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Immunological studies on albino rats against crude endotoxins of *Aeromonas hydrophila*

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ABSTRACT

The genus *Aeromonas* comprises a group of organisms widely distributed in the environment, isolated from water, shallow tube wells, dug wells, piped water supply from sewage, children and adults with diarrhoea, and associated with disease in animals and man. The present work studied the effect of crude endotoxin injection of such bacteria on the immunological response (innate, humoral and cellular) of albino rats and histochemical changes of the nucleoprotein content within the frozen sections of spleen and thymus. Animals were grouped into two groups; the first group was injected intraperiteonally once every 3 days for 21 days with a crude endotoxin of *Aeromonas hydrophila* (AhE) as a dose of 8 mg protein/ 0.5 ml PBS/ rat, while the second group remained as control group, injected at the same intervals with an equivalent volume (0.5 ml) of PBS. The results showed a depletion in the total WBCs and lymphocytes, while the neutorphils and monocytes fluctuated. The innate immune response was greatly increased. The endotoxin increased the humoral immune response, while the cell-mediated immune response was decreased.

KEYWORDS*: Aeromonas,* endotoxins, and immunity.

INTRODUCTION

Aeromonas sp. are members of the true Gram-negative bacteria (Eubacteria) with rod-shaped structure (Popoff 1984). The genus *Aeromonas* comprises a group of organisms widely distributed in the environment and isolated from water, shallow tube wells, dug wells, piped water supply from sewage (Ho *et al.* 1990), children and adults with diarrhea (Singh & Sanyal 1993), and associated with disease in animals and man (Majeed & Macrae 1994). Motile aeromonads, on the other hand, have been reported to cause various infections in man being most commonly implicated in diarrhoea wound infections, septicemia, meningitis, pneumonia and gastroenteritis (Xu *et al.* 1998). The bacterial components released in the bloodstream can lead to a fatal syndrome known as septic shock. There are about 500,000 new episodes of septic shock each year in the USA, with an associated mortality of 35% (death rate of 7.9 per 100,000 population). Approximately 50-60% of septic-shock episodes are associated with Gram-negative bacteria, and particularly with their LPS component. The exaggerated response leads to a cascade of pathophysiological events termed sepsis and in the USA alone, the annual number of deaths caused by sepsis (~ 70,000) is comparable with that caused by AIDS (Chaby 1999). Todar (1997) and Salton & Kim (2000) described the endotoxins as a part of the outer cell wall of Gram-negative bacteria. The biological activity of endotoxin is associated with the lipopolysaccharide complex, which envelops Gram-negative bacteria by forming part of the outer leaflet of the outer membrane structure. Usually, LPS molecules have three regions: the lipid A structure required for insertion in the outer leaflet of the outer membrane bilayer; a covalently attached core; and polysaccharide chains linked to the core. The later constitute the O-antigens of the Gram-negative bacteria, while the individual monosaccharide constituents confer serological specificity on these components.

The present work was intiated to study the effect of the bacterial toxic antigens through haematological (white blood cells; total and differential count), and immunological studies (innate, humoral and cellular effects) together with the histochemical changes of the nucleoprotein content within frozen sections of spleen and thymus

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MATERIALS AND METHODS

Experimental Animals*:* White male albino rats (Rattus norvegicus) weighing 140-180 g were kept under observation for 15 days prior to the experiment to exclude any intercurrent infection.

Antigen Preparation: The strain used in this study was identified as A. hydrophila (A-47) on the basis of morphology and biochemical characteristics by the Microbiology Dept., Faculty of Science, Al-Azhar University. The crude endotoxin of Aeromonas hydrophila-47 was estimated according to Schill et al. (1984). The total crude protein content of the obtained endotoxin was estimated as described by Layne (1963).

Experimental Design: Animals were grouped into two groups; the first group was injected intraperitoneally, once every 3 days for 21 days with 8 mg of crude endotoxin of Aeromonas hydrophila (AhE) as (8 mg protein/ 0.5 ml phosphate buffer saline solution (PBS) /rat). While the second group was remained as control group and injected by equivalent volume (0.5 ml) of PBS. Six animals from each group were scarified one every 3days for 21 days.

Haematological Studies: Total and Differential White Blood Cell (WBCs) Counts: Counting of the total white blood cell (WBCs) as well as the differential leukocytic count was calculated according to Dacie & Lewis (1991).

Immunological Investigations:

Innate Immune Response:

Phagocytosis (*in vitro*): Phagocytosis of polymorphnuclear cells using Candida albicans yeast was performed according to the method described by Wilkinson (1981). Radial immunodiffusion (RID) plates for quantitative determination of complement 3 (C3) in serum:The plate used in the present study was purchased from Bioscientifica CO. Argentina. The logarithm of the C_3 concentration is proportionate to the diameter of the ring.

Humoral Immune Response:

Detection of agglutinating antibodies: Agglutinating antibody titers to the microbial antigens were assessed using the passive haemagglutination technique described by Hudson & Hay (1980).

Detection of precipitating antibodies: Precipitating antibodies were detected in control as well as treated serum using the immuno-double-diffusion technique described by Hudson & Hay (1980).

Preparation of conditioned media (CM): Conditioned medium (CM) for the isolated splenocytes and lymphocytes was prepared according to the method of Gospondarowicz (1975).

Preparation of cell lysates (CL): Cell lysate (CL) for the isolated splenocytes and lymphocytes of treated cultures was prepared according to the method of Mimuro *et al.* (1987). Turbidity test for estimation of total immunoglobulins levels: The technique was carried out according to Pfeiffer *et al.* (1977). The calibration curve was done by preparing a series of known immunoglobulin concentrations of human sera (INCSTAR corporation, Science Tehcnology Stillwater, MN 55082, Lot No.97148;1-5 Cat. No., 86100). Also, this method was applied for the estimation of the total immunoglobulin levels in the conditioned and cell lysate media for both splenocytic and lymphocytic cell cultures.

Cell-mediated immune response

Immunocytoadherance rosette forming cells (RFC): The ability of treated T-cells to form a rosette shape with sensitized sheep red blood cells was estimated as described by Hudson & Hay (1980).

Migration inhibition factor (M.I.F):- It was estimated as described by (Rocklin 1975) and expressed migration index:

$$\frac{\text{Average area of migration with antigen}}{\text{Average area of migration without antigen}}$$

Histochemical staining for nucleoproteins (DNA &RNA): This is carried out by using Unna-papenheim (Methylgreen-pyronin) stain. Methyl green is regarded as being specific for DNA and the pyronin, if controlled by ribonuclease extraction, specifically demonstrates DNA (Bancroft & Cook 1994). DNA stains with green colour & RNA will stain red colour.
Statistical analysis: Data obtained within the present work were statistically evaluated for the mean, and standard error (S. E.) of the mean for each group through the experimental time using Student's "t" test according to Snedecor & Cochran (1980).

RESULTS

It was found that the crude bacterial endotoxin injection caused a decrease effect upon the total count of WBCs at all periods of injection (Table 1). The "t" test analysis indicated that the AhE injection produced significant decrease of total WBCs at the 3rd, 18th and 21st days (with $P<0.05$) and highly significant decrease at the 12th (with $P<0.01$) days only. Regarding the changes in the differential lymphocytic count, the data showed a decrease effect as a result of injection of the toxin and the change was significant at 15th and 18th day's post-injection (with $P< 0.05$). On the other hand, the crude endotoxin (AhE) caused a contrary effect since it causes an elevation of monocytes percentage till the 9th day, then decreased as the period extended beyond this point. This effect was non-significant through the experimental period.
The percentage of the neutrophils exhibited an increase as a result of treatment of AhE from the 9th day till the end of the experiment. These changes were significant at 12th days (with $P<0.05$) and highly significant at 3rd and 15th days (with $P<0.01$). Also eosinophils percentage showed a decreased effect at all experimental periods of crude AhE treatment except at the 3rd and 6th days. Moreover, the effect of endotoxin injection was only significant at 6th days (with $P<0.05$). On the other hand, basophils percentage was not detected allover the experimental time during the injection of the bacterial toxins.

In all tables: AhE = *Aeromonas hydrophila* endotoxins. N =6 (mean ± S.E.), * Significant ($P<0.05$), ** Highly significant ($P<0.01$) and *** Very highly significant ($P<0.001$). L: Lymphocytes, M: Monocytes; N: Neutrophils; E:Eosinophils and B: Basophils N.D. not detected.

Table 1: Effect of intraperitoneal (i.p.) injection of bacterial endotoxins (8 mg/ 0.5ml AhE) on the total (cells $x10^3$) and differential (cell %) leucocytic counts of albino rats.

Time/ days	Group	Total WBCs Cellx10^3	L (cell %)	M (cell %)	N (cell %)	E (cell %)	B (cell %)
0	Control	6.644±1.393	84.30±4.969	0.98±0.102	14.11±0.861	0.61±0.129	N. D
3	Control	7.520±1.109	83.78±5.273	0.95±0.849	13.88±1.109	0.68±0.400	N. D
	AhE	3.408±0.775*	90.00±1.523	1.00±0.707	8.60±1.091**	0.40±0.224	N. D
6	Control	6.0±1.273	82.75±1.744	1.25±0.334	14.67±1.763	0.33±0.129	N. D
	AhE	4.475±1.072	83.80±1.483	1.80±0.532	11.40±0.939	3.20±1.095*	N. D
9	Control	6.125± 1.613	84.30±4.680	1.07±0.333	13.36±3.413	0.97±0.409	N. D
	AhE	2.87±0.404	83.60±2.276	1.40±0.550	14.60±2.310	1.40±0.551	N. D
12	Control	7.315±0.460	83.00±0.454	1.17±0.151	14.42±1.706	0.78±0.073	N. D
	AhE	3.68±0.768**	78.57±2.664	0.75±0.680	22.428±2.632*	0.714±0.308	N. D
15	Control	7.115± 1.102	82.97±1.677	1.13±0.306	12.52±0.306	0.81±0.077	N. D
	AhE	5.020±0.533	76.00±2.553*	0.69±0.817	22.25±2.512**	0.62±0.102	N. D
18	Control	6.980±0.831	84.65±1.332	1.08±0.192	14.98±0.906	0.53±0.496	N. D
	AhE	4.250±0.459*	80.57±1.005*	0.60±0.480	18.47±2.735	0.62±0.644	N. D
21	Control	6.820±1.284	84.37±1.688	1.48±0.239	13.73±0.861	0.45±0.481	N. D
	AhE	3.270±0.402*	80.37±2.015	0.386±0.740	17.00±2.592	0.60±0.575	N. D

Immunological Investigations

Innate immune response: The data represented the neutrophils phagocytic activity *in vitro* are presented in Table 2. The microbial antigen produced non-significant decrease effect that

reached its peak at the 21st days of injection. The data of serum complement 3 (C_3) of albino rats given interperitonealy AhE were represented in Table 2. On contrast to the change in phagocytic activity in tested animals, C3 was increased post-AhE injection and the maximum effect was pointed at the end of the experimental period (21st day) with a very highly significance increase ($P<0.001$).

Humoral immune response: The recorded values of the agglutination titre of control and treated groups are represented in Table 3. The estimated value of control rats was increased as a result of intraperitoneal injection of AhE. Moreover, the administration of AhE crude toxin produced significant increase at the 9th day ($P<0.05$) and very highly significant increase at the 21st day ($P<0.001$). The agar double diffusion precipitation technique was represented in Table 3 and showed that injection with AhE injection increase the precipitating antibodies till the 12th day post-injection as compared with the control group.

The crude bacterial toxin produced an increase effect on the total level of serum immunoglobulins and the obtained data are presented in Table 4. AhE toxin caused a significant increase at the 12th, 15th, and 18th days post-injection, but the maximum effect was produced at the 12th day. Data of total immunoglobulin concentration in the conditioned medium of splenocytic and the lymphocytic cell cultures of albino rats are represented in Table 5. Concerning the conditioned medium of the splenocytic cell culture, total immunoglobulin level exhibited an increase at all experimental periods of AhE injection. Of all these changes, only the injection with AhE at the 3rd day produced a significant increase ($P<0.05$). Regarding the conditioned medium of the lymphocytic cell culture, the injection with AhE resulted in an increase effect at all experimental periods. Injection with AhE for 3 days produced a significant increase ($P<0.05$).

The total immunoglobulin concentration in the cell lysate medium of splenocytes and the lymphocytic cell cultures of albino rats are presented in Table 6. Concerning splenocytic cell culture, the injection of AhE caused marked elevation of total immunoglobulin concentration at the 3rd and 21st days. Furthermore, the most potent effect was obtained at the 21st day post-AhE injection. It was also found that all changes of total immunoglobulin level in splenocytic cell lysate were non-significant as compared with their control except at the 15th day post-AhE toxin injection, it was to be significant increase ($P<0.05$). On the other hand, immunoglobulin concentration in the cell lysate of the lymphocytic cell culture, were increased at all experimental periods after treatment with the AhE Also, it was noticed that the increased effect was more pronounced as the experimental period extended after treatment with AhE. The "t" test analysis indicated that the AhE treatment caused non-significant effect ($p>0.05$) at the 15th day, significant ($P<0.05$) at the 3rd day, highly significant ($P<0.01$) effect at the 9th day and very highly significant ($P<0.001$) effect at the 21st the experiment.

Table 2: Effect of i. p. injection of bacterial endotoxins (8 mg/ 0.5ml AhE) on neutrophil phagocytic activity *in vitro* and the level of complement 3 in serum of albino rats.

Time/ days	Group	Neutrophil phagocytic activity (%)	Complement 3 (C_3) (mg %)
0	Control	67.951 ± 3.398	160.000 ± 2.243
3	AhE	70.259 ± 2.191	174.333 ± 4.299*
6	AhE	73.684 ± 3.941	167.333 ± 1.018*
9	AhE	74.219 ± 4.596	173.676 ± 1.202***
12	AhE	76.357 ± 1.698	170.000 ± 1.475**
15	AhE	70.267 ± 3.013	168.333 ± 1.389*
18	AhE	66.354 ± 2.628	170.528 ± 2.023**
21	AhE	58.952 ± 3.409	175.000 ± 1.527***

Table 3: Effect of i.p. injection of bacterial endotoxins (8 mg/ 0.5ml AhE) on the humoral response; agglutination titre (- log2) and the precipitating antibodies of albino rats.

Time/ days	Group	Agglutination Titre (-Log2)	Precipitating antibodies
0	Control	1.666 ± 0.235	---
3	AhE	2.500 ± 0.707	+
6	AhE	2.350 ± 0.390	++
9	AhE	2.300 ±0.144*	++
12	AhE	1.715 ± 0.499	+++
15	AhE	2.000 ± 0.577	++
18	AhE	2.100 ± 0.559	++
21	AhE	2.23 ±0.333**	+

Cell-mediated immune response: The results of cell mediated response (Table 7) showed that enumeration of the rosette forming cells was greatly affected as a result of intraperitoneal injection AhE toxin. It was markedly decreased at all experimental periods of AhE endotoxin injection except at the 12th and 21st days. Of these changes, only the group treated with AhE endotoxin for 15 days significantly decreased ($P<0.05$) as compared with control.

The data of migration inhibition factor (MIF) released by the activated leucocytes (Table 7) and showed that the tested toxin produced a potent decrease effect at 3rd and 6th days. After the 6th day of injection, the change was tolerated as the experimental period extended to the 18th day. At the end of the experimental period, while AhE toxin caused an increase of MIF, it was significant ($P<0.05$) only at the 6th day of AhE toxin injection.

Concerning the methyl-green pyronine stain for nucleoproteins within the frozen sections of spleen and thymus for both treated and control groups, the obtained data showed that spleen sections of the *Aeromonas hydrophila* endotoxin (AhE) group showed a wide hyperactivation and hyperplasia of the germinal center that associated with more pyroninophilic cells (Figs. 3 & 4). The activated lymphoid follicle, mental zone and germinal centers returned to resemble the control sections at the 15th day post-injection (Fig. 5). Regarding to the thymus sections, methyl-green pyronine stain showed that the injection with *Aeromonas hydrophila* endotoxin (AhE) showed a wide active germinal center associated by the presence of many of pyroniphilic lymphoblasts (Figs. 6, 7 and 8). This character was diminished gradually and resembles the control section at the 15th post-injection (Fig .9).

Table 4: Effect of i.p. injection of bacterial endotoxins (8 mg/ 0.5 ml AhE) on total immunoglobulins in serum of albino rats.

Time/ days	Control	AhE
0-day	1114.3 ± 88.99	1114.3± 88.99
3	1191.1± 52.37	1141.5± 30.13
6	1293.1± 71.67	1390.3± 75.24
9	1280.3± 75.71	1432.8± 59.37
12	1175.2± 80.29	1513.7±63.89*
15	1248.5± 68.38	1485.5±71.61*
18	1067.4± 61.42	1325.0±87.54*
21	1079.7± 50.39	1235.0±69.12

Table 5: Effect of i.p. injection of bacterial endotoxins (8 mg/ 0.5ml AhE) on total immunoglobulins level (mg %) in conditioned medium (CM) of (A) splenocytic cell culture and (B) lymphocytic cell culture of albino rats

Time/ days	Group	(A) Splenocytic Conditioned medium	(B) Lymphocytic Conditioned medium
0	Control	14.902 ± 2.175	19.678 ± 3.416
3	Control	13.364 ± 2.090	21.415 ± 2.917
	AhE	25.606 ± 3.437*	31.739 ± 2.838*
9	Control	15.768 ± 2.654	23.319 ± 2.903
	AhE	18.932 ± 6.299	29.175 ± 1.759
15	Control	18.103 ± 4.262	26.721 ± 2.089
	AhE	20.046 ± 3.608	35.081 ±4.773
21	Control	16.918 ± 2.610	23.502 ± 2.654
	AhE	16.705 ± 2.727	30.387 ± 2.956*

Table 6: Effect of i.p. injection of bacterial endotoxins on total immunoglobulins level (mg %) in cell lysate (CL) of (A) splenocytic cell culture and (B) lymphocytic cell culture of albino rats.

Time/ days	Group	(A) Splenocytic Cell lysate	(B) Lymphocytic Cell lysate
0	Control	12.5 ±2.1	11.3 ±1.3
3	Control	13.5 ±1.7	10.0 ±1.7
	AhE	17.8±2.8	16.6 ± 1.3*
9	Control	14.5 ±1.3	11.2 ±1.2
	AhE	16.0 ±3.4	21.2 ±2.8**
15	Control	13.5 ±1.4	16.6 ±1.6
	AhE	19.6 ± 1.9*	21.3 ±3.4
21	Control	15.6 ±1.7	13.5 ± 1.6
	AhE	21.3 ±3.9	23.3 ±1.3***

Table 7: Effect of i.p. injection of bacterial endotoxins on cell-mediated response; rosette forming cells (RFC) and migration inhibition factor (MIF) of albino rats.

Time/ days	Group	Rosette forming cells (cells/ H.P.F)	MIF
0	Control	5.00 ± 1.07	1.0 ± 0.32
3	AhE	3.03 ± 0.55	0.29 ± 0.02
6	AhE	3.11 ± 0.38	0.20 ± 0.01*
9	AhE	3.57 ± 0.34	0.38 ± 0.01
12	AhE	5.00 ± 0.76	0.65 ± 0.09
15	AhE	2.00 ± 0.38*	0.91 ± 0.02
18	AhE	2.98 ± 0.41	0.89 ± 0.023
21	AhE	4.94 ± 0.66	1.41 ± 0.13

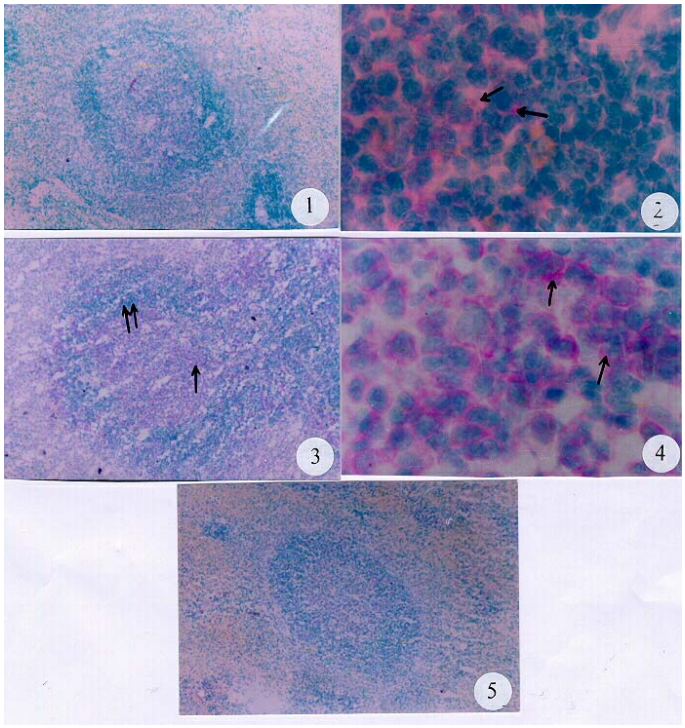

Fig. 1: Photograph of spleen frozen section of control male albino rat shows the normal distribution of splenocytes through the whit pulp (MGPx10).
Fig. 2: Photograph of spleen frozen section of control male albino rat shows the mild reaction of some pyrinophilic splenocytes (▲) (MGPx100).
Fig. 3: Photograph of spleen frozen section of male albino rat experimentally i.p. injected with AHE at 3rd day shows a hyperactivation (▲) and hyperplasia (▲▲) represented by more pyroniphilic cells (MGPx10).
Fig. 4: Photograph of spleen frozen section of male albino rat experimentally i.p. injected with AHE at 3rd day shows a germinal center rich with pyroninophilic (▲) (MGPx100).
Fig. 5: Photograph of spleen frozen section of male albino rat experimentally i.p. injected with AHE at 15rthday shows the lymphoid follicle resembling the normal sections (MGPx10).

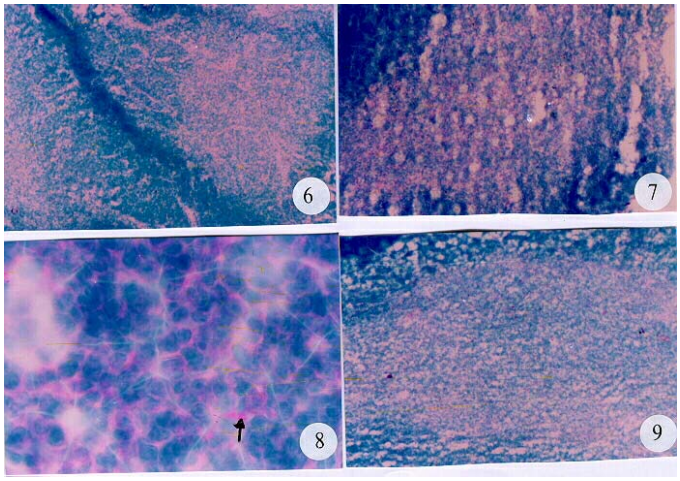

Fig. 6: Photograph of thymus frozen section of control male albino rat shows the normal distribution of thymocytes through the cortical (MGPx10).
Fig. 7: Photograph of thymus frozen section of male albino rat experimentally i.p. injected with AHE at 3rd day miled active germinal center rich with pyroninophilic cells (MGPx10).
Fig. 8: Photograph of thymus frozen section of male albino rat experimentally i.p. injected with AHE at 3rd day miled active germinal center rich with pyroninophilic cells (MGPx100).
Fig. 9: Photograph of thymus frozen section of male albino rat experimentally i.p. injected with AHE at 15rthday shows the lymphoid follicle resembling the normal sections (MGPx10).

DISCUSSION

The depletion in the Total WBCs count may be due to a direct result of WBC destruction during the killing of bacterial or to the exposure to the toxic effect of the bacterial endotoxins (El-Feki *et al.* 1993) The depletion in the differential lymphocytic count in *Aeromonas hydrophila* endotoxin group may be due to lymphocyte arrest, i.e. an inhibition of their release into the blood for two reasons: (1): increase of lymphocyte-like cell density and (2): a massive proliferation that could cause the increase in their mean percentage (Sovenyl *et al.* 1990). Data on mammals showed that LPS induce a depletion of lymphocytes in the lymphoid organs in LPS-sensitive mice but not in LPS-resistant mice (Norimatsu *et al.* 1995).
The recorded temporally increase in the monocytes at the 3rd, 6th, and 9th days post-endotoxin injection, may be due to the chemotactic response to the lipid rich material of LPS of the bacterial endotoxin (Benjamine 1984). Monocytes have the ability to modify, deacylate and dephosphorylate the chemical nature of the three enterobacterial LPS moieties (Lipid A,

oligosaccharide core and O-chain) and as a consequence alter the electrophoretic mobility of these molecules and their reactivity against specific antibodies (Forestier *et al.* 1999).
Recent data showed that the LPS increases some chemokine proteins such as macrophage inflammatory proteins (MIP-1α & MIP-2β), and monocyte chemoattractant protein (MCP-1) (Luster 1998). MCP-1 is considered as prototypic chemokine with chemoattractant activity for mononuclear cells, and may be have an anti-inflammatory role during endotoxemia (Olszyna *et al.* 2000). This may explain the sharp decline in the monocytic count in the endotoxin group due to the tissue demand in the inflammatory regions.
The prompt neutropenia that was recorded in *Aeromonas hydrophila* endotoxin group may be due to the migration of neutrophils across the epithelium from the blood pool to tissue damaged area (Haraoka *et al.* 1999). The recorded neutrophilia in the same group from the 9th day post-injection till the end of the experiment may be due to the stress of the associated rapid neutrophils proliferation: the neutrophils have azurophilic granules containing bactericidal-permeability increasing protein (BPI) which inhibits the biological activity of LPS (Schultz *et al.* 2000). The elevation in eosinophilic count at the 6th day post-endotoxin injection, may be due to the fact that eosinophils neutrilize the effect of histamine that is release through tissue destruction as a result of the toxic effect of the endotoxin (Benjamine 1984). Despite this, basophils were not detected at all during experiment.
The increase in phagocytic activity in the present study may be related to the high affinity, binding and phagocytosis of red blood cells' debris by Kupffer cells pre-incubated with bacterial endotoxin (Steffan & Kirn 1986). The elevation in the phagocytic index may also be due to an increase in the endotoxin-coated RBCs debris as a result of the action of bacterial haemolysin or other bacterial factors, such as phospholipase (Brenden & Huizinga 1986). Abu El-Saad (1996) suggested that the fixed reticulocytes within spleen sections injected with *Aeromonas hydrophila* pathogen will transformed into migrating macrophages, thus increasing the phagocytic activity. The elevated monocytes and neutrophils have a surface anchor protein CD14, that plays a role in the following biological actions and finally enhances the phagocytic activity: 1) attachment and internalization of Gram-negative bacteria (Wright *et al.* 1990); 2) an important component in LPS-signaling cascade (Ingalls *et al.* 1999), and 3) assists the attachment of LPS to LPS-binding protein via its N-terminal domain; the resting C-terminal domain promotes bacterial attachment to neutrophils and monocyte cell surface, leading to elevation in their phagocytic activity (Hancock & Scott 2000).
The mild elevation in phagocytic activity and C3 level recorded in endotoxin group is similar to that reported by Wang *et al.* (1998) and Chaby (1999). The former find that binding and phagocytosis of opsonized RBCs with C3 by pre-incubated Kupffer cells with bacterial endotoxins, revealed an increase in the phagocytic index related to an increase in the number of phagocytic cells. They added that C3-mediated phagocytosis, together with the intrinsic and extrinsic activities and interferon synthesis that take place in the activated cells, may play an important role in the non-specific immunity. The obvious decrease of the neutrophil phagocytic activity against *Candida albicans* in the treated group may be due to a release of immunologically incompetent leucocytes into the peripheral blood and the enhanced migration of activated neutrophils towards the in vivo injected *Aeromonas hydrophila* site (Gad 1999). Wang *et al.* (1998) found an increase in the acute phase protein C3 and serum amyloid A protein (SAA) in the hepatocytes, enterocytes and plasma after injection of endotoxin and they supported the role of C3 in the convergising the classical and alternative pathways to the final common pathway in the complement cascade. Also, the elevation in C3 may be regulated by the secreted cytokines in endotoxemia (e.g. TNF, IL-1) rather than a direct effect of endotoxin itself.

The passive haemagglutinating assay showed a high response against the bacterial endotoxin allover the experimental intervals except at the 9th day post-injection. This

elevation may be due to the elevation in the total immunoglobulins level demonstrated in the present study. The elevated agglutinating titres following the bacterial endotoxin injection may be due to the presence of agglutinin in the injected animals which is specially secreted and actively reacted against the cellular and the biological antigens (e.g. bacteria) (Hudson & Hay 1980).

Furthermore, Klein (1990) noticed that LPS has the ability to stimulate B cell proliferation if given *in vivo* in appropriate doses. On the other hand, LPS readily binds to the surface of cells and therefore, could be trapped at the site of infection, and this may explain the lower response because less antigen could be released into the blood circulation for transport to the lymphoid organs to stimulate antibody production (Ingram & Alexander 1980).

The observed precipitating antibodies in the sera of the injected group may be due to the presence of high molecular weight LPS which is strongly mitogenic (Aakre *et al.* 1994). Also, Magnadottir *et al.* (1995) added that the continuous exposure to certain bacterial antigens, like the mitogenic LPS, might exert a pressure on the immune system. So, the elevated level of total immunoglobulins in endotoxin-treated group may be due to their binding to the surface component of the Gram-negative bacteria, and their ability to overcome the serum resistance phenomena in some cases i.e. resistance to serum killing activity of the complement. Also, this elevated level of total immunoglobulins in endotoxin-treated group either in serum or in conditioned and cell lysate media of splenocytic and lymphocytic cultures, may be due to the increase in γ-globulin fraction or due to the fact that LPS interaction with the cellular components leads to the polyclonally activation of the B-cell subsets and secretion of the immunoglobulins (Chaby 1999).

The decline in the migration inhibition factor resulted in the endotoxin-group may be due to the prompt elevation in the monocytic count in the chemotactic response to the LPS components (Forestier *et al.* 1999). While, the re-increase in the MIF index during endotoxemia may due to the effect of LPS on the functional cells to release pro-inflammatory cytokines e.g. tumor necrosis factor (TNF- α) that acts in autocrine/paracrine fashion with MIF i.e. MIF increases the macrophages-TNF- α releasing which in turn increases MIF release (Bozza *et al.* 1999).

Regarding the hisotchemical studies upon the activities of the nucleoprotein secretions, the noticed lymphoid hyperplasia and inflammaroty cell infiltration may be related to the interaction of LPS with the cellular components that leads to polyclonally activation of B-cells to secrete immunoglobulins, or to confirm that endotoxin possesses immunomodulatory activities capable of stimulation of macrophages and B-cells differentiation (Chaby 1999). These findings were also agreed with the recorded of elevation of total immunoglobulins levels in the present study and with the feature of spleen sections that showed a wide hyperactivation and hyperplasia of the germinal center associated with more pyroniphilic cells when the frozen sections of the infected spleen were stained with the methyl-green pyronine. Also, Norimatsu *et al.* (1995) noticed depletion of lymphocytes in the lymphoid organs in LPS-sensitive mice but not in LPS-resistant mice. While the noticed wide active germinal centers that were associated with the presence of many of pyroninophilic lymphoblasts may be related to the stress factors related to LPS administration (Chaby 1999).

In conclusion, the present study showed that the bacterial toxin (endotoxin of *Aeromonas hydrophila*) is considered as a member of the most factors that attenuate the immune response of the body, thus leading to increase the susceptibility of the animal and fish mass production to the infection with different diseases.

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الملخص العربى

دراسة مناعية لتأثير سموم بكتريا *الايروموناس* هيدروفيلا فى الجرذان البيضاء

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يشكل جنس *الأيروموناس* مجموعة من الكائنات الواسعة الانتشار والتى تم عزلها من المياه والآبار والمصارف والأطفال والكبار المصابين بالاسهال. يستهدف هذا البحث تأثير حقن السموم البكتيرية لبكتريا *الايروموناس هيدروفيلا* على المناعة الذاتية والخلوية واللاخلوية للجرذان البيضاء , وكذلك التغيرات الهستوكيميائية المصاحبة لكل من الطحال والغدة التيموسية. وقد قسمت الحيوانات الى مجموعتين: المجموعة الأولى حقنت فى التجويف البريتونى مرة كل ثلاثة أيام ولمدة واحد وعشرين يوما بجرعة مقدارها 8 مللى جرام بروتين من السموم البكتيرية / نصف مللىPBS , أما المجموعة الثانية حقنت نصف مللىPBS كمجموعة ضابطة فى نفس مواعيد لمجموعة الأولى . وقد أظهرت النتائج نقص فى العدد الكلى لكرات الدم البيضاء وكذلك الكرات الليمفاوية , أما الخلايا البيضاء المتعادلة ووحيدة الخلية فقد أظهرت نتائج متغيرة . زادت المناعة الذاتية بشكل واضح وكذلك المناعة اللاخلوية على الرغم من حدوث نقص فى المناعة الخلوية.