Leishmania* suspension containing 2×10^7 cells/ml of virulence *L.donovani* and All groups were sacrificed at day 30 , 60 day post challenge for measurement the weight of liver and spleen. And internal organ were taken and fixed in 10% formalin for histopathological examination according to⁽²⁴⁾

Immunological tests:

Delayed type hypersensitivity test DTH (skin test):

This test was done according to⁽²⁰⁾

Passive haemagglutination test (PHA):

This test was done as described by⁽¹³⁾

Results

The immunological tests:

1-Skin test: Delay Type Hyper sensitivity (DTH):

The results showed that the mean values of skin thickness against SSLAgs (3.96 ± 0.36) in first group compared with these

values in second group (3.82 ± 0.21) and third group(3.76 ± 0.11)at 24 hours post test .and these values were decline in first group (3.82 ± 0.39) (3.46 ± 0.36) ,second group (3.62 ± 0.26) (3.16 ± 0.21) and third group (3.54 ± 0.15) (2.8 ± 0.18)at 48, 72 hours

respectively post examine Table (1). There is no statistical significant differences between 3 antigens in 24 ,48 hours, but there is significant difference $p < 0.05$

between LPG Ag and the two other antigens at 72 hours after injection . But, there is no change in the right footpad injected with PBS for all groups.

Table (1): The means thickness of the left footpads of mice during DTH test.

Periods after injection	First group LPGAg	Second group AL Ag	Third group WLAG	Fourth Control-group Inoculated with PBS
24 hours	3.96 ± 0.36 A	3.82 ± 0.21 A	3.76 ± 0.11 A	1.99 ± 0.01 B
48 hours	3.82 ± 0.39 A	3.62 ± 0.26 A	3.54 ± 0.15 A	2 ± 0.01 B
72 hours	3.46 ± 0.36 A	3.16 ± 0.21 AB	2.8 ± 0.18 B	2 ± 0.01 C

L.S.D= 0.6

*The thickness of footpads was measured by millimeter unit (mm). ** Mean \pm Standard error .The capital letter showed that there are differences in account, and there is no significant at 24,48 hours ,but there is significant differences among antigens $p < 0.05$ at 72 hours .

2-Passive Haemagglutination test:

The Abs titer in the first group (112 ± 32), second group (96 ± 36.95) and third group (80 ± 32). These results showed increased Abs titer in LPGAg, then AL Ag and WL Ag but there is no statistical significant

differences between them but there is statistical significant $p < 0.05$ with control group compared with all other antigen. Table (2).

Table (2):The mean of Abs titers and SE of different groups:

groups	Antibody titer mean \pm SE
First (LPG Ag)	112 ± 32 A
Second (AL Ag)	96 ± 36.95 A
Third (WL Ag)	80 ± 32 A
Fourth Control ve+	40 ± 16 AB
Fifth Control ve-	0 B

L.S.D=67.3

The capital letter refers that there is differences in account and there is no statistical significant in LPGAg ,ALAg and WLAG but there is statistical significant compared with Control ve- $p < 0.05$.

The result of skin test explained that all *leishmanial* antigens (LPG, AL, WL) at the present study were elicited cellular and humoral immune response was variable according of type of Ags these results indicated these Ags elicited CMI, due to associated with activated CD4+ T cells and CD8+ T cells which play important role in DTH these evidence was in consistence with Noazin, (2011)³¹ who explained that both CD4+ T cells and CD8+ T recognized, small peptide Ags which expressed on MHCs displayed by APCs such as macrophages and dendritic cell. Our results showed DTH reaction against SSLAgs. The (LPG, AL, WL) antigens were response at 24 hours 48 hours and 72 hours after antigen injection, these observation suggested that the *leishmania* antigen elicited DTH in 24 hours, 48 hours and 72 hours after antigen injection. This agree with Abbas and Lichtman, (2004)³² explained that the term "delayed" signifies the length of time (24 to 48 hours after challenge with the antigen) for circulating effector T cells to accumulate at the site. And investigated that the DTH was measured. at 24, 48 and 72 hours and The data collected at 72 hours were more apparent and were presented. Result in present study showed that LPG Ag generation high level of Abs titer comparatively when immunized with ALAg, WLAG due to anigen specifity, LPG consider virulence factor and good immunogen and this observation was supported by previous observation was supported by previous observation of Spath *et al.*, (2003)³⁸ who explained that LPG is the major surface antigen of all *leishmania* spp, also reported that LPG is known to interact with host's cell receptors during phagocytosis of the parasite, and LPG induce a Th1 immune response in the host is necessary for a cell mediated immune response against intracellular pathogens such as *leishmania* and the LPG good stimulator candidate when used alone or in combination with adjuvants LPG Ag elicited

cell mediated immune response and this data was in agreement with Gifawesen and Farrell, (1989)¹⁸ who reported good immune response in hamster, however, Russo *et al.*, (1992)³⁴ reported that the T-cell responses to evaluate the immunestimulatory potential of *Leishmania* antigens. Data in present study showed the Abs titer and data was high in LPGAg compared with that in mice immunized by ALAg and WLAG, this supported the idea of Th1 cell act as with B cells to induce Abs producing cell IFN- γ , the Th1 cytokine, upregulates the isotypes IgG2a and IgG3 in mice and probably IgG1 and IgG3 in humans. IL-4 and IL-5, Th2 cytokines, stimulate the production of high levels of IgM, IgE, and IgG isotypes such as IgG1 in mice and IgG4 in humans.⁽³⁵⁾

3-pathology study

3.1: Gross pathological changes:

Gross examination showed no clear pathological changes in examined organs of non immunized infected animal that died during 30,60 day post inoculation, except congestion of some organs. no gross lesions were seen in examined organ of immunized infected animals.

A-Percentage of weight of liver / weight of body :

The results of the study indicate increased weight of liver of mice immunized with antigens and infected with *L.donovani* compared with control negative mice, at 30 days, 60 days. Sacrificed (5) mice from each group of antigens (LPG, AL, WL, C+, C-), and weight per/ gram has been measured respectively at 30 days (5.499 ± 0.673 , 5.497 ± 0.13 , 5.579 ± 0.429 , 5.647 ± 0.650 , 4.773 ± 0.537) also weight has been measured respectively at 60 days (5.999 ± 0.47 , 6.128 ± 1.636 , 6.261 ± 0.68 , 6.875 ± 1.99 , 4.76 ± 0.11) there is no statistical significant differences among these groups at 30 days $p > 0.05$ but there is statistical significant differences between these groups at 60 days $p < 0.05$ Table (3).

Table (3) : The effect of different antigens in weight of liver

Time ps Groups	30days %weight of liver /body weight gram mean±SE	60days %weight of liver/ body weight gram mean±SE
First (LPG Ag)	5.499 ± 0.673	5.999 ± 0.47 AB
Second (AL Ag)	5.497 ± 0.13	6.128 ± 1.636 AB
Third (WL Ag)	5.579 ± 0.429	6.261 ± 0.68 AB
Fourth Control ve+	5.647 ± 0.650	6.875 ± 1.99 A
Fifth Control ve-	4.773 ± 0.537	4.76 ± 0.11 B

L.S.D=2.1

B-Percentage of weight of spleen /weight of body :

The results of the study indicated that weight per /gram of spleen of mice immunized with antigens and infected with *L.donovani* at 30 days,60 days sacrificed (5) mice from each group of antigens (LPG ,AL ,WL ,C+ ,C-), and weight has been measured respectively at 30 days (0.517 ± 0.186 , 0.530 ± 0.1 , 0.65 ± 0.11,0.707 ±

0.17 , 0.404 ± 0.08) also weight has been measured respectively at 60 days (0.46 ± 0.13 , 0.534 ± 0.16 ,0.540 ± 0.17 , 0.8 ± 0.27,0.46 ± 0.04). There is no statistical significant differences between these groups at 30 days $p>0.05$ also no statistical significant differences between these groups at 60 days $p>0.05$. Table (4) .

Table (4) :The effect of different antigens in weight of spleen .

Time Groups	30 days %weight of spleen/body weight mean ±SE	60 days% weight of spleen/body weight mean ±SE
First (LPG Ag)	0.517 ± 0.186	0.46 ± 0.13
Second (AL Ag)	0.530 ± 0.1	0.534 ± 0.16
Third (WL Ag)	0.65 ± 0.11	0.540 ± 0.17
Fourth Control ve+	0.707 ± 0.17	0.8 ± 0.27
Fifth Control ve-	0.404 ± 0.08	0.46 ± 0.04

L.S.D=0.4

The results of the present study indicate that there is slight enlargement in liver and spleen in all mice immunized with LPG Ag ,ALAg ,and WLAg respectively but there is no significant

differences at day 30 . this observation in acceptance with singh *et al* .,(2002)⁴³ who said that The principle pathological lesions are the result of reticuloendothelial cell hyperplasia especially in spleen, liver,

invasion of mucosa and submucosa in digestive tract leading to hypertrophic congestion and small ulceration and bleeding may occur. It may be many reasons such as increased in defence cells or parasite aggregation or amyloid aggregation and this agreement with ^(6; 8,9). This study revealed less increase in weight of liver and spleen of mice inoculated with LPG Ag, AL Ag, WL Ag respectively and this indicated that LPG Ag give immune response than other due to parasite load which directly correlated with weight and size of liver and spleen. This agree with Kumari *et al.*, (2008) ²¹, who explained that hepatosplenomegaly associated with challenge infection was virtually in the potential subfractions vaccinated group. Results in this study showed the increase in liver weight in day 60 compared with spleen, this supported the idea mentioned by Wilson *et al.*, 1996⁴⁸, who said that parasites grow slowly in the spleen of mice infected with *L. donovani* amastigotes, but the pattern of rapid growth and spontaneous resolution in the liver is similar. also Engwerda and Kaye, (2000)²³ observed that in contrast to the liver, parasites grow more slowly in the spleen and bone marrow, where they can persist for life in the animal.

4-Histopathological examination :-

A.1) Non immunized infected animal :-

Histopathological changes of mice killing during 30,60 day post inoculation.

1-Bone marrow:

Histopathological examination of bone marrow of mice infected with *L. donovani* after 60 days showed congestion of blood vessel with moderate depletion cellularity (Fig:1).

2-Liver :mononuclear cells and neutrophil infiltrate in the liver parenchyma and around central vein together with necrotic area characterized by pyknotic cell of nuclei or disappear (Fig: 2). In liver at 30 days showed small granulomatous lesion composed from aggregation of

macrophages in liver parenchyma together with vacular swelling of hepatocyte (Fig:3). Result in current study showed necrosis of hepatocytes due to occlusion of the blood vessels by *L. donovani* protozoa parasite and inflammatory reaction, which may account for ischemic effect on hepatic cell, ischemic cause loss of oxidative phosphorylation by mitochondria and generation of ATP slow or stopped ⁽³⁶⁾. Also depletion of ATP is primarily responsible for acute cellular degeneration and necrosis ⁽¹²⁾. Other possibility relating to pathogenesis of necrosis may be due to acid phosphatase release from *L. donovani* which lysis the cell membrane ⁽⁴⁰⁾ and the cellular debris attracted PMNs to infected area causing suppurative reaction and release PMNs enzymes cause necrosis of tissue ⁽²⁵⁾. The pathology of VL is dominated by the specific suppression of cell-mediated immunity; permitting the dissemination and uncontrolled multiplication of the parasite resulting in various complications, if untreated. Results in this study showed amyloid like substance deposition in the spleen these result was agree with Rica-Capela *et al.*, (2003)³³, who explained that the amyloid progenitor number was selectively increased in the spleen, in comparison to similar increases in amyloid and erythroid hematopoiesis observed in the bone marrow and characterized for the first time the local changes in hematopoietic activity in tissues, which harbor persistent parasites and compared it with both parasite dynamics and the evolution of cytokine and chemokine responses in these organs. In this study showed granulomas the liver at 30 days and this agree with observation that Amastigote of leishmania parasite were observed in two main anatomical locations: within granulomas, where they were predominantly associated with the core, and within parenchyma, granulomas provide focus to the ensuing immune response, helping to contain parasite dissemination and providing the major

effector site responsible for parasites elimination from the liver. although granulomas are believed to form around infected resident liver macrophages (Kupffer cells), the role of these cells in intra-granuloma antigen presentation is currently unknown⁽¹¹⁾. Also Nieto *et al.*, (2011)³⁰ explained that granulomas become fully evolved by 2-4 weeks the over all antimicrobial efficacy of the granulomatous response appears to be variable, and only mature granulomas develop efficient leishmanicidal mechanisms to kill parasites. In contrast, developing granulomas have been reported to be less efficient at killing *Leishmania* parasites, among other factors, granuloma development has been found to vary depending on the initial inoculum size.

A.2) Histopathological changes of immunized animal.

1- Animal immunized with LPG Ag and infected with *L.donovani*.

histopathological examination after 60 days post infection in liver no clear pathological except proliferation of kupffer cell and few mononuclear cell in the wall of central vein and sinusoid (Fig:4).

The main pathological lesion in examined organs of mice in spleen of animal immunized with LPG Ag after 60 days post infection showed moderate hyperplasia of white pulp (Fig:5).

2- Animal immunized with ALAg and infected with *L.donovani*.

Histopathological examination of liver immunized with AL Ag after 30 days post challenge liver microscope examination showed aggregation of mononuclear cell in liver parenchyma together with the vacular degeneration of hepatocyte and inflammatory cell in the dilated sinusoid mostly neutrophil (Fig:6). Spleen showed deposition amyloid like substance around depletion pulp figure (Fig:7).

3-Animal immunized with WLAg and infected with *L.donovani*.

At 30 days the histopathological examination of liver in immunized animal with WL Ag showed single necrosis of hepatocyte which characterized by eosinophilic cytoplasm and dense nuclei together with mononuclear cell infiltrate around blood vessel (Fig:8). in spleen amyloid like substance deposition is the main lesion.(Fig:9).

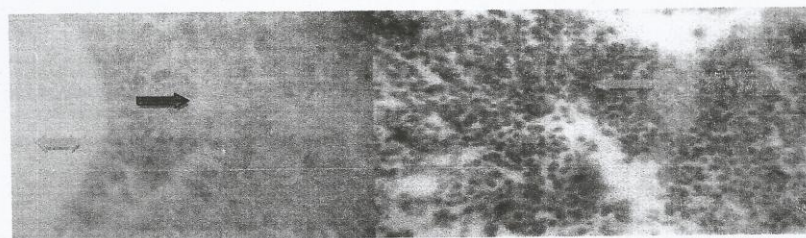


Fig 2: Histological section in liver of animal infected with *leishmania donovani* Showed inflammatory cell aggregation around central vein with necrosis of hepatocyte (H&E 40X).

Fig 1: Histological section in the bone marrow of animal infected with *leishmania donovani* showed congestion of blood vessels and moderate depletion of Cellularity (H&E40X).

Fig 3: Histological section in liver of animal infected with *leishmania donovani* Showed granulomatous lesion in liver parenchyma together with vacuolar degeneration of hepatocytes (H&E 40X).

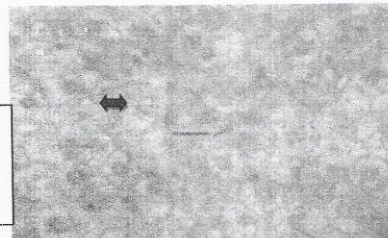


Fig 5: Histological section in spleen of animal at 2 month postimmunized with LpG Ag showed moderate hyperplasia of white pulp (H&E40X).

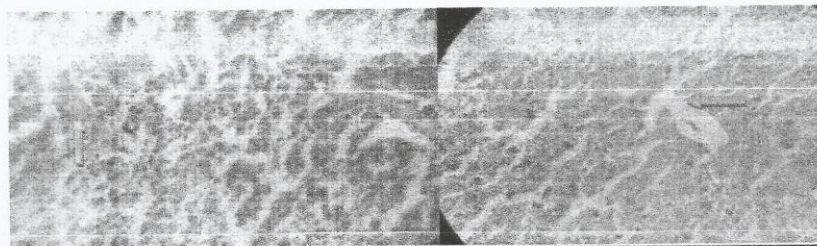


figure 4 : Histological section in liver the of animal immunized with LPG Ag a er 2 month post infec on Ag showed moderate proliferation of kupffur cells and few mononuclear in the wall of blood vessel (H&E40X).

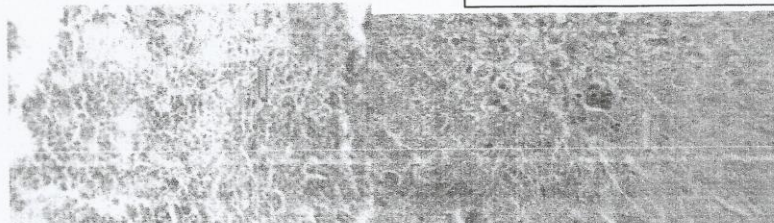


Fig 7 : Histological section in the spleen of animal immunized with A L Ag and challenge after two month showed amyloid like substance deposition around white pulp (H&E40X).

Fig 6: Histological section in the liver of animal immunized with A L Ag and challenge after one month showed small granulomatous lesion in the liver parenchyma (H&E40X).

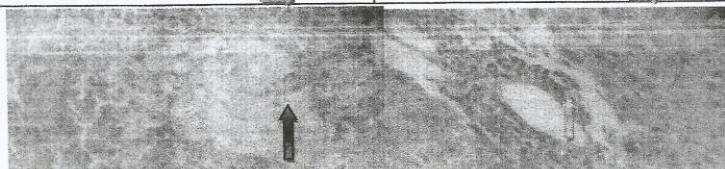


Fig 9: Histological section of the spleen of animal immunized with WL Ag. 2 month pos- challenge showed amyloid like substance deposi on in red pulp (H&E40X).

Fig 8: Histological section in the liver of immunized animal with W L Ag at 1 month post infection showed inflammatory cells aggregation around blood vessels in portal area and bile duct (H&E 40X).

The pathological results in animal immunized with LPGAg and infected with *L.donovani* coincided with no clinical signs this results confirmed that Ags provide good protection against *L.donovani* infected. this evidence is supported by marked aggregation of mononuclear cell in the wall of central vein and sinusoid of liver with hyperplasia of white pulp in spleen and this according to these finding the LPG Ag of *L.donovani* activated the peritoneal macrophage that kill all or most *L.donovani* at site of inoculation and this agree with Stanley and Engwerda (2007)⁴¹ who explained that the anti leishmanial immunity is mediated by both innate and adaptive immune responses and requires effective activation of macrophages, dendritic cells (DCs), and antigen-specific CD4+ and CD8+ T cells. Other authors explained that other cell like Th2 cells help antibody production of IgG1, IgE and IgA (28). According to this idea the present study suggested that the primary immune response by LPGAg induced active memory immune cell and exposed the animal to inoculation of the virulent *L.donovani*, rapidly stimulate effective secondary immune response that activate phagocytic cell, by CD4 + and CD8+, this evidence is in coincidence with previous investigation that mentioned by several workers (16). The presence of neutrophil in the liver of mice inoculated with ALAg may be due to active production of TNF α which is a proximal mediator for neutrophils chemotactic factor (30) and neutrophil are essential for host defence against protozoa. neutrophil can produce inflammatory mediators such as IL-12 (14) also TNF α play role in the activation of DCs and increase the ability in Ag-presenting which correlated with magnitude and quality of the T-cell immune response (27). The presence of granulomatous lesion in liver of animal immunized with ALAg at 30day post inoculation of virulence *L.donovani* indicated no sufficient immunity elicited during early stage of

infection. some protozoa disseminated from the site of inoculation to internal organs and were sequestered by activated macrophages forming granulomatous lesion which reduce the growth rate of *L.donovani* and destroy them during 30 day post challenge. This idea may be explained the reason of the diminished size of granulomatous lesion in examined organ at 60 day post inoculation and this agree with Kaye *et al.* (2004)²³ and Wilson *et al.* (2005)⁴⁹, who explained that the Parasite persistence is accompanied by the failure of granuloma formation and by a variety of pathologic changes, including splenomegaly, disruption of lymphoid tissue micro architecture, and enhanced hematopoietic activity. The salient features of these distinct tissue responses and high light the varied roles that cytokines of the tumor necrosis factor family play in immunity to this infection. The heavily infected mononuclear cell core of the granuloma is composed almost entirely of Kupffer cells, many having migrated from the surrounding sinusoids and this agree with Beattie *et al.* (2010)¹¹, who showed that only kupffer cells laden with intracellular amastigotes are able to form long -lasting antigen-specific interactions with CD8 T cells within the granuloma microenvironment may have important implications for the development of vaccines to *Leishmania* that are designed to induce CD8⁺ T cell responses.

Fractionation of LPG Ag :-

Column chromatography for LPG Ag, was done to characterize protein peaks here molecular weight, and results showed the existence of (3 peaks). The first one appears between tube (8 to 20), the second peak appears between tube (24 to 38), the third peak appears between tube (46 to 64). The separated protein in (P 1, P 2, P 3), was collected each in a single test tube. The molecular weight of (P 1) were 42.561 dalton, (P 2) 30.600 dalton and (P 3) 18.320 dalton Figure (23)

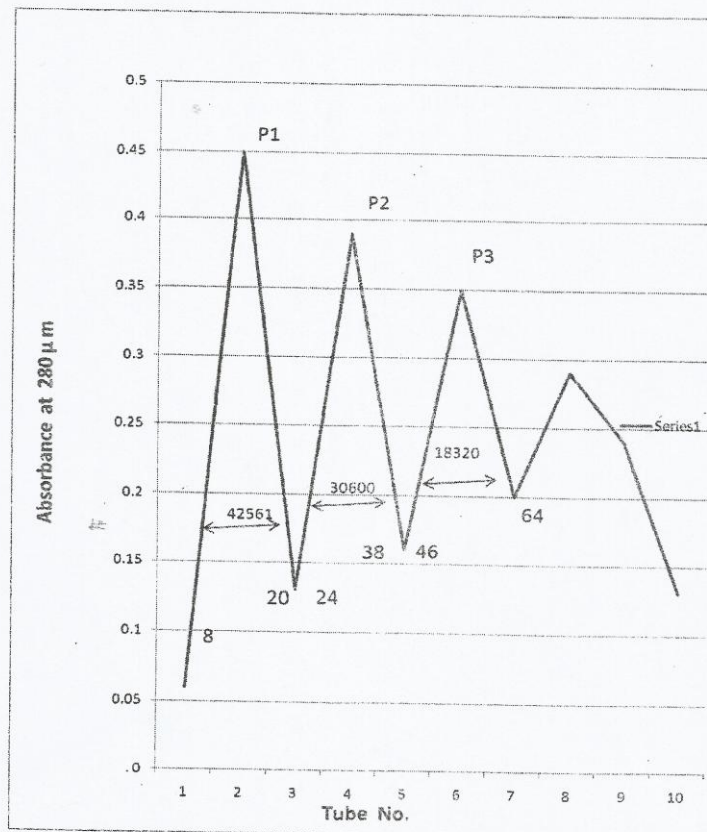


Fig.(23):fractionation of LPG Ag of *L.donovani* by column chromatography in to (3 peak) .
Speed of separation 150 ml/hr.

In the study (Fig 23) showed the peaks appears in column chromatography technique generally these results agree with AL-obaidi ,(1999)⁵ , who isolated (3 peaks) antigens with molecular weight 65 , 14 , 12 kilo Daltons from soluble extract of *L.donovani* promastigote by sephadex G-

200column.Also Sacks *e t al.*, (1995)³⁹ reported fractionation by G-150 Sephadex chromatography of a single broad peak of procyclic LPG fractions between (38 and 80) with single broad high molecular weight peak .

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تحضير مستضدات اللشمانيا الحشوية ودراسة استجابتها المناعية في الفئران المصابة

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الخلاصة

تضمنت هذه الدراسة ، معرفة تأثير مستضدات اللشمانيا الحشوية في تحفيز المناعة الخلوية والخلطية ضد *L.donovani* في الفئران ، ودراسة للتغيرات المرضية في الاعضاء الداخلية ، وفصل افضل مستضد بطريقة الترشيح الهلامي ودراسة الاوزان الجزيئية . استخدمت في هذه الدراسة مئة من الفئران البيض نوع Balb/c من كلا الجنسين قسمت الى خمس مجاميع :-

المجموعة الاولى (حقنت في التجويف الخلي مستضد (Lipophosphoglycan Ag) المجموعة الثانية حقنت في التجويف الخلي مستضد (Autoclaved *Leishmania* Ag) المجموعة الثالثة حقنت في التجويف الخلي مستضد (Whole *Leishmania* Ag) حقنت جميع هذه المجاميع بالجرعة الثانية بعد ٤ ايام من اعطاء الجرعة الاولى. المجموعة الرابعة مجموعة سيطرة موجبة. وبعد ٢١ يوم تم اجراء الفحص الجلدي للمجاميع كافة لتحديد الاستجابة الخلوية باستخدام مستضد المكسر لنفس الطفيلي، بعد ٢٨ يوم تم قتل ١٠ فأر من كل المجاميع لاجراء فحص التلازن الدموي الغير مباشر لتحديد الاستجابة المناعية الخلطية. اما الحيوانات المتبقية من كافة المجاميع فقد حقنت بجرعة التحدي من العترة الضارية لطفيلي اللشمانيا الحشوية والتي تم الحصول عليها من مركز البحوث التابع الى كلية طب النهرين. المجموعة الخامسة (مجموعة سيطرة سالبة فقد حقنت المحلول التسلسلي الملحي المتعادل. تم قتل كل الحيوانات بعد ٣٠ ، ٦٠ يوم من الإصابة. بينت النتائج وجود فروق غير معنوية ($p > 0.05$) في معدل نتخن الجلد في الحيوانات الممنعة بمستضد LPG بمقارنة بالمستضد AL والمستضد WL خلال ٢٤ ، ٤٨ ساعة على التوالي. كذلك بينت النتائج وجود فروق معنوية ($p < 0.05$) في معدل نتخن الجلد في الحيوانات الممنعة بمستضد LPG مقارنة بالمستضد AL ومستضد WL خلال ٧٢ ساعة لم تظهر حيوانات السيطرة اي نتخن في الجلد . كذلك مستوى الأجسام المضادة ضد مستضد LPG اعلى من مستضد AL ومستضد WL. لوحظ تضخم طفيف للكبد والطحال في الحيوانات المصابة والممنعة بينت النتائج وجود فروق غير معنوية ($p > 0.05$) في اليوم ٣٠ في اوزان الكبد واوزان الطحال في اليوم ٣٠ ، ٦٠ وجود فروق معنوية ($p < 0.05$) بين الحيوانات المصابة والممنعة والحيوانات الغير مصابة في اليوم 60 في اوزان الكبد. بالنسبة للافات المرضية كانت طفيفة للحيوانات الممنعة بمستضد LPG واعطى افضل استجابة للمناعة. تم فصل مستضد LPG بطريقة الترشيح الهلامي الى ٣ ذروات بالتعاقب بأوزان جزيئية (42561 ، 30600 ، 18320) دالتون .