Immunological Effect of Polysaccharide Extracted from Pseudomonas aeruginosa against Leishmania donovani in mice

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ABSTRACT:

BACKGROUND:

Visceral leishmaniasis is a serious disease with high pathoogenicity. It was noticed that Polysaccharide extracted from Pseudomonas aeruginosa had the ability to induce both cellular & humoral immunity response against bacteria, fungi & parasites.

OBJECTIVE:

The aim of this study was to know the immunological effects of polysaccharide extracted from Pseudomonas aeruginosa before & after the infection of BALB/c mice with Leishmania donovani in trial to provide a method for prevention of the disease in human.

METHODS:

174 male BALB/c mice were divided into control & experimental groups with each group consisting of six animals. The experimental groups injected intraperitoneally (i.p) with 0.2 ml phosphate buffer saline (PBS) containing 1×108 parasite. Cerdain groups were injected intraperitoneally by polysaccharide (PS) at doses of 100, 150, 250 µg on day 3, 6, and daily for 6 days before infection with Leishmania donovani. These animals were sacrified after 8 weeks. Other groups were injected intraperitoneally with polysaccharides on day 7 post infection at the doses of 100, 150, 250, µg, 6 animals were sacrified after 2, 4, 6, 8 weeks. Total & differential count of leukocytes, measurement of spleen & liver weight and index, measurement of cellular immune response, histopathological changes liver and spleen were carried out.

RESULTS:

This study showed an increase in the total count of leukocyte accompanied by an increase in lymphocyte with decrease in monocyte percent in mice treated with polysaccharide before and after infection with Leishmania donovani which were statistically significant. Reduction in liver & spleen weights & their index in all treated groups was noticed before & after infection. The cellular immune response represented by delayed hypersensitivity has been enhanced in the mice treated with polysaccharide before &after infection. In treated groups, histopathological studies of mice showed hepatocyte hypertrophy, infiltration of the inflammatory cells in liver before & after infection. In spleen showed an increase in lymphocytes & monocytes in red pulp, while the infected mice (non treated) showed a necrotic foci, loss of architecture of both liver & spleen & obvious granulomatous inflammation in liver.

CONCLUSION:

Polysaccharidde extracted from Pseudomonas aeruginosa induced immunological activity by activation of cell mediated responses, stimulated an increase in WBC count & lymphocytes with minor histoptholgical changes in liver and spleen.

KEYWORDS: Immunological effect, Polysaccharides, Leishmania, mice.

INTRODUCTION:

Visceral leishmaniasis is a serious systemic disease caused by an intracellular protozoan parasites of reticuloendothelial cells, called Leishmania donovani complex Spp.[1]. The life cycle of Leishmania Spp involves an alternate existence in a vertebrate and an insect host. Human and other vertebrate hosts are infected through bite of infected female sandfly (phlebotomus Spp.).

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Two stages (amastigate and promastigote) are recognized during the development of this parasite[2]. The world health organization (WHO) considered the leishmaniasis to be among the six more important infectious disease of human world wide[3]. The disease is endemic in Mediterranean region, central and south Asia, Russia and eastern china[4]. In the last decades, there was four to six fold rise in the number of cases of this disease in Iraq [4].

The disease is mainly manifested by fever, enlarged spleen liver, a marked lenkopenia and anemia developed.

The white blood cell count is generally below 4000/ cu mm, accompanied by progressive monocytosis[5]. The anemia is due to a reduced red cell life span and a mild degree of reduction of erythropoiesis. The plasma proteins undergo marked changes, the gamma globulins may constitute 60 to 70 percent of the total protein. Immunity with the leishmanial infection is associated with the stimulation of protective T cell, which produce lymphokines that activate macrophages resulting in the elimination of the intracellular parasite[6]. Interferom – gamma [IFN. γ] also is an important promoter to activated macrophages[7]. In the recent years a considerable attention has been focused on the effect of polysaccharides extracted from bacteria, which have immunological properties.

Polysaccharide antigens have the ability to induce both cellular & humoral immunity response is considered T – independent antigen[8].

MATERIALS & METHODS :

MATERIALS:

Solutions & stains:

Normal saline (0.9% NaCl), phosphate buffer saline (PBS), Hank's Balance salt solution (HBSS), 5% phenol, concentrated H_2SO_4, Glucose (different concentration), Methanol (absolute), Giemsa stain, Türks solution (white blood cell diluted fluid), Bouin's solution (for fixation of tissues), Harris Hematoxylin & Eosin, stain. Media: Nutrient agar (According to Barron[9] et al 1994), Semi-solid media (Al-Bashir[10] 1990), Liquid media (AL-Bashir [11] et al., 1992). Animals :- 174 Male BALB/c mice, 6-8 weeks of age and of 18-20 gm body weight. These animals were obtained from Al-Nahrain medical college. Instruments :- Autoclave, water bath, incubator, centrifuge, improved Neubour chamber, refrigerator & freezer, Magnetic stirrer, Balance, spectrophotometer. Methods : Extraction of polysaccharides from Pseudomonas aeruginosa : Pseudomonas aeruginosa culture obtained from Department of biology – college of science, Al – Mustansiyria University. Polysaccharides (Ps) extraction was prepared according to Taei[12](1992). Quantitative estimation of polysaccharides (Ps) was carried out according to Dubois method[13].

Different concentrations of polysaccharides in 0.5 ml distilled water with 0.5 ml (5% phenol) and 2.5 ml concentrated H_2So_4 mixed and heated in a boiling water bath for 15 minutes. The optical density was read by spectrophotometer at 490nm against blank, which was contained 0.5 ml (5% phenol), 2.5 concentrated H_2So_4 and 0.5 ml Saline.

Different concentrations of Glucose were used to prepare the standard curve. After estimated polysaccharides quantitive, which is 600 µg per ml, sterilized by passing through millipore filter 0.22, stored at – $10C^0$. Parasites and their maintenance Culture of Leishmania donovani [strain MHOM / IQ / BRCI (AA_3)] was obtained from department of biology – college of science, Baghdad – University. Leishmania donovani promastigotes were cultured at $26C^0$ in sterile semi – solid media until they transformed to metacyclic promastigotes. Such promastigote culture were normally ready for use within six to ten days. When adequate numbers of cultured promastigotes were obtained they were harvested and used for infection into mice.

Virulent Parasites:-

These were obtained by inoculating intraperitoneal (i.p) into BALB/c mice and the infection left to develop for one month animals were sacrified and biopsies were taken from the liver and spleen inoculated at stationary phase and washed in sterile PBS by centrifugation. The parasites were counted in a chamber (Improved neubauer chamber) and parasites densities adjusted to give a concentration of 1×10^8 in a 0.2 ml volume of BPS for (i.p) injection into mice. Experimental Protocol :

Note:- each group contains 6 animals.

1. Control groups:-

1.1 Group injected intraperitoneal with 100µg/20gm b. wt. of PS.

1.2 Group was injected i.p. with 150 µg/20g b. wt. of PS.

1.3 Group was injected i.p. with 250 µg/20g b. wt. of PS.

1.4 Groups infected by 1×10^8 i.p. Leishmania donovani. Six animals were sacrified after 2,4,6,8 weeks.

1.5 Group was injected with 0.2 ml PBS i.p.

2. Experimental groups:

2.1 Animal groups treated before infection.

2.1.1 Group of BALB/ C mice treated with 100 µg / 20 g. b wt. of PS on day three before infection.

2.1.2 Group was treated with 100 µg /20 gm. b. wt of PS i.p. on day six before infection.

2.1.3 Group was treated with 100 µg / 20gm. b. wt. of PS i.p. daily for 6 days.

2.1.4 Group was treated with 150 µg / 20 gm. b. wt. of PS i.p. on day 3 before infection.

2.1.5 Group was treated with 150 µg / 20 gm. b. wt. of PS i.p. on day 6 before infection.

2.1.6 Group was treated with 150 µg / 20 gm. b. wt. of PS i.p. daily for 6 days.

2.1.7 Group was treated with 250 µg / 20 gm. b. wt. of PS i.p. on day 3 before infection.

2.1.8 Group was treated with 250 µg / 20 gm. b. wt. of PS i.p. on day 6 before infection.

2.1.9 Group was treated with 250 µg / 20 gm. b. wt. of PS daily for 6 days.

Note: Animals were sacrified after 8 weeks.

2.1 Animal groups were treated after infection.

2.1.1 Groups of BALB/c mice infected with Leishmania donovani were treated with 100 µg / 20 gm. b. wt. of PS i.p. on day 7 post-infection. 6 animals were sacrified after 2,4,6,8 weeks.

2.1.2 Groups of BALB/c mice infected with Leishmania donovani were treated with 150 µg / 20 gm. b. wt. of PS i.p. on day 7 post-infection. 6 animals sacrified after 2,4,6,8 weeks.

2.2.3 Groups of BALB/c mice infected with Leishmania donovani were treated with 250 µg / 20 gm. b. wt. of PS i.p. on day 7 post-infection. 6 animals were sacrified after 2,4,6,8 weeks.

Whit Blood Cell Count & Differential Count:-

1 ml of blood was collected from axillary vein of each mouse into sterile EDTA tubes, and white blood cell counts were done. A blood smears were made & stained with Giemsa stain for differential white blood cell count. These were carried out, according to (Green[14] 1989). Measurement of Spleen & Liver **weight :-** All animals were weighed at the end of experimental time, their spleens and livers aseptically harvested and weighed and organ index was calculated according to (Kroeze & Tanner[15] 1985) as the following:-

$$\text{Organ (liver \& spleen) index} = \frac{Organ\ \text{wt} \times 1000}{Animal\ \text{wt}}$$

Cellular Immune Response:-

Leishmanial skin test antigens were prepared by suspending washed promastigotes of Leishmania donovani (1×10^8 /ml) in a solution of 0.5% phenol saline. For the Leishmanian skin test, mice were injected intradermally with (0.1) ml of Leishmania donovani 1×10^8 /ml in the left leg. The right leg was injected with (0.1) ml phenol saline (control). The footpad thickness was measured with a vernier caliper at 24hr after the injection of Leishmanian antigens, and the difference in thickness between the control and antigen – injected foot was considered to represent antigen specific reactivity[16].

Histopathological Study:-

Samples of tissues from spleen and liver were prepared for histopathological studies, after sacrifing of the mice, fixed in Bouin's solution for several hours, then processed and sections were cut by a microtome 4 – 5 µm in thickness and stained with Haematoxylin and Eosin method according to (Bancroft & stevens[17] 1982). Statistical analysis:- Data were expressed as mean ± standard deviation and a statistical significance was calculated by mean of the student's T – test[18].

RESULTS:-

Total and Differential count of Leukocytes :-

Table (1) shows the changes of WBC & differential count for mice treated with polysaccharides 100, 150, 250 µg PS on (3, 6, daily for six days) before infection with Leishmania donovani, for infected mice and negative mice. WBC were increased for all treated groups in comparison with infected mice.

The higher level of WBC count were obtained in the group immunized with 100 µg of PS daily for six days. Also mice treated with 100, 150, 250 µg of PS alone showed an increase in WBC count in comparison with control negative. While WBC count were decreased in infected mice and reached to (1920). The count were significant in all pre – infection groups ($P < 0.05$). as shown in Fig.1(A). Differential count showed an increase in lymphocyte percent for all treated groups ($P<0.05$), and reached to (87.7%) in group treated with 100 µg daily for six days, while lymphocyte were decreased in infected mice and reached to (74.7%).

Monocyte were decreased ($P<0.05$) for all treated groups, except those treated with 250 µg of polysaccharides on 3,6 days before infection which were increased. Table 2, and Fig. 1 (B) shows the changes in mice groups treated with Polysaccharid. On day seven after infection with Leishmania donovani. The leukocyte count for mice treated with 100 µg PS, 150 µg PS , 250 µg PS on day seven after infection at (2, 4) weeks showed significant increase ($P < 0.05$). Further more lymphocyte percent showed significant increase for the same groups. Weight and Organ Index for spleen and liver :- Table 3, Fig. 2 (A) and Fig. 3 (A) show the change in weight and organ index for spleen & liver in mice groups treated before infection with Leishmania donovani the spleen weight and its index were decreased for all treated groups and reached to (0.118), (4.82) in group immunized with (100) µg. PS daily for six days.

Also the liver and its index were decreased for all treated groups, and reached to (1.13), (46.6) in group immunized with (100) μg PS daily for six days, which was statistically significant ($p<0.05$) for all treated groups in comparison with positive control. The spleen and its index for infected mice were (0.169), (7.08) and for liver were (1.91), (78.14). Table 4, Fig. 2(B), and Fig 3, (B) show the changes in weight and organ index for spleen, and liver in mice treated on day seven after infection with Leishmania donovani. The changes in liver and spleen weight and their organ index for mice treated with (100) μg PS, 150 μg PS, 250 μg PS on day seven-post infection and scarified after (2, 4, 6, 8) weeks, were slightly increased.

Cellular Immune Response :-

This parameter, investigated the potential of polysaccharides extracted from Pseudomonas auroginosa to induce a protective immune response against infection with Leishmania donovani in BALB/c mice. As shown in Table (5), the delayed type hypersensitivity (DTH) response showed no significant increase in infected mice.

The footpad swelling for 100 μg PS treated on day 3, 6, daily for six days before infection were (2.91, 3.54, 3.77), For 150 μg PS were (3.08, 3.06, 3.42) and for 250 μg PS (2.7, 3.64, 3.64), which were significant ($p<0.05$) for all treated groups as shown in Table (5) & Fig. 4 (A).

The footpad swelling for mice treated with 100 μg of polysaccharides on day seven after infection with Leishmania donovani, measured at (2, 4, 6, 8) weeks were (2.128, 2.036, 1.96, 1.608), for 150 μg PS were (2.168, 1.923, 1.53, 1.143).

For 250 μg PS were (1.778, 152, 1.306, 1.15) and for infected mice (1.45, 1.24, 1.15, 1.12) as shown in Table(6)&Fig.4 (B).

Histopathological Study :-

Histological pictures of infected liver for 8 weeks were shown. At 2 weeks, there were granuloma like formation, slight congestion, infiltration of inflammatory cells, hyperplasia of kupffer cells.

At 4 weeks: hepatic granulomas were formed with the same other changes on 2 weeks.

By the 6 and 8th weeks, granulomas increased, fibroblastic and infiltration of mononuclear cells as shown in Fig. (5) and Fig. (6).

In groups treated with 100 μg PS, before infection with Leishmania donovani , liver showed slight congestion, focal aggregation, hyperplasia of kupffer cells and decrease in Leishmania donovani bodies number as shown in Fig. (7).

On the other hands the histological changes of liver in post infection groups treated with PS were slight hypertrophy, little congestion, focal aggregation, granuloma like forming (6, 8 weeks) and hyperplasia of macrophages as shown in Fig. (8).

Infected spleen for 8 weeks showed: an increase in the number of parasitized macrophages and widening of white pulp at 2 week.

At 4 weeks, the splenic architecture was preserved, and there was an increased number of parasitized macrophages in red and white pulp, while at 8 weeks spleen were lost it architecture, severe necrosis, widening of white and red pulp, many collection of macrophages (immature granulomes) containing abundant number of parasites, and fibrosis.

Plasma cells were present in the sinusoids, as shown in Fig. (9).

Treated groups with PS before infection the histological picture of spleen showed a slight hypertrophy, congestion, diffuse hyperplasia, widening of white pulp and Leishmania donovani bodies were less than in infected mice.

Histological changes in spleen traeted with 100 μg daily for six days before infection showed that architecture of it was look like normal as shown in Fig. (10) In treated groups after infection which sacarified after (2, 4, 6, 8) weeks, the histological picture of spleen showed widening of white pulp, hyperplasia of macrophages, congestion, and diffuse hyperplasia. The parasite number and immature granuloma especially at 8 weeks were more than in pre infection groups.

Table (1): Leukocyte and differential count for BALB/ c mice treated with PS on different periods before infection with Lieshmania donovani and compassion with infected mice.

PS concentration	Time before Infection	Leukocyte count		Differential count					
				Lymphocyte%		Neutrophil%		Monocyte %	
		Mean	± SD	Mean	± SD	Mean	± SD	Mean	±SD
100	3	*5920	± 271.29	*86.94	± 0.31	*10.96	± 1.10	*1.82	± 0.69
150	3	*4233.33	± 150.55	*86.9	± 35.48	*11.61	± 4.8	*1.10	± 0.5
250	3	*4016.67	± 204.12	*76.79	± 0.58	*12.64	± 0.176	*0.82	± 0.91
100	6	*7033.33	± 454.72	*87.96	± 0.72	*10.57	± 0.84	*1.20	± 0.53
150	6	*6500	± 275.78	*87.82	± 35.89	*10.17	± 4.2	*1.94	± 0.81
250	6	*4800	± 178.8	*72.64	± 31.27	*12.66	± 0.64	*11.11	± 0.74
100	Daily -6 day	*7983.33	± 222.4	*87.7	± 0.55	*11.89	± 0.65	*0.34	± 0.14
150	Daily -6 day	*6033.33	± 445.72	*85.24	± 34.8	*13.5	± 5.56	*1.16	± 0.54
250	Daily -6 day	*4383	± 299.4	*76.03	± 0.7	*11.40	± 0.98	*12.24	± 1.42
C+	-	1920	± 576.19	74.7	± 2.04	13.11	± 2.1	11.1	± 2.54
C-	-	8697.8	± 635.29	83.9	± 1.02	14.2	±1.09	1.7	± 0.9

C+ Control Positive
C- Control Negative
****P<0.05***
Mean ± standard deviation for six mice

Table (2): Leukocyte and differential count for BALB/ c mice treated with PS on day 7 after infection with Lieshmania donovani and compassion with infected mice.

PS concentration	Weeks after infection	Leukocyte count		Differential count					
				Lymphocyte%		Neutrophil%		Monocyte %	
		Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
100	2	*5610	± 1.54	*88.66	± 0.72	10	± 4.35	1.34	± 1.31
150	2	*5540	± 1.34	*87.7	± 0.54	10.96	± 2.00	1.44	± 0.98
250	2	*5100	± 1.67	*86.94	± 1.00	11.4	± 2.00	1.66	± 1.25
C+	2	3110	± 1.54	81	± 0.81	13.9	± 7.02	6.1	± 1.31
100	4	*5120	± 0.89	*87.7	± 0.51	9.3	± 0.57	2	± 1.51
150	4	*4980	± 0.78	*86.9	± 1.25	9.00	± 4.00	4.1	± 0.51
250	4	*4980	± 0.45	*86.1	± 1.62	12.6	± 2.3	1.3	± 0.48
C+	4	3080	± 1.31	77.67	± 0.81	13.0	± 1.5	9.33	± 0.57
100	6	4900	± 3.21	8524	± 0.91	12.6	± 6.6	2.16	± 0.81
150	6	3090	± 1.34	83.6	± 1.21	13.0	± 3.6	3.4	± 0.4
250	6	3000	± 1.51	83.9	± 3.21	13.66	± 3.21	2.44	± 3.98
C+	6	2420	± 1.41	76.6	± 0.30	13.5	± 2.2	9.9	± 4.00
100	8	3880	± 1.35	83.66	± 0.81	13.00	± 3.2	3.44	± 2.98
150	8	2780	± 1.41	81	± 0.45	13.66	± 3.46	5.34	± 2.31
250	8	2700	± 0.22	80	± 1.2	15.00	± 0.5	5	± 2.31
C+	8	1920	± 0.31	74.7	± 1.22	12.6	± 0.57	12.7	± 3.00

C^+ Control Positive
****P<0.05***
Mean ± standard deviation for six mice

Table (3): Effect of PS on Liver and spleen weight and their index in BALB/ c mice treated with PS on different periods, and comparison with infected mice.

PS concentration	Time before Infection	Spleen weight		Spleen index		Liver weight		Liver index	
		Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
100	3	*0.138	± 0.004	*6.33	± 0.41	*1.33	± 0.064	*56.12	± 2.87
150	3	*0.147	± 0.004	*6.12	± 1.37	*1.37	± 0.08	*58.23	± 4.2
250	3	*0.147	± 1.002	*6.19	± 1.216	*1.45	± 0.03	*61.05	± 2.55
100	6	*0.135	± 0.03	*5.71	± 1.45	*1.16	± 0.02	*45.07	± 2.65
150	6	*0.139	± 0.007	*5.84	± 0.39	*1.26	± 0.03	*47.5	± 0.163
250	6	*0.144	± 0.003	*6.02	± 0.12	*1.53	± 0.03	*61.93	± 4.14
100	Daily -6 day	*0.118	± 0.01	*4.82	± 0.3	*1.13	± 0.66	*46.6	± 2.4
150	Daily -6 day	*0.12	± 1.005	*5.25	± 0.27	*1.27	± 0.05	*48.09	± 1.75
250	Daily -6 day	*0.144	± 0.006	*5.86	± 0.45	*1.46	± 0.03	*50.01	± 1.6
100	activated	*0.147	± 4.0	*6.73	± 0.13	*1.34	± 0.07	*57.37	± 4.31
150	activated	*0.149	± 0.55	*6.58	± 0.63	*.1.23	± 0.01	*55.21	± 3.13
250	activated	*0.149	± 4.3	*6.00	± 0.29	*1.20	± 0.1	*54.07	± 1.70
C+	-	0.169	± 0.01	7.08	± 0.44	1.91	± 0.06	78.14	± 3.3
C-	-	0.09	± 0.001	5.01	± 1.6	1.09	± 0.1	49.5	± 17.7

C+ Control Positive
C- Control Negative
****P<0.05***
Mean ± standard deviation for six mice

Table(4): Effect of PS on spleen and liver weight and their index for BALB / c mice treated with PS on day 7 post infection with leishmania donovani ,and comparison with infected mice .

PS Concentration	Weeks after infection	Spleen weight Mean ± SD	Spleen index Mean ± SD	Liver weight Mean ± SD	Liver index Mean ± SD
100	2	*٠,١٢٩±0.002	*٥,٢±0.45	*٠,٩٩±0.05	*٤٤,٦١+3.02
150	2	*٠,١٣٥±0.002	*٦,٢٣٦+1.87	*١,٠٨±0.03	*٤٩,١٧+1.41
250	2	*٠,١٤±0.001	*٦,٣٦+0.06	*١,١٤±0.04	*٥١,٧٩+2.06
C+	2	٠,١٣٧±0.02	٦,٤١٤±0.099	١,١٣٧±0.16	٥٤,٥٢±1.11
100	4	*٠,١٣٨+0.112	*٥,٦٥+0.98	*١,١٣+0.01	*٥١,٩٧+1.49
150	4	*٠,١٤٥+0.004	*٦,١٥+0.18	*١,١٥+0.007	*٤٨,٨١+1.04
250	4	*٠,١٤١٤+0.003	*٦,٣٦+0.06	*١,٢٤+0.04	*٥١,٧+2.05
C+	4	٠,١٥١+0.04	٦,٥٧+0.16	١,١٩+0.006	٥٣,٣٤+2.31
100	6	*٠,١٥٣±0.115	*٦,٣٤+0.187	*١,٣٥+0.08	*٥٦,٥±1.82
150	6	*٠,١٤٩+0.001	*٦,١٨+0.117	*١,٣٧±0.04	*٥٧,٣١±1.8
250	6	*٠,١٥٢+0.002	*٦,٢١+0.25	*١,٤٥±0.01	*٦٠,٤٦+1.26
C+	6	٠,١٥٨+0.002	٦,٥١+0.057	١,٥±0.017	٦٢,٦٥+0.283
100	8	*٠,١٤٣+0.007	*٦,٤٢+0.32	*١,٥٧+0.03	*٥٦,٦٨+1.9
150	8	*٠,١٤٩±0.001	*٦,١٤±0.21	*١,٤٦+0.03	*٥٩,٥±1.87
250	8	*٠,١٥٧±0.005	*٦,٢+0.49	*١,٤٦+0.03	*٦٠,٠٥±0.61
C+	8	٠,١٥٨±0.002	٦,٧٢±0.314	١,٥١+0.058	٦٣,٢+0.98

C+ Control Positive
*P<0.05
Mean ± standard deviation for six mice

Table (5): Delayed hypersensitivity (mm) in BALB/c mice treated with PS in different periods before infected with Leishmania donovani, and comparison with infected mice.

PS concentration	Time before Infection	Footpad swelling	
		Mean	±SD
100	3	*2.91	± 0.211
150	3	*3.08	± 0.15
250	3	*2.7	± 0.11
100	6	*3.54	± 0.132
150	6	*3.06	± 0.08
250	6	*3.64	± 0.214
100	Daily for 6 day	*3.77	± 0.182
150	Daily for 6 day	*3.42	± 0.145
250	Daily for 6 day	*3.64	± 0.22
100	activated	*2.80	± 0.05
150	activated	*2.64	± 0.17
250	activated	*2.70	± 0.01
C+		1.15	± 0.10
C-		1.35	± 0.13

C+ Control Positive
C- Control Negative
****P<0.05*** ***Mean ± standard deviation for six mice***

Table (6): Delayed hypersensitivity (mm) in BALB/c mice treated with PS on day 7 post infection with Leishmania donovani, and comparison with infected mice.

PS concentration	Weeks post infection	Footpad swelling (mm)	
		Mean	±SD
100	2	*2.128	± 0.158
150	2	*2.168	± 1.646
250	2	*1.778	± 0.05
C+	2	1.45	± 0.05
100	4	*2.036	± 0.042
150	4	*1.923	± 0.065
250	4	*1.52	± 0.04
C+	4	1.242	± 0.01
100	6	*1.96	± 0.038
150	6	*1.53	± 0.83
250	6	*1.306	± 0.01
C+	6	1.154	± 0.32
100	8	*1.608	± 0.044
150	8	*1.143	± 0.124
250	8	*1.15	± 0.10
C+	8	1.12	± 0.04

C+ Control Positive
****P<0.05*** ***Mean ± standard deviation for six mice***

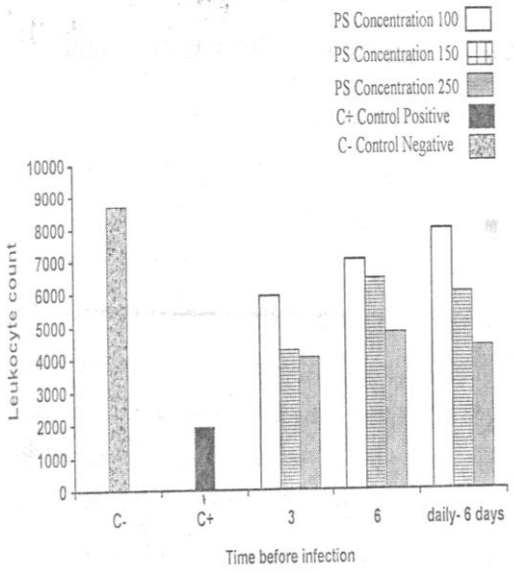

Fig. 1 (A): Leukocyte (WBC) count for BALB/ c mice treated with PS on different periods before infection with *Leishmania donovani* and comparison with infected mice.

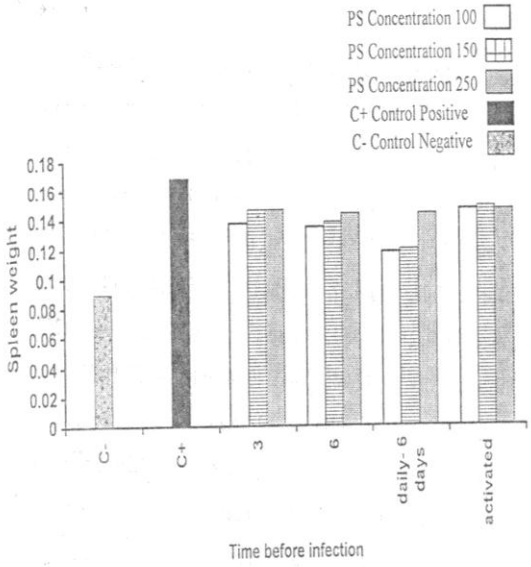

Fig. 2(A): Effect of PS on spleen weight in BALB/ c mice treated with PS on different periods, and comparison with infected mice.

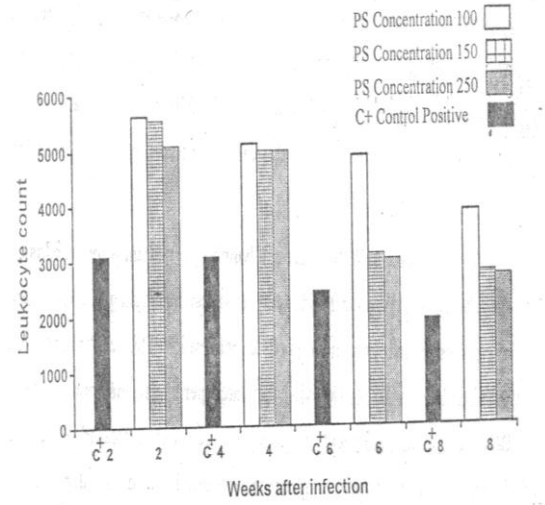

Fig. 1 (B): Leukocyte (WBC) Count for BALB /c mice treated with PS on day 7 after infection with *Leishmania donovani*, and comparison with infected mice

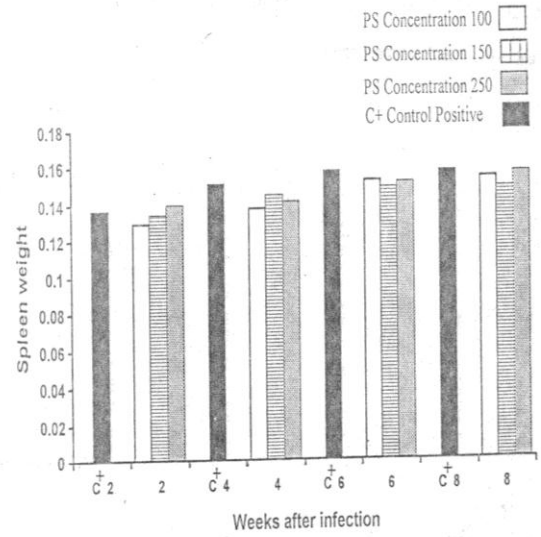

Fig. 2(B): Effect of PS on spleen weight in BALB/c mice , treated with PS on day 7 post infection with *Leishmania donovani*, and comparison with infected mice

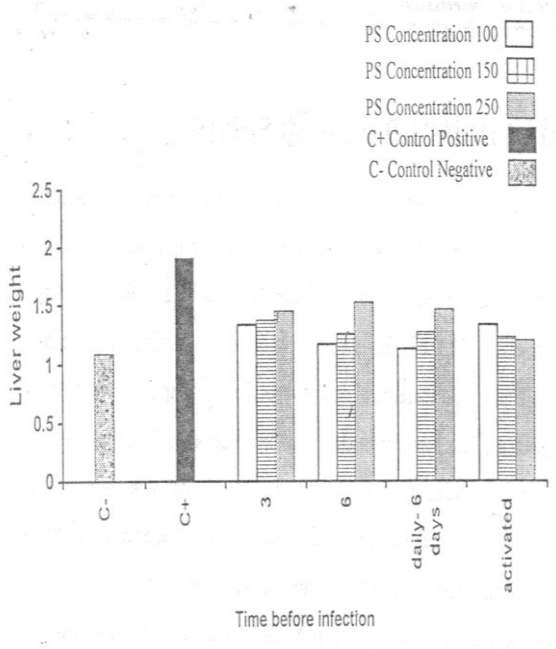

Fig. 3(A): Effect of PS on Liver weight in BALB/ c mice treated with PS on different periods, and comparison with infected mice.

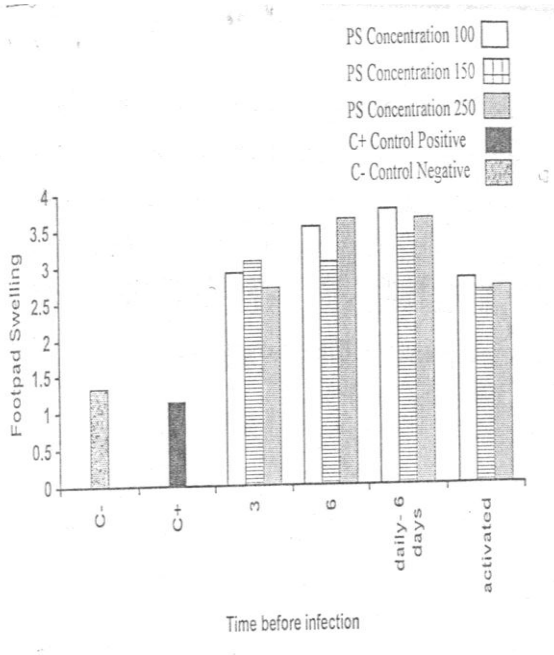

Fig. 4(A): Delayed hypersensitivity (mm) in BALB/c mice treated with PS in different periods before infected with *Leishmania donovani*, and comparison with infected mice.

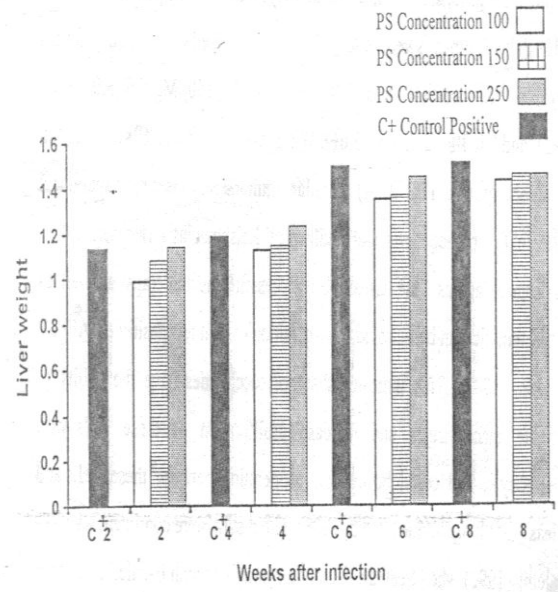

Fig. 3(B): Effect of PS on Liver weight in BALB/c mice treated with PS on day 7 post infection with *Leishmania donovani*, and comparison with infected mice

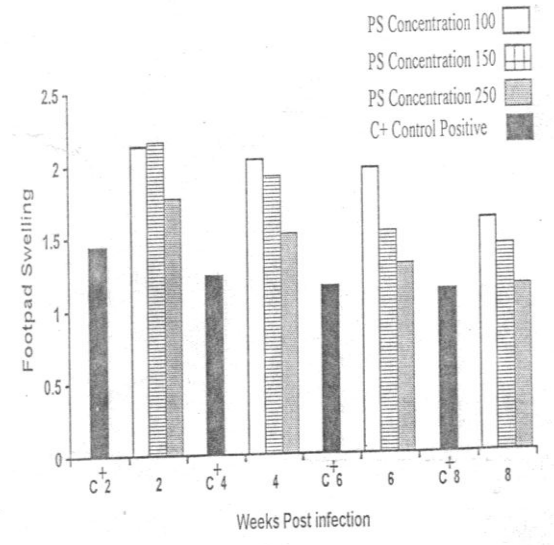

Fig. 4(B): Delayed hypersensitivity (mm) in BALB/c mice treated with PS on day 7 post infection with *Leishmania- donovani*, and comparison with infected mice.

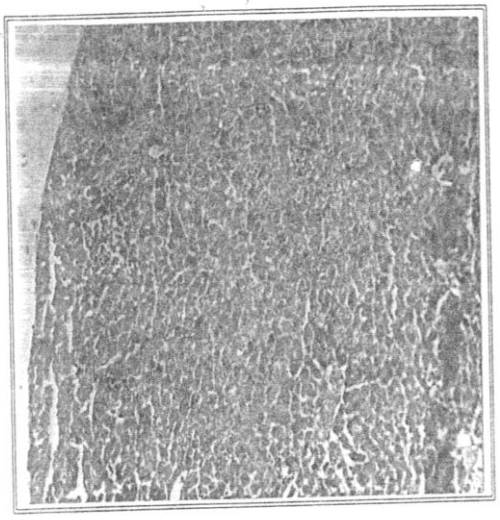

Fig. 5 Section of liver, infected with L. donvoni after 4 weeks, showing : slight necrosis with few inflamentary cell infiltration with early formed granuloma. X(240), H & E stain

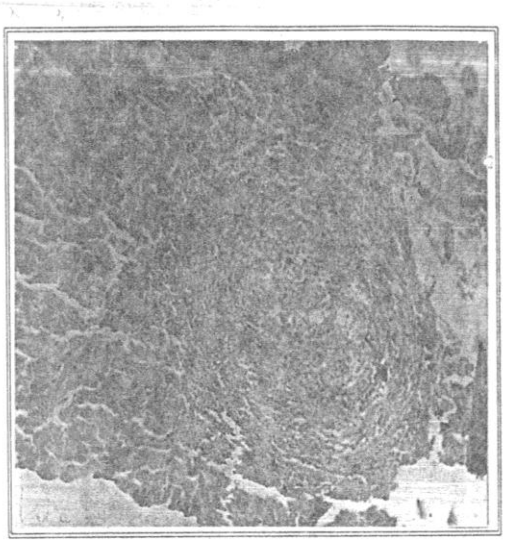

Fig. 6 Section of liver, infected with L. donvoni after 8 weeks, showing : granuloma forming . X(240), H & E stain.

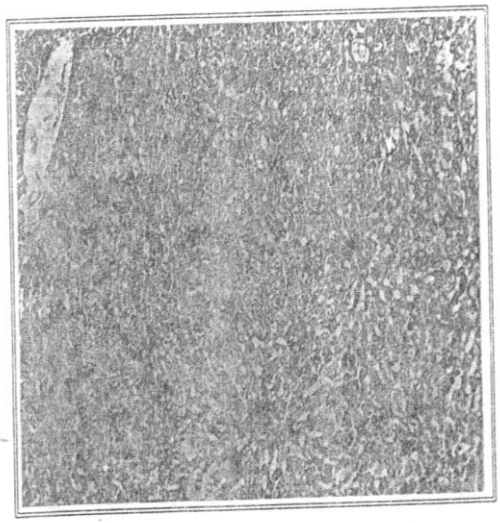

Fig. 8 Section of liver, treated with 100 mg ps. On day 7 after infection , showing : slight sinusoidal dilatation & look normal . X (240) H & E stain

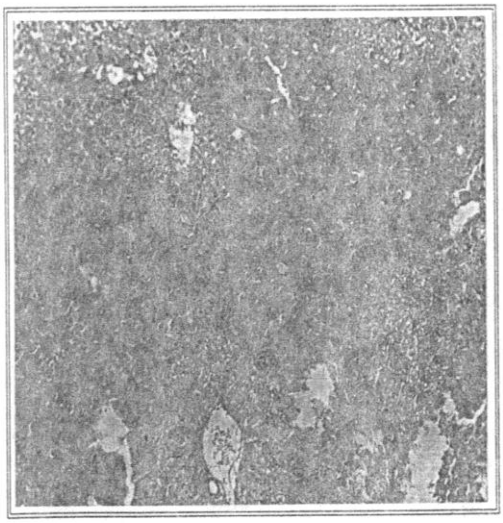

Fig. 7 Section of liver, treated with 100 mg ps. Daily for 6 days, before infection showing : hypertrophy of hepatocyte, hyperplasia of kupffer cells X 240 .H an E stain

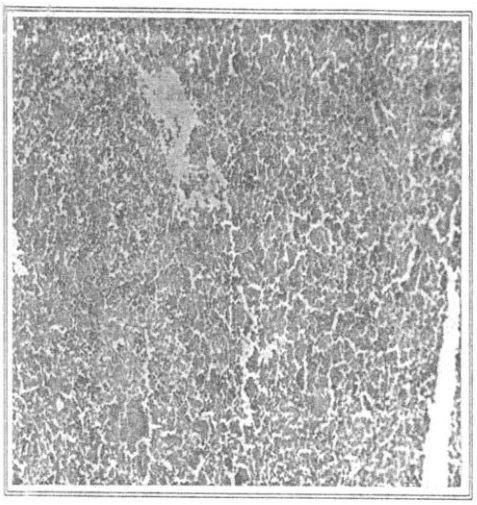

Fig. 9 Section of spleen, infected with L. donvoni after 8 weeks, showing : multiple focal necrosis. X(240), H & E stain.

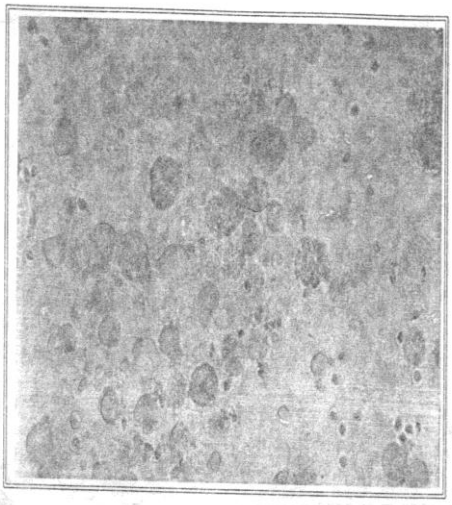

Fig. (11)

Impression smear showing : amastigotes of L. donovani. X (330) H & E stain

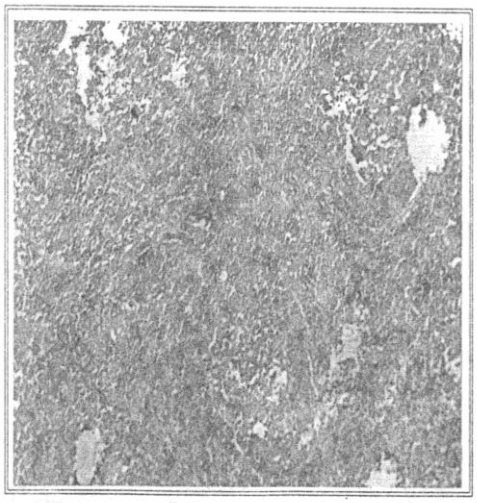

Fig. 10 Section of spleen, treated with 100 mg ps. Daily for 6 days, before infection
showing : widening red pulp activation (hyperplasia) X 240 .H & E stain

DISCUSSION:

The increase in WBC count and lymphocyte percent were dependant on the ability of polysaccharides on activation macrophages and secrete monokines which help in lymphocyte proliferation. These results were consistent with Reuben[19] et al., (1978) when activated peritoneal macrophages with BCG, the WBC count and lymphocyte percent were increased, While the decrease in WBC count & lymphocyte percent in infected mice depends on the infiltration of WBC from the blood to parasite position[20].

The decrease in monocyte was attribute to immigration of it from blood to parasite position and confirm to macrophages[21] by effective immunomodulator, while the immigration of monocyte decreased in infected mice.

The neutrophil persent was not significant as compared to infected mice. This was consistent with Kroese[22] & Tanner (1987) when they found mild increase and not significant for neutrophil percent in infected mice for 60 days. The results of this study showed that treatment with PS before infection caused more changes in total and differential counts of leukocytes than post-infection, which agreed with a previous study[19]. The decreased weight & index of liver and spleen in pre-infection groups was consistent with Al – Delemi[23] (1992) when pre-infection mice treated with Esculetin daily for six days, the spleen and liver weight were decreased. Also spleen and liver weight reduced to nearly normal weight when treated the visceral Leishmaniasis with interferon gamma conjucate muramyltripeptid daily for five days[24].

The increased weights of liver and spleen in infected mice, noticed in this study, were also observed by Ali & Afrin[25] (1998) when reported that liver and spleen weight had increased by 1.5 and 4.7 fold, correlating with visceral proliferation of amastigotes in these organs. Also in this study, the liver and spleen weights of the mice groups treated with PS after infection, were decreased. The same results reported by Riffat[26] et al., (1989) when treated visceral Leishmaniasis with ivermectin. The absence of parasite-specific cellular immune responses in infected mice, apparently related to an antigen-specific suppressor T-cell, and an indomethacin-sensitive adherent cell that suppresses both specific and non-specific responses. This result was consistent with Reiner[27] (1982) when examined immunological changes in BALB/c mice infected with *Leishmania donovani*.

The low level of parasite-specific delayed type hyper sensitivity (DTH) responses in positive control correlated with disease progression in mice[28], While the groups immunized with polysaccharides expressed a strong DTH. Cellular immune responses play an important role in the pathogenesis and healing of leishmaniasis[29]. Resistance against the disease by successful chemotherapy a well as asymptomatic can be assessed by acquisition of skin test positivity[28]. This indicated that polysaccharides have the ability to activate macrophages and increase proliferation of these cells with lymphocyte, and could be cellular cooperation between these cells and releases of lymphokines[30]. The results were consistent with Al-Delemi[23] (1992) when treated visceral Leishmaniasis with Esculetin daily for six days, the DTH were increased. The histopathological changes of infected liver reported in this study were consistent with Squires[32] et al (1989) when described granuloma changes in liver for 24 weeks. The increase in mature granuloma were correlated with cell-immune responses as shown in our results when DTH were decreased along the infected period. This was consistent with Turk & Bryceson[33], (1971). Also the increase of mature granuloma were correlated with liver weight which increased along infected period as shown in our results. Histogical pictures of mice liver study treated with PS before infection were consistent with consistent with Squires[34] et al., (1990) when treated BALB/c mice with cytokines before infected with *Leishmania donovani*. These hepatic changes were correlated with increase in immune-cell response which shown as an increase in DTH in our results. These agreed also with Al-Delemi[23] (1992) when treated pre infected mice with Esculetin. The histopathological changes in liver of treated groups with PS after infection i.e little congestion, hyperplasia of macrophages, granuloma like forming. These results were correlated with increase in liver weight and reduction in parasites number in post infection groups. And also correlated with decrease in DTH reaction at 6^{th} and 8^{th} weeks.

These results were consistent with Squires[32] et al., (1989) when treated visceral Leishmaniasis with interferon gamma. Histological pictures showed that treatment especially with 100 μg of PS daily for six days pre-infection were of more protective effects.

The histopathological changes in infected spleen for 8 weeks were consistent with Gutierres[35] et al., (1984).

The cause of necrosis depends on immune reaction against lymphocyte and Leishmanial antigens.

The decrease in lymphocyte will lead to inhibition of immune cell response[36]. The decrease in B & T lymphocyte leads to decrease in cell–mediated immune response, which determine whether the infection remains localized or become generalized. The same results were shown by Gutierres[35] et al (1984). The histological changes (immature granuloma) and decrease in cell-mediated immunity are correlated with increase in parasite number, infection percent, and increase in spleen weight Al – Shanawi[37] et al (1993) reported the same changes after 8 weeks of infected golden hamster with Leishmania major. The aggregation of macrophages and lymphocyte depends on activation by PS, which raise the cell mediated immune response[23].

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