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A STUDY ON THE BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS ASSOCIATED WITH *PASTEURELLA MULTOCIDA* OUTER MEMBRANE PROTEINS IMMUNIZATION IN RABBITS

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ABSTRACT
This study was included extraction and purification of outer membrane proteins (OMP) of *Pasteurella multocida* by using sepharose – 6B as well as chemical analysis of OMP content. Half lethal dose (LD50) was 250 μ/ml of the purified OMP. The rabbits is considered more susceptible animal to *Pasteurella multocida* infection, so, (15) rabbits were injected S/C by 25 μ/ml. The other (15) rabbits were injected S/C by phosphate buffer saline (PBS) as a control group. 2 doses at 2 weeks intervals. At 15th and 27th days immunization the hematological and biochemical parameters were measured and it is found that there is not significant differences in the hematological and biochemical parameters in the OMP group of Immunization comparable to the control group. Although slight increase the level of both hematological and biochemical parameters in the group of Immunization with OMP, but still with normal level of control group.

KEYWORDS: outer membrane proteins, LD50, Immunization, biochemical parameters.

INTRODUCTION

Outer membrane proteins (OMP) are the major part of the outer membrane of the cell wall of the Gram negative bacteria and play a critical role in the pathogenesis of the disease caused by Gram negative bacterial species[1]. OMP are the major part of the capsule of the *Pasteurella multocida* has a pivotal role in the determination of serogroups types of the bacteria and play important role in pathogenesis[2]. Also the OMP are involved in the resistance to phagocytosis by the peritoneal macrophages and possessed Immunological properties [3,4] and useful in vaccine preparation, through their contribution to the pathogenic potential of disease causing bacteria , it act at an interface between the host and the Gram negative bacteria[5]. Through the importance of *Pasteurella multocida*, causing the disease in the most of animal species, this study aimed at:

1- Study the biochemical parameters (total serum protein, Albumin, globulin, Blood Urea and serum creatinine in rabbits following their immunization with OMP.
2- Study the hematological parameters (Total leukocytes count, erythrocytes count, differential leukocytes count and hemoglobin) in rabbits following their Immunization with OMP.

MATERIAL & METHODS

Pasteurella multocida strain was supplied by Alkindi Company for veterinary drugs and vaccines production, Baghdad, Iraq. These bacterial agents were reidentified to be sure *Pasteurella multocida* using cultural, biochemical, API – 20 NE kit (Biomerieux. USA)[6].

Outer membrane proteins extraction and purification

According to[7] following culturing of *Pasteurella multocida* in brain heart infusion broth Centrifugation, the pellets, washed with PBS and resuspended with hepes buffer, then subjected for sonication, then centrifugation and bacterial Debris (pellets) were removed and supernatant were taken (containing outer membrane proteins), then by ultracentrifugation the pellets were resuspended in the Hepes buffer containing 2% sodium N-Laurylsarcosinate and incubated for 1hr. The detergent insoluble outer membrane protein enriched fraction were pelleted out by ultracentrifugation, the pellets were resuspended with distilled water and stored at–20C°, for purification of OMP, the carbohydrate estimation according to [8] and protein estimation by using Biuret method [9].

Determination of the LD 50 of OMP

According to[10] method, using serial concentration of OMP (500, 375 , 250, 125 μ/ml). The 15 rabbits were inoculated S/C with 25 u/kg body weigh of rabbit at a dose of 1ml /rabbit , followed by booster dose after 15days. Other 15 rabbits were injected with PBS as a control group , at 15th and 27th days post inoculation , blood samples were taken for hematological parameters using[11] method , and for biochemical parameters by using (Agape, Switzerland[9], linear chemical, Spain[9] Biosystem, Spain [12].

RESULTS & DISCUSSION

Outer membrane protein of *Pasteurella multocida* were 250 μ/ml extracted from 25 gm (wet weight) of *Pasteurella multocida* and carbohydrate 36.8 μ/ml , according to phenol sulphuric acid procedure . The LD50 of OMP were 250 μ/ml.

The biochemical parameters

The results revealed that there is not significant differences (P< 0.05) between values of OMP immunized group and control group after 15th and 27th days post immunization, but still slight high level in OMP group compared to the control group (Table -1) of total protein , albumin, globulin , S. creatinine and B. urea (table – 1) but still with normal level

Hematological parameters

The results revealed that there is not significant differences (P< 0.05) between values of OMP immunized group and control group after 15th, 27th days post immunization, but there is slight increase in the level of all hematological parameter in the OMP group compared to the control of total leukocytes count, differential leukocytes count, erythrocytes count and hemoglobin count (Table –2), but still with the normal level range. The results of this study revealed that the all biochemical and hematological parameters in the OMP group of Immunization and control group showed no significant differences with slight elevation of all the biochemical and hematological in Immunized group with OMP compared to the control group, but generally still with normal level range. A similar finding reported by[13] that Immunization with OMP and LPS gave good Immune response, and the slight elevation in all biochemical and hematological parameters but still with normal level which showed in this study were also reported by [14] on the basis that the state of immunization enhance slight effect on all biochemical and hematological parameters , similary reported by[14] that the immunization stimulate erythropoiesis state in the bone marrow and lead to slight elevation of all the hematological parameters in peripheral circulation, similarly with slight elevation of all the biochemical parameters in OMP group of immunization in this study. A similar finding reported by[15,16] that the immunization don't effect on biochemical and hematological parameters levels strongly .

TABLE 1: Biochemical levels in immunized (OMP) group and in control group of Rabbits

Groups	Total protein (gm/dl) mean ± SE		Albumin (gm/dl) mean ± SE		Globulin (gm/dl) mean ± SE		S. creatinine (mg/dl) mean ± SE		B. Urea (mg/dl) mean ± SE	
OMP	15th. Day	27th day	15th day	27th. Day	15th. Day	27th. Day	15th. day	Day 27th	15th. Day	27th day
	5.52 ± 0.222	5.56 ±0.166	3.1 ±0.213	3.1 ± 0.213	2.38 ±0.404	2.40 ±0.374	0. 6 ±0.10	1.68 ±0.08	24 ±1.41	24.6 ±1.32
Control group	5.46 ±0.132	5.48 ±0.146	3.0 ±0.143	3.0 ±0.100	2.42 ±0.220	2.48 ±0.226	0.67 ±0.05	0.63 ±0.05	24.2 ±0.66	23.6 ±1.43

There is not significant differences between groups and between periods (P< 0.05)

TABLE 2: Hematological parameters in immunized OMP and control groups of Rabbits

Groups	Hb. Content (gm/dl) mean ± SE		Leukocyte count / mean ± SE		Neut. % mean± SE		Lymph % mean ± SE		Mono % mean ± SE		Eosin.% mean ±SE		RBcs count / mean ± SE	
OMP group	15th. Day	27th day	15th day	27th Day	15th Day	27th Day	15th Day	27th Day	15th Day	27th Day	15th Day	27th day	15th Day	27th day
	10. 8 ± 0.67	10.6 ± 0.43	6.44 ± 0.56	6.66 ± 0.65	51.6 ± 5.3	50 ± 5.54	47.2 ± 2.08	47.6 ± 4.11	2.4 ± 0.50	2.2 ± 0.58	3.2 ± 0.37	3 ± 0.31	6.02 ± 0.56	5.8 ± 0.58
Control group	11.22 ± 0.62	11.18 ± 0.81	6.6 ±0.12	6.48 ± 0.41	50.6 ± 3.10	49 ± 2.93	45.8 ± 2.2	45 ± 2.82	1.8 ± 0.37	2 ± 0.31	3 ± 0.44	3.4 ± 0.67	5.98 ± 0.36	6.24 ± 0.22

There is not significant differences between groups and between periods (P< 0.05)

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