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Protective Effect Of Oral And Intranasal Bacterial Lysates In Mice

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Abstract

In this study albino mice were used to test the beneficial effect of bacterial lysates vaccination against lethal dose of *Pseudomonas aeroginosa*. *Escherichia coli lysate, Pseudomonas aeroginosa lysate, Staphylococcus aureus lysate* and mixed bacterial lysates were administered through the oral and the intranasal routes, both in the presence and absence of Freund's adjuvant versus a placebo. *Pseudomonas aeroginosa* fifty percent lethal dose (LD50) was injected intraperitonealy following intranasal and oral vaccination. The placebo and the four bacterial lysates were also used in association with Freund's adjuvant. The results of the LD50 in intranasal vaccinated groups were 50%, 37.5%, 100%, 0% and 12.5%, and those with Freund's adjuvant were 25%, 25% , 12.5%, 62.5%, 0% and 0% respectively. The results of LD50 in oral vaccination were 50%, 25%, 62.5% 0% and 37.5%, and those treated with Freund's adjuvants were 12.5%, 12.5 %, 12.5%, 37.5 %, 0% and 25% respectively. The bacterial lysates vaccinated groups were studied for the total body weight (T), liver (L), spleen (S), thymus (Th) weights and the L+S+Th/T ratio. Besides, the peripheral blood and the peritoneal fluid total and differential leucocytic counts were determined and the bone marrow lymphocyte percentage. The serum immunoglobulins G and M were assessed using the immundiffusion plates. Our conclusion is: Bacterial lysates can play an important role as immunomodulators when used by oral or intranasal routes.

Introduction

Few substances have a greater positive impact upon health care management than antibodies, vaccines and adjuvants . For most of this century, these immunological agents have enjoyed widespread medical applications, predominantly for the treatment, prevention of infectious diseases. Technologies are evolving that are leading to safer, more effective and more cost- efficient vaccines. In Europe, a killed bacterial is referred to as a bacterial vaccine, while in the United States a killed bacterial product is referred to as a bacterin and the term vaccine is reserved for an immunizing agent that contains live inactivated microbial components (Bey *et al*, 1997) . Inactivated whole bacterial cell suspensions are probably the most common type of vaccine used in domestic animals. These vaccines proved to be extremely effective and safe even in young or pregnant animals ,an example is *Leptospira* vaccines (Kurstak, 1994).

Rutishauser *et al.* (1998) showed that the use of an oral bacterial lysate was effective in patients with recurrent respiratory tract bacterial infections. Their study demonstrated that the use of an oral bacterial lysate consisting of the antigens of seven bacteria commonly involved in respiratory tract infections has been developed for the induction of specific and non specific immune responses of the mucosa –associated lymphoid tissue. Tablet formulation were taken once daily during two periods of four weeks each. The clinical severity score was significantly lower in patients treated with bacterial lysate compared to patients given placebo. The infection rates revealed reduction of 39% in children and reduction of 44% in

adolescents and adults.On the other hand, bacterial vaccines can be applied effectivly through the intranasal route (Orr *et al*, 1993 and Ambrosino, 1996). Barackman *et al*, (1999) showed that intranasal immunization of mice with influenza virus vaccine in combination with the adjuvant LT- R72 induced potent mucosal and serum immunity which was stronger than that with traditional intramuscular immunization . This potently observed immunostimulatory effect can be explained by the natural role of mucosal immune system in defense against inhaled infections (Kiyono *et al*, 1992).

Bacterial enterotoxin can play a role in vaccination as a mucosal adjuvant also other adjuvants could stimulate mucosal and antibody response (Jackson *et al*, 1993 and Giuliani *et al*, 1998).

This study aimed at assessing and comparing the immunoprotective effect of repeated oral and intranasal bacterial lysates using *Escherichia coli, Pseudomonas aeroginosa, Staph. aureus,* and mixed bacterial lysates.

Material And Methods

Bacteria:

1. *Escherichia coli (CAIM- 1357);*
2. *Pseudomonas aeroginosa (clinical isolate);*
3. *Staphylococcus aureus (CAIM- 1352);*
4. *Salmonella typhi;*
5. *Shigella sp. (NMRO)* and
6. *Bacillus subtilis* (CAIM- 1007).

Bacterial strains used in this study were obtained from the Microbiology Department in NODCAR.

Animals:

Four hundred male albino mice weighing 18- 25 gm each were used in the present study. The animals were obtained from the animal house of (NODCAR). The animals were divided into four equal groups, control, placebo, intranasal vaccinated and oral vaccinated.

Intranasal vaccinated group:

This group was subdivided into

I- Bacterial lysate vaccinated group which recived *Esch. coli lysate, Ps. aeroginosa lysate, Staph. aureus lysate* and mixed bacterial lysates (Corthesy- Theulaz *et al*, 1998 and Bonenfant *et al*, 2001).

II- Bacterial lysate mixed with incomplete Freund's adjuvant (Behriµngwerke A. G., Marburg. Germany) in the first dose followed by complete Freund's adjuvant in the next three booster doses (Hanaa,1999 and O'Brien *et al*, 2000).

Each animal had received 5 µl of the vaccine in each nostril, once weekly repeated for four weeks (Sramek *et al*, 1986 and Raghavan *et al*, 2002).

Oral vaccinated group

This group was subdivided as what was previously mentioned before in the intranasal vaccinated animal group.

Each animal had received 10 µl of the vaccine orally once weekly repeated for four weeks(Kuenen *et al*, 1994).

Bacterial lysate preparation :

A 24 hours bacterial growth of *Escherichia coli, Pseudomonas aeroginosa, Salmonella typhi, Staphylococcus aureus* and *Bacillus subtilis* that had viable counts $2.72x10^8$, $7.56x10^8$, $13.91x10^8$, $7.33x10^8$ and $2.91x10^8$ CFU/ml respectively was used to prepare the bacterial lysate used in this study. A volume of 25 ml of the 24 hours bacterial growth was lysed using high speed 4000 rpm homogenizer [variable GKH- GT MOTOR control- Glas- Col (USA)]. 1/100 v/v of 10% formalin was added . Then a subculture of the lysates on nutrient agar (Oxoid) were observed for 24 hours to ensure that the bacterial lysates did not include any viable bacteria (Hugo and Russel, 1993 and Raghavan *et al*, 2002).

Mixed bacterial lysates were prepared from equal volumes of the different bacterial lysates used in this study (Ruah *et al*, 2001).

Bacterial challenge test :

In the preliminary work of this study, the *Pseudomonas aeroginosa* LD50 was detrmined. Then the different animal test groups were injected intraperitoneal with LD50 of 24 hours growth of *Pseudomonas aeroginosa.* The number of mice survivals was recorded in the 48 hours following

bacterial challenge.(Hamid, 1989 and Bennett- Guerrero *et al*, 2000).

Immunoglobulins assay :

Serum was obtained from control animals and bacterial lysates treated animals. Serum was divided into aliquots and stored in the freezer until processed. Immunoglobulins G and M assays were done using [Mouse Immunoglobulin 'LL' NANORID- BIND A RID- NANORID PRODUCTS- immundiffusion plates which were supplied by THE BINDING SITE LIMITED Co. UK]. The precipitation rings were measured to an accuracy of 0.1 mm . The assay results were obtained from the calibration table values given by the manufacturer (Fahey and Mc Kelvey, 1965 and Sadeq *et al*, 1992).

Haematology assays:

About 0.25 ml blood samples were drawn by capillary tubes from the retro-orbital plexus from each mouse, being added to EDTA for peripheral blood total leucocyte count and differntial leucocyte count. Direct smears were withdrawn from the peritoneal fluid for peritoneal total and differential leucocyte counts.Bone marrow smears that were obtained from the femur bone were spreaded for bone marrow lymphocyte count. Leishman stain was used for the different leucocyte counts.

Physiological parameters:

The total body weight (T) of each animal was recorded, followed by determining the liver(L), spleen(S) and thymus (Th) weights; the (L+S+Th)/T ratio was determined.

The results were statistically evaluated using the student t- test where the significance of the differences between treated and respective control groups was analysed (Goldstein, 1964) .

Results

Bacterial challenge test results :

Both oral and intranasal vaccinations could effectively protect the vaccinated mice against *Pseudomonas aeroginosa* challenge dose. One exception was detected when *Pseudomonas aeroginosa* bacterial lysate was used for vaccination. Freund's adjuvant alone it could protect against *Ps. aerog*. challenge dose, and actively potentiated the immunoprotective effect of both oral and intranasal bacterial lysates vaccinations (Table 1 and Fig. 1).

Escherichia coli bacterial lysate vaccination :

It raised the L+S+Th/T ratio both in the presence and absence of bacterial challenge when compared with normal control group and placebo group. Besides, the bacterially challenged intranasal vaccinated animal group showed a significant increase in the splenic weight, mean spleen weight ± SD was 0.398 ± 0.223 (Table 2). Oral and intranasal *Escherichia coli* lysate vaccination caused significant increase in the eosinophil counts when compared with normal control and placebo groups (Table 3). Vaccination was associated with increased peritoneal fluid lymphocyte counts (Table 4) and a drop of bone marrow lymphocyte percent-age that were significant when associated with bacterial challenge (Table 5).

Pseudomonas aeroginosa bacterial lysate vaccination :

It caused an increase in the splenic and thymus weights. Sometimes these changes were associated with significant increase of L+S+Th/T ratio (Table 6). The peripheral blood haematological findings showed increased eosinophilic counts that were significant when compared with normal control and placebo groups. A significant drop of monocyte count was detected in intranasal vaccinated –bacterial challenged animals (Table 7). Intranasal vaccination with *Pseudomonas aeroginosa* lysate caused an increase of peritoneal fluid neutrophile count (Table 8) and a drop of bone marrow lymphocyte count (Table 9). It seemed that the different changes recorded in the reticuloendothelial system organs represented by liver, spleen, thymus and L+S+Th/T ratio and changes observed in haematological findings and bone marrow lymphocytic percentage played a role in increasing lethality rate in intranasal and oral *Ps. aerog*.- treated mice.

Staphylococcus aureus bacterial lysate vaccination :

Both oral and intranasal vaccination caused a significant increase of peripheral

blood total leucocyte count, also a significant increase of thymus weight was observed after intranasal vaccination (Table 10). Peripheral blood differential leucocyte count showed a significant increase of eosinophile count when compared with normal control and placebo groups, this was associated with a significant increase of monocyte count after intranasal vaccination (Table 11). Oral and intranasal vaccinations caused a significant increase of peritoneal fluid leucocyte counts , that was associated with increased monocyte count (Table 12). *Staphylococcus aureus* lysate orally vaccinated animals showed a drop of bone marrow lymphocyte percentage (Table 13).

Mixed bacterial lysates vaccination :

Mixed bacterial lysates vaccinations caused an increase of splenic weights (Table 14). Peripheral blood showed a significant increase of total leucocyte count and eosinophile count after intranasal and oral vaccinated animal groups. A signifycant drop of neutrophilic percentage associated with a significant increase of lymphocyte count (Table 15). Similar observations were detected in the peritoneal fluid cell counts (Table 16). Bone marrow showed a drop of lymphocytic percentages in the different vaccinated animal groups.

Serum immunoglobulins G and M :

All bacterial lysate vaccinations led to significant elevation of serum Ig G and Ig M, whether after oral or in intranasal vaccination. This response was highly magnified in the presence of bacterial infection due to challenge with *Ps. aerog.* LD50 (Tables 18-21 and figures 2-5). It seemed that the reported elevation of serum Igs G and M were inversely correlated to the death rates reported in (Table 1 and Fig. 1)

Table (1): *Pseudomonas aeroginosa* LD_{50} * bacterial challenge in intranasal and oral bacterial lysate weekly vaccinated mice groups for (four weeks).

***Ps aeroginosa* bacterial infection -challenged mice groups**	**Intranasal vaccin. (LD)***	**Oral Vaccin. (LD)***
Control group (CG)	50%	50%
Freund's adjuv. (FA)	25%	12.5%
Placebo (PT).	50%	50%
Placebo-Freund's adjuv (PF)	25%	12.5%
Esch. coli lysate vaccin. (EL)	37.5%	25%
Esch. coli lysate-Freund's adjuv (EF)	12.5%	12.5%
Pseud. aerog. lysate vaccin. (PL)	100%	62.5%
Pseud. aerog. lysate-Freund's adjuv (PLF)	62.5%	37.5%
Staph. aureus lysate vaccin (SL)	0%	0%
Staph. aureus lysate-Freund's adjuv. (SF)	0%	0%
Mixed bact. Lysate vaccin. (ML)	12.5%	37.5%
Mixed bact. lysate-Freund's adjuv. (MF)	0%	25%

*LD : Lethal dose

Table (2): Total body weight (T), liver weight (L), spleen weight (S), thymus weight (Th) and (L+S+Th/ T) ratio in mice vaccinated with *Esch. coli* lysate weekly for four weeks.

Animal group	Total body weight (T) in grams	Liver weight (L) in grams	Spleen weight (S) in grams	Thymus weight (Th) in grams	(L+S+Th)/ T ratio
1- Normal control	22.1 ± 5.85	1.097± 0.374	0.10 ± 0.047	0.039 ± 0.014	0.055 ± 0.003
2- Placebo	23.3 ± 4.52	1.056 ± 0.249	0.076 ± 0.022	0.041 ± 0.014	0.057 ± 0.004
3- *Ps. aerog* bact infect.	18.5 ± 3.75	1.159 ± 0.452	0.113 ± 0.049	0.026 ± 0.011	0.069±0.002***, ooo
4- *Esch coli lys.* IN vaccin.	21.6 ± 1.51	0.871 ± 0.138	0.090 ± 0.015	0.039 ± 0.013	0.046 ± 0.003*,oo
5- *Esch.coli lys.* IN vaccin – *Ps. aerog*. bact. infect.	26.2 ± 5.17	1.486 ± 0.363	0.398 ± 0.223*oo	0.034 ± 0.008	0.071±0.005***,ooo
6- *Esch.coli lys.* oral vaccin.	21.9 ± 1.43	0.968 ± 0.167	0.307 ± 0.395	0.040 ± 0.004	0.066 ± 0.008
7- *Esch.coli lys.* oral vaccin - *Ps. aerog*. bact. infect.	23.4 ± 2.64	1.289 ± 0.174	0.124 ± 0.051	0.034 ± 0.012	0.064 ± 0.004*

*,**,***Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
o, oo, ooo Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxxSignificant at P<0.05, P<0.01, P<0.001 when compared with gp (3).

Table (3): Mice peripheral blood haematological findings after four weekly vaccinations with *Esch. coli* lysate .

Animal group	Total Leucocyte count $X10^3$/cmm	Eosinophil %	Neutrophils %	Lymphocytes %	Monocytes %
1- Normal control	3.86 ± 1.68	0.40 ± 1.8	49.20 ± 7.1	49.40 ± 6.1	1.90 ± 0.7
2- Placebo	3.83 ± 1.28	0.40 ± 1.8	48.70 ± 5.3	48.10 ± 6.8	1.80 ± 0.8
3- *Ps. aerog* bact infect.	4.20 ± 2.47	3.70 ± 2.3***,ooo	50.70 ± 2.5	51.50 ± 3.7	2.70 ± 1.6
4- *Esch.coli lys.* IN vaccin.	4.32 ± 1.17	4.20 ± 2.5***,ooo	45.20 ± 11.4	46.00 ± 13.6	3.40 ± 1.7
5- *Esch.coli lys.* IN vaccin – *Ps. aerog*. bact. infect.	4.64 ± 2.52	3.40 ± 0.6***,ooo	44.80 ± 6.3	50.00 ± 6.4	2.00 ± 1.4
6- *Esch.coli lys.* oral vaccin.	3.90 ± 0.89	3.20 ± 2.7**,oo	44.00 ± 3.8	50.60 ± 2.1	2.00 ± 1.4
7- *Esch.coli lys.* oral vaccin – *Ps. aerog*. bact. infect.	5.55 ± 2.02	2.80 ± 1.8*,o	47.30 ± 9.3	46.30 ± 10.4	3.50 ± 2.3

*,**,***Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
o, oo, ooo Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxxSignificant at P<0.05, P<0.01, P<0.001 when compared with gp (3).

Table (4): Peritoneal fluid haematological findings in mice after four weekly vaccinations with *Esch. coli* lysate.

Animal group	Total Leucocyte count $X10^3$/cmm	Eosinophil %	Neutrophils %	Lymphocytes %	Monocytes %
1- Normal control	3.51 ± 2.1	0.20 ± 1.94	52.6 ± 5.1	43.00 ± 3.8	1.30 ± 0.26
2- Placebo	3.08 ± 1.2	0.10 ± 1.67	52.8 ± 5.4	44.10 ± 3.6	1.10 ± 0.14
3- *Ps. aerog* bact infect.	3.35 ± 1.8	2.80 ± 3.11	39.8 ± 5.3**,ºº	57.80 ± 8.1***,ººº	0.33 ± 0.84
4- *Esch.coli lys.* IN vaccin.	3.10 ± 1.3	3.40 ± 0.97	41.00 ± 5.7**,ºº	58.00 ± 6.2***,ººº	1.00 ± 1.15
5- *Esch.coli lys.* IN vaccin – *Ps. aerog*. bact. infect.	3.54 ± 2.6	4.10 ± 1.36	46.20 ± 9.3	50.40 ± 10.6	3.00 ± 3.74
6- *Esch.coli lys.* oral vaccin.	4.70 ± 1.9	2.80 ± 1.21	38.5 ± 7.7***,ººº	56.50 ± 9.0**,ºº	3.50 ± 1.92*,ºº,xx
7- *Esch.coli lys.* oral vaccin - *Ps. aerog*. bact. infect.	7.32 ± 2.6*,º,xx	4.70 ± 2.19	49.8 ± 9.4	46.80 ± 10.9	1.70 ± 1.51

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxx Significant at P<0.05, P<0.01, P<0.001 when compared with gp (3).

Table (5): Mice bone marrow , peripheral blood and peritoneal fluid films lymphocyte percentage after four weekly vaccinations with *Esch. coli* lysate .

Animal group	Bone marrow lymphocytes %	Peripheral blood lymphocytes %	Peritoneal fleuid lymphocytes %
1- Normal control	**41.50 ± 6.1**	**49.40 ± 6.1**	**43.00 ± 3.8**
2- Placebo	**42.00 ± 5.8**	**48.10 ± 6.8**	**44.10 ± 3.6**
3- *Ps. aerog* bact infect.	**26.50 ± 2.8***,ººº**	**51.50 ± 3.7**	**57.80 ± 8.1***,ººº**
4- *Esch.coli lys.* IN vaccin.	**32.50 ± 9.1**	**46.00 ± 13.6**	**58.00 ± 6.2***,ººº**
5- *Esch.coli lys.* IN vaccin – *Ps. aerog*. bact. infect.	**29.80 ± 2.1***,ººº**	**50.00 ± 6.4**	**50.40 ± 10.6**
6- *Esch.coli lys.* oral vaccin.	**32.00 ± 2.9*,º**	**50.60 ± 2.1**	**56.50 ± 9.0**,ºº**
7- *Esch.coli lys.* oral vaccin - *Ps. aerog*. bact. infect.	**27.70 ± 4.6*,º**	**46.30 ± 10.4**	**46.80 ± 10.9**

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxx Significant at P<0.05, P<0.01, P<0.001 when compared with gp (3).
–,– –,– – – Significant at P<0.05, P<0.01, P<0.001 when compared with previous group.

Table (6): Total body weight (T), liver weight (L), spleen weight (S), thymus weight (Th) and (L+S+Th/ T) ratio in mice vaccinated with *Pseudomonas aeroginosa* lysate for four weeks.

Animal group	Total body weight (T) in grams	Liver weight (L) in grams	Spleen weight (S) in grams	Thymus weight (Th) in grams	(L+S+Th)/ T ratio
1- Normal control	22.7 ± 5.4	1.08± 0.35	0.101 ± 0.03	0.032 ± 0.02	0.055 ± 0.007
2- Placebo	23.1 ± 4.7	1.08 ± 0.29	0.096 ± 0.02	0.034 ± 0.01	0.052 ± 0.006
3- *Ps. Aerog.* bact infect.	20.2 ± 3.8	1.16 ± 0.45	0.113 ± 0.04	0.026 ± 0.01	0.069±0.004*,o
4- *Ps. Aerog.lys.* IN vaccin.	24.2 ± 2.3	1.11 ± 0.09	0.133 ± 0.02*,oo	0.062 ± 0.03***,ooo	0.046 ± 0.002
5- *Ps. aerog.lys.* IN vaccin – *Ps. aerog*. bact. infect.	24.6 ± 2.7	1.49 ± 0.35	0.112 ± 0.03	0.044 ± 0.02 -,xx	0.071±0.003**,oo
6- *Ps. aerog.lys.* oral vaccin.	27.3 ± 2.1	1.42 ± 0.17	0.14 ± 0.04*,oo	0.049 ± 0.01*,o	0.066 ± 0.014
7- *Ps. aerog.lys.* oral vaccin - *PS. aerog*. bact. infect.	23.2 ± 4.1	1.25 ± 0.17	0.122 ± 0.04*,o	0.056 ± 0.03	0.064 ± 0.009

*,**,***Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
o, oo, ooo Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxxSignificant at P<0.05, P<0.01, P<0.001 when compared with gp (3).
–,– –,– – – Significant at P<0.05, P<0.01, P<0.001 when compared with its previous gps.

Table (7): Mice peripheral blood haematological findings after four weekly vaccinations with *Pseudomonas aeroginosa* lysate.

Animal group	Total Leucocyte count $X10^3$/cmm	Eosinophil %	Neutrophils %	Lymphocytes %	Monocytes %
1- Normal control	3.89 ± 1.46	0.40 ± 1.4	48.70 ± 9.1	46.70 ± 5.3	1.86 ± 0.6
2- Placebo	4.01 ± 1.19	0.40 ± 1.2	48.20 ± 6.1	49.20 ± 7.3	1.81 ± 0.7
3- *Ps. aerog.* bact infect.	4.20 ± 2.47	3.7 ± 2.3***,ooo	50.70 ± 2.5	45.50 ± 7.4	2.70 ± 1.6
4- *Ps aerog. lys.* IN vaccin.	5.83 ± 1.8	2.00 ± 0.0***,ooo	52.80 ± 10.2	43.80 ± 9.7	2.00 ± 0.0
5- *Ps. aerog. lys.* IN vaccin – *Ps aerog*. bact. infect.	5.90 ± 2.9	1.80 ± 2.3	51.30 ± 4.5	48.40 ± 4.4	0.50 ± 0.2*,o
6- *Ps. aerog. lys.* oral vaccin.	5.35 ± 1.3	2.00 ± 0.0***,ooo	52.50 ± 5.7	43.50 ± 5.7	2.00 ± 0.0
7- *Ps. aerog.lys.* oral vaccin - *Ps. aerog*. bact. infect.	9.38 ± 6.9	0.80 ± 1.0	52.00 ± 9.2	47.60 ± 9.2	0.80 ± 1.1

*,**,***Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
o, oo, ooo Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).

Table (8): Peritoneal fluid haematological findings in mice after four weekly vaccinations with *Pseudomonas aeroginosa* lysate.

Animal group	Total Leucocyte count $X10^3$/cmm	Eosinophil %	Neutrophils %	Lymphocytes %	Monocytes %
1- Normal control	3.71 ± 0.9	0.20 ± 1.87	53.20 ± 4.8	44.10 ± 4.3	1.40 ± 0.31
2- Placebo	3.26 ± 1.7	0.10 ± 2.3	52.90 ± 5.6	42.6 ± 3.3	1.10 ± 0.17
3- *Ps.aerog.* bact infect.	3.84 ± 2.2	3.30 ± 3.61	39.80 ± 5.3**,ºº	58.30 ± 7.8***,ººº	0.33 ± 0.79
4- *Ps. aerog.lys.* IN vaccin.	6.41 ± 2.1	0.00 ± 0.0	61.00 ± 2.0**,ºº	38.50 ± 1.9*,º	0.50 ± 1.0
5- *Ps. aerog.lys.* IN vaccin – *Ps. aerog*. bact. infect.	5.73 ± 1.9	2.80 ± 3.09	61.40 ± 12.9	37.50 ± 11.6	0.50 ± 0.93
6- *Ps. aerog.lys.* oral vaccin.	7.28 ± 3.7	1.50 ± 2.1	44.00 ± 8.00	55.50 ± 14.6	0.50 ± 1.0
7- *Ps. aerog.lys.* oral vaccin - *Ps. aerog*. bact. infect.	5.23 ± 4.3	1.20 ± 1.7	66.10 ± 9.1	33.10 ± 8.4	0.75 ± 1.04

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).

Table (9): Mice bone marrow , peripheral blood and peritoneal fluid films lymphocyte percentage after four weekly vaccinations with *Pseudomonas aeroginosa lysate* .

Animal group	Bone marrow lymphocytes %	Peripheral blood lymphocytes %	Peritoneal fleuid lymphocytes %
1- Normal control	43.10 ± 7.1	46.70 ± 5.3	44.10 ± 4.3
2- Placebo	42.90 ± 6.3	49.20 ± 7.3	42.60 ± 3.3
3- *Ps. aerog.* bact infect.	26.50 ± 2.8***,ººº	45.50 ±	58.30 ± 7.8***,ººº
4- *Ps. aerog.lys.* IN vaccin.	39.80 ± 5.8	43.80 ± 9.7	38.50 ± 1.9*,º
5- *Ps. aerog.lys.* IN vaccin - *PS aerog*. bact. infect.	38.50 ± 16.5	48.40 ± 4.4	37.50 ± 11.6
6- *Ps. aerog.lys.* oral vaccin.	31.60 ± 1.6**,ºº	43.50 ± 5.7	55.50 ± 14.6
7- *Ps. aerog.lys.* oral vaccin - *Ps. aerog*. bact. Infect.	30.50 ± 5.8*,º	47.60 ± 9.2	33.10 ± 8.4

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxx Significant at P<0.05, P<0.01, P<0.001 when compared with gp (3).
–,– –,– – – Significant at P<0.05, P<0.01, P<0.001 when compared with previous group.

Table (10): Total body weight (T), liver weight (L), spleen weight (S), thymus weight (Th) and (L+S+Th/ T) ratio in mice vaccinated with *Staph. aureus lysate* for four weeks.

Animal group	Total body weight (T) in grams	Liver weight (L) in grams	Spleen weight (S) in grams	Thymus weight (Th) in grams	(L+S+Th)/ T ratio
1- Normal control	23.8 ± 4.63	1.102± 0.401	0.103 ± 0.036	0.041 ± 0.013	0.054 ± 0.003
2- Placebo	23.6 ± 4.12	1.059 ± 0.273	0.081 ± 0.007	0.041 ± 0.014	0.056 ± 0.003
3- *Ps. aerog.* bact infect.	18.7 ± 3.47	1.169 ± 0.431	0.117 ± 0.032	0.026 ± 0.013	0.069±0.002
4- *Staph. aureus lys.* IN vaccin.	27.7 ± 2.79	1.18 ± 0.286	0.117 ± 0.027	0.061 ± 0.004*,º	0.048 ± 0.006
5- *Staph. aureus lys.* IN vaccin – *Ps. aerog*. bact. infect.	27.4 ± 1.5	1.29 ± 0.18	0.133 ± 0.032	0.054 ± 0.008	0.053±0.004
6-*Staph.aureus lys.* oral vaccin	26.3 ± 1.73	1.26 ± 0.17	0.127 ± 0.02	0.051 ± 0.011	0.054 ± 0.001
7- *Staph. aureus lys.* oral vaccin – *Ps. aerog*. bact. Infect	26.6 ± 2.85	1.13 ± 0.21	0.131 ± 0.027	0.046 ± 0.012	0.051 ± 0.005

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxx Significant at P<0.05, P<0.01, P<0.001 when compared with gp (3).
–,– –,– – – Significant at P<0.05, P<0.01, P<0.001 when compared with its previous gp

Table (11): Mice peripheral blood haematological findings after four weekly vaccinations with *Staph. aureus lysate.*

Animal group	Total Leucocyte count $X10^3$/cmm	Eosinophil %	Neutrophils %	Lymphocytes %	Monocytes %
1- Normal control	3.91 ± 1.26	0.37 ± 1.2	48.70 ± 8.7	45.20 ± 6.1	1.66 ± 0.31
2- Placebo	4.03 ± 1.08	0.39 ± 1.1	46.90 ± 8.2	48.10 ± 7.6	1.63 ± 0.42
3- *Ps. aerog* bact infect.	4.41 ± 1.98	3.9 ± 2.2***,ººº	52.10 ± 3.1	44.90 ± 6.4	2.91 ± 1.46
4- *Staph. aureus lys.* IN vaccin.	8.35 ± 2.41**,ºº	2.14 ± 1.35***,ººº	37.60 ± 4.2	55.10 ± 4.9	4.51 ± 2.93*,º
5- *Staph. aureus lys.* IN vaccin – *Ps. aerog*. bact. infect.	6.29 ± 2.37	1.25 ± 1.49**,ºº	48.30 ± 2.8	48.90 ± 2.0	1.63 ± 1.51
6-*Staph.aureus lys.* oral vaccin	8.43 ± 3.27*,º	2.38 ± 0.74***,ººº	45.60 ± 8.5	50.50 ± 9.4	0.51 ± 0.76
7- *Staph. aureus lys.* oral vaccin – *Ps. aerog*. bact. infect.	5.71 ± 2.13	2.51 ± 1.41***,ººº	41.30 ± 6.5	52.90 ± 10.6	3.00 ± 3.62

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxx Significant at P<0.05, P<0.01, P<0.001 when compared with gp (3).
–,– –,– – – Significant at P<0.05, P<0.01, P<0.001 when compared with its previous gp

Table (12): Peritoneal fluid haematological findings in mice after four weekly vaccinations with *Staph. aurerus* lysate

Animal group	Total Leucocyte count $X10^3$/cmm	Eosinophil %	Neutrophils %	Lymphocytes %	Monocytes %
1- Normal control	3.41 ± 0.87	0.21 ± 1.77	52.70 ± 4.1	44.96 ± 4.7	1.31 ± 0.42
2- Placebo	3.05 ± 0.91	0.17 ± 1.91	52.81 ± 4.7	43.23 ± 3.9	1.11 ± 0.57
3- *PS aerrog* bact infect.	3.94 ± 2.01	3.21 ± 3.49	39.92 ± 4.9	58.39 ± 6.2***,ooo	0.37 ± 0.791
4- *Staph. aureus lys.* IN vaccin.	3.86 ± 1.10	0.00 ± 0.0	56.63 ± 9.3	39.63 ± 8.2	3.50 ± 2.39
5- *Staph. aureus lys.* IN vaccin – *Ps. aerog*. bact. infect.	8.89 ± 4.3**,oo	0.25 ± 0.66	48.63 ± 7.9	48.38 ± 8.9	3.00 ± 1.19*,o,x
6-*Staph.aureus lys.* oral vaccin	7.40 ± 2.5**,oo	0.00 ± 0.0	53.00 ± 7.7	42.11 ± 7.89	3.00 ± 0.93**,oo
7- *Staph. aureus lys.* oral vaccin – *Ps. aerog*. bact. infect.	11.63±4.5***,ooo,xxx	0.25 ± 0.66	51.38 ± 11.7	45.13 ± 11.14	3.25 ± 1.82 x

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
o, oo, ooo Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxx Significant at P<0.05, P<0.01, P<0.001 when compared with gp (3).

Table (13): Mice bone marrow , peripheral blood and peritoneal fluid films lymphocyte percentage after four weekly vaccinations with *Staph. aureus* lysate.

Animal group	Bone marrow lymphocytes %	Peripheral blood lymphocytes %	Peritoneal fleuid lymphocytes %
1- Normal control	42.10 ± 4.8	45.20 ± 6.1	44.90 ± 4.7
2- Placebo	43.10 ± 5.1	48.10 ± 7.6	43.20 ± 3.9
3- *Ps. aerog* bact infect.	25.60 ± 3.01	44.90 ± 6.4	58.40 ± 6.2***,ooo
4- *Staph. aureus lys.* IN vaccin.	40.30 ± 2.32	55.10 ± 4.9	39.60 ± 8.2
5- *Staph. aureus lys.* IN vaccin – *Ps. aerog*. bact. infect.	44.10 ± 2.48	48.90 ± 2.0	48.40 ± 8.9
6- *Staph. aureus lys.* oral vaccin.	33.80 ± 2.12*,o	50.50 ± 9.4	42.10 ± 7.9
7- *Staph. aureus lys.* oral vaccin – *Ps.. aerog*. bact. infect.	35.40 ± 7.3	52.90 ± 10.6	45.10 ± 11.1

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
o, oo, ooo Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
–,– –,– – – Significant at P<0.05, P<0.01, P<0.001 when compared with gp (3).
x,xx,xxx Significant at P<0.05, P<0.01, P<0.001 when compared with previous group.

Table (14): Total body weight (T), liver weight (L), spleen weight (S), thymus weight (Th) and (L+S+Th/ T) ratio in mice vaccinated with mixed bacterial lysates weekly for four weeks .

Animal group	Total body weight (T) in grams	Liver weight (L) in grams	Spleen weight (S) in grams	Thymus weight (Th) in grams	(L+S+Th)/ T ratio
1- Normal control	23.2 ± 4.65	1.19± 0.34	0.102 ± 0.042	0.039 ± 0.014	0.057 ± 0.007
2- Placebo	23.6 ± 4.13	1.09 ± 0.27	0.089 ± 0.022	0.046 ± 0.016	0.054 ± 0.006
3- *Ps. aerog* bact infect.	18.5 ± 3.74	1.16 ± 0.45	0.113 ± 0.048	0.026 ± 0.01	0.069±0.004*,º
4- Mixed bact. Lys. IN vaccin.	25.1 ± 2.84	1.06 ± 0.07	0.117 ± 0.023	0.072 ± 0.112	0.049 ± 0.01
5- Mixed bact. Lys. IN vaccin – *Ps. aerog*. Bact. Infect.	22.3 ± 3.44	1.23 ± 0.22	0.127 ± 0.037	0.030 ± 0.012	0.062±0.003
6- Mixed bact. Lys. oral vaccin.	26.4 ± 3.56	1.18 ± 0.18	0.139 ± 0.039	0.034 ± 0.012	0.051 ± 0.006
7- Mixed bact. Lys. oral vaccin-*Ps. aerog*. bact. infect.	22.0 ± 2.58	0.93 ± 0.22	0.123 ± 0.032	0.036 ± 0.008	0.049 ± 0.005

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxx Significant at P<0.05, P<0.01, P<0.001 when compared with gp (3).
–,– –,– – – Significant at P<0.05, P<0.01, P<0.001 when compared with its previous gp

Table (15): Mice peripheral blood haematological findings after four weekly vaccinations with mixed bacterial lysates .

Animal group	Total Leucocyte count $X10^3$/cmm	Eosinophil %	Neutrophils %	Lymphocytes %	Monocytes %
1- Normal control	3.86 ± 1.27	0.40 ± 1.6	48.70 ± 8.1	50.10 ± 5.91	1.90 ± 0.87
2- Placebo	3.86 ± 1.19	0.40 ± 1.6	47.10 ± 9.6	49.30 ± 6.43	1.80 ± 0.66
3- *Ps. aerog* bact infect.	4.12 ± 2.33	3.67 ± 2.34***,ººº	50.70 ± 2.48	51.50 ± 3.62	2.67 ± 1.63
4- Mixed bact. Lys. IN vaccin.	6.61 ± 0.39***,ººº	2.00 ± 1.19**,ºº	32.80 ± 6.64**,ºº	63.60 ± 6.05***,ººº	1.50 ± 0.76
5- Mixed bact. Lys. IN vaccin – *Ps. aerog*. bact. infect.	6.76 ± 2.38***,ººº,x	1.14 ± 1.57–	46.10 ± 5.8–	49.80 ± 7.36– –	2.86 ± 2.55
6- Mixed bact. Lys. oral vaccin.	4.84 ± 1.81	3.00 ± 1.77***,ººº	42.50 ± 2.93**,ºº	50.60 ± 4.77	5.00 ± 2.0
7- Mixed bact. Lys. oral vaccin- *Ps. aerog*. bact. infect.	7.37 ± 2.51***,ººº,x	3.60 ± 2.16***,ººº	46.80 ± 1.04– –,xx	46.80 ± 2.13	2.83 ± 0.98

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxx Significant at P<0.05, P<0.01, P<0.001 when compared with gp (3).
–,– –,– – – Significant at P<0.05, P<0.01, P<0.001 when compared with its previous gp

Table (16): Peritoneal fluid haematological findings in mice after four weekly vaccinations with mixed bacterial lysates.

Animal group	Total Leucocyte count $X10^3$/cmm	Eosinophil %	Neutrophils %	Lymphocytes %	Monocytes %
1- Normal control	3.61 ± 1.81	0.38 ± 1.02	54.70 ± 4.31	41.70 ± 4.11	1.30 ± 0.6
2- Placebo	3.20 ± 1.22	0.38 ± 1.02	52.80 ± 3.16	42.90 ± 5.16	1.10 ± 0.7
3- *Ps. aerog* bact infect.	3.35 ± 2.06	0.50 ± 3.40	39.80 ± 8.75**,^{oo}	57.80 ± 8.92***,^{ooo}	0.33 ± 0.82
4- Mixed bact. Lys. IN vaccin.	1.89 ± 0.84*,^{o}	0.00 ± 0.0	55.50 ± 6.99	42.00 ± 6.53	2.13 ± 1.25
5- Mixed bact. Lys. IN vaccin – *Ps. aerog*. bact. infect.	4.46 ± 2.66	0.25 ± 0.04	56.40 ± 11.92^{x}	43.10 ± 12.32	0.75 ± 1.04,
6-Mixed bact. Lys. oral vaccn.	6.68 ± 1.21**,^{oo}	0.00 ± 0.0	64.50 ± 5.33***,^{ooo}	33.00 ± 7.62	2.13 ± 1.25
7- Mixed bact. Lys. oral vaccin- *Ps. aerog*. bact. infect.	5.23 ± 3.76	0.25 ± 0.02	60.00 ± 8.39*,^{o,x}	38.90 ± 9.71^{xx}	0.89 ± 1.45

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
^{o}, ^{oo}, ^{ooo} Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxx Significant at P<0.05, P<0.01, P<0.001 when compared with gp (3).
–,– –,– – – Significant at P<0.05, P<0.01, P<0.001 when compared with its previous gp

Table (17):Mice bone marrow , peripheral blood and peritoneal fluid films lymphocyte percentage after four weekly vaccinations with mixed bacterial lysates .

Animal group	Bone marrow lymphocytes %	Peripheral blood lymphocytes %	Peritoneal fleuid lymphocytes %
1- Normal control	41.90 ± 5.9	50.10 ± 5.91	41.70 ± 4.11
2- Placebo	42.80 ± 5.4	49.30 ± 6.43	42.90 ± 5.16
3- *Ps. aerrog* bact infect.	25.90 ± 2.92***,^{ooo}	51.50 ± 3.62	57.80 ± 8.92***,^{ooo}
4- Mixed bact. lys. IN vaccin.	30.10 ± 1.64***,^{ooo}	63.60 ± 6.05***,^{ooo}	42.00 ± 6.53
5- Mixed bact. lys. IN vaccin – *Ps. aerog*. bact. infect.	36.30 ± 4.31$^{– – –,xx}$	49.80 ± 7.63$^{– –}$	43.10 ± 12.32
6- Mixed bact. lys. oral vaccin.	40.40 ± 4.63	50.60 ± 4.77	33.00 ± 7.62
7- Mixed bact. lys. oral vaccin - *PS. aerog*. bact. infect.	35.30 ± 5.77^{xx}	46.80 ± 2.13	38.90 ± 9.71^{xx}

*,**,*** Significant at P<0.05, P<0.01, P<0.001 when compared with gp. (1)
^{o}, ^{oo}, ^{ooo} Significant at P<0.05, P<0.01, P<0.001 when compared with gp (2).
x,xx,xxx Significant at P<0.05, P<0.01, P<0.001 when compared with gp (3).
–,– –,– – – Significant at P<0.05, P<0.01, P<0.001 when compared with its previous gp

Table (18): Mice serum IgM and IgG levels after four weekly vaccinations with *Esch. coli* lysate ▲.

Animal group	Serum IgM (mg/L)▲	Serum IgG (mg/L)▲
1- Normal control (C)	16.70 ± 0.58	25.7 ± 8.51
2- Placebo (P)	16.5 ± 0.56	24.3 ± 7.81
3- *Ps. aerog.* bact infect. (PSI)	25.4 ±2.21***,ººº	39.5 ± 2.43**,ºº
4- *Esch. coli lys.* IN vaccin. (EIN)	26.80 ± 1.84***,ººº	22.50 ± 4.57
5- *Esch. coli lys.* IN vaccin – *Ps. aerog*. bact. infect. (EINI)	28.50 ± 1.04***,ººº	79.10 ± 4.08***,ººº
6- *Esch. coli lys.* oral vaccin. (EO)	24.2 ± 2.49**,ººº	38.4 ± 2.89**,ººº
7- *Esch. coli lys.* oral vaccin - *Ps. aerog*. bact. infect. (EOI)	28.60 ± 1.09***,ººº	75.7 ± 6.57***,ººº

*,**,***Significant at P<0.05, P<0.01, P<0.001, respectively, when compared with normal control group.
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001, respectively, when compared with placebo treated group

▲ Using plate diffusion method.

Table (19): Mice serum IgM and IgG levels after four weekly vaccinations with *Pseudomona aeroginosa* lysate▲ .

Animal group	Serum IgM (mg/L)▲	Serum IgG (mg/L)▲
1- Normal control (C)	16.70 ± 0.58	25.7 ± 8.51
2- Placebo (P)	16.5 ± 0.56	24.3 ± 7.81
3- *Ps. aerog.* bact infect. (PSI)	25.4 ±2.21***,ººº	39.5 ± 2.43**,ºº
4- *Ps. aerog lys.* IN vaccin. (PSIN)	21.4 ± 1.48**,ºº	43.1 ± 3.66***,ººº
5-*Ps aerog.lys.* IN vaccin – *Ps. aerog*. bact. infect. (PSINI)	26.2 ± 2.51***,ººº	79.40 ± 3.10***,ººº
6-*Ps. aerog. lys.* oral vaccin. (PSO)	24.60 ± 2.69***,ººº	43.0 ± 3.42***,ººº
7-*Ps aerog. lys.* oral vaccin - *Ps. aerog*. bact. infect. (PSOI)	27.6 ± 3.05***,ººº	85.6 ± 3.23***,ººº

*,**,***Significant at P<0.05, P<0.01, P<0.001, respectively, when compared with normal control group.
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001, respectively, when compared with placebo treated group

▲ Using plate diffusion method.

Table (20): Mice serum IgM and IgG levels after four weekly vaccinations with *Staph. aureus* lysate ▲.

Animal group	Serum IgM (mg/L)▲	Serum IgG (mg/L)▲
1- Normal control (C)	16.70 ± 0.58	25.7 ± 8.51
2- Placebo (P)	16.5 ± 0.56	24.3 ± 7.81
3- *Ps. aerog.* bact infect. (PSI)	25.4 ±2.21***,ººº	39.5 ± 2.43**,ºº
4- *Staph. aureus lys.* IN vaccin. (SIN)	22.9 ± 6.61***,ººº	42.9 ± 4.19**,ºº
5- *Staph. aureus lys.* IN vaccin – *Ps. aerog*. bact. infect. SINI)	29.4 ± 4.11***,ººº	49.70 ± 4.81***,ººº
6- *Staph. aureus lys.* oral vaccin. (SO)	21.70 ± 4.73***,ººº	43.90 ± 5.02***,ººº
7- *Staph. aureus lys.* oral vaccin - *Ps. aerog*. bact. Infect. (SOI)	32.4 ± 5.64***,ººº	65.6 ± 5.23***,ººº

*,**,*** Significant at P<0.05, P<0.01, P<0.001, respectively, when compared with normal control group.
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001, respectively, when compared with placebo treated group

▲ Using plate diffusion method.

Table (21): Mice serum IgM and IgG levels after four weekly vaccinations with mixed bacterial lysates▲.

Animal group	Serum IgM (mg/L)▲	Serum IgG (mg/L)▲
1- Normal control (C)	16.70 ± 0.58	25.7 ± 8.51
2- Placebo (P)	16.5 ± 0.56	24.3 ± 7.81
3- *Ps. aerog.* bact infect. (PSI)	25.4 ±2.21***,ººº	39.5 ± 2.43**,ºº
4- Mixed bact. lys. IN vaccin. (MIN)	20.90 ± 3.64*,º	39.2 ± 4.17*,º
5- Mixed bact. lys. IN vaccin – *Ps. aerog*. bact. infect. (MINI)	26.30 ± 3.91***,ººº	83.0 ± 6.28***,ººº
6- Mixed bact. lys. oral vaccin. (MO)	22.40 ± 3.29**,ºº	42.1 ± 5.89**,ºº
7- Mixed bact. lys. oral vaccin - *Ps. aerog*. bact. infect. (MOI)	28.6 ± 4.72***,ººº	65.6 ± 7.82***,ººº

*,**,*** Significant at P<0.05, P<0.01, P<0.001, respectively, when compared with normal control group.
º, ºº, ººº Significant at P<0.05, P<0.01, P<0.001, respectively, when compared with placebo treated group.
▲ Using plate diffusion method.

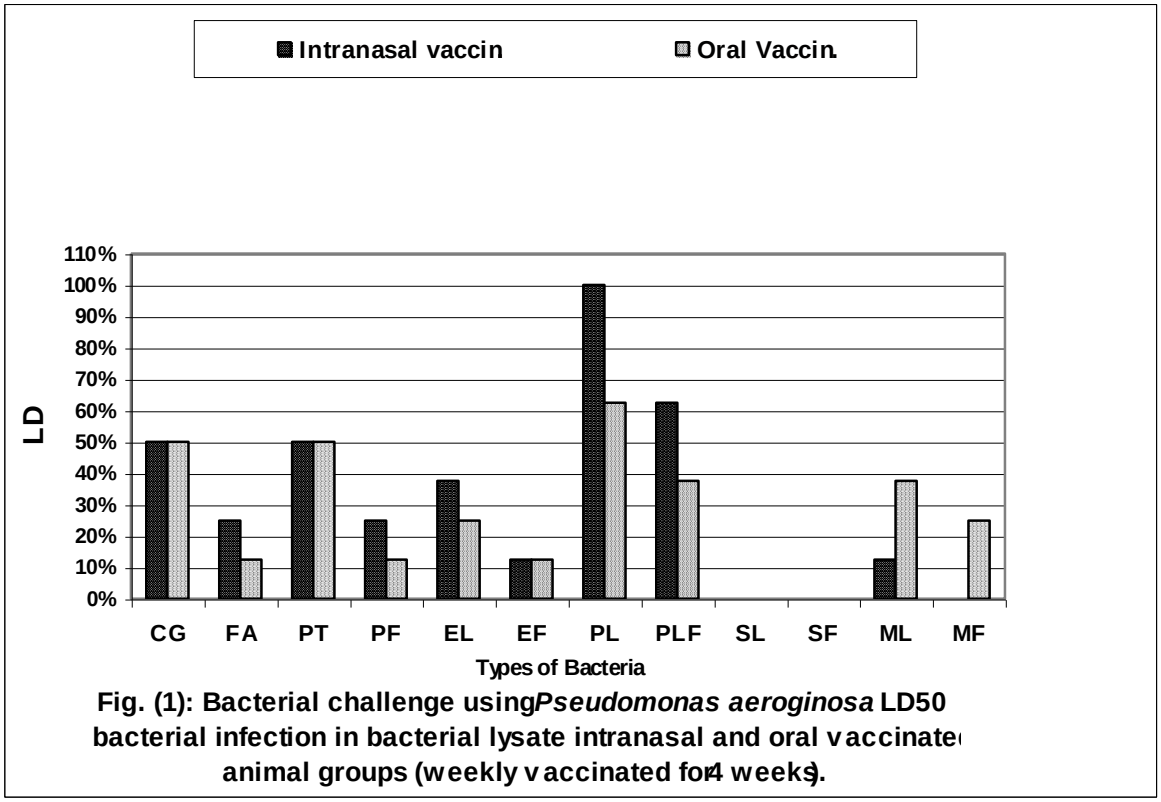

Fig. (1): Bacterial challenge using *Pseudomonas aeroginosa* LD50 bacterial infection in bacterial lysate intranasal and oral vaccinated animal groups (weekly vaccinated for 4 weeks).

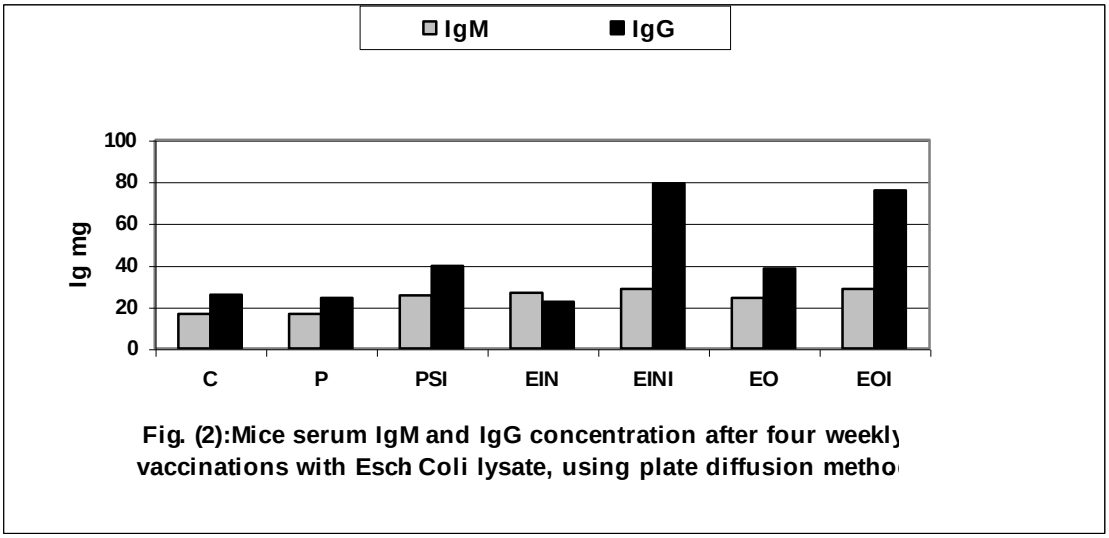

Fig. (2):Mice serum IgM and IgG concentration after four weekly vaccinations with Esch Coli lysate, using plate diffusion metho

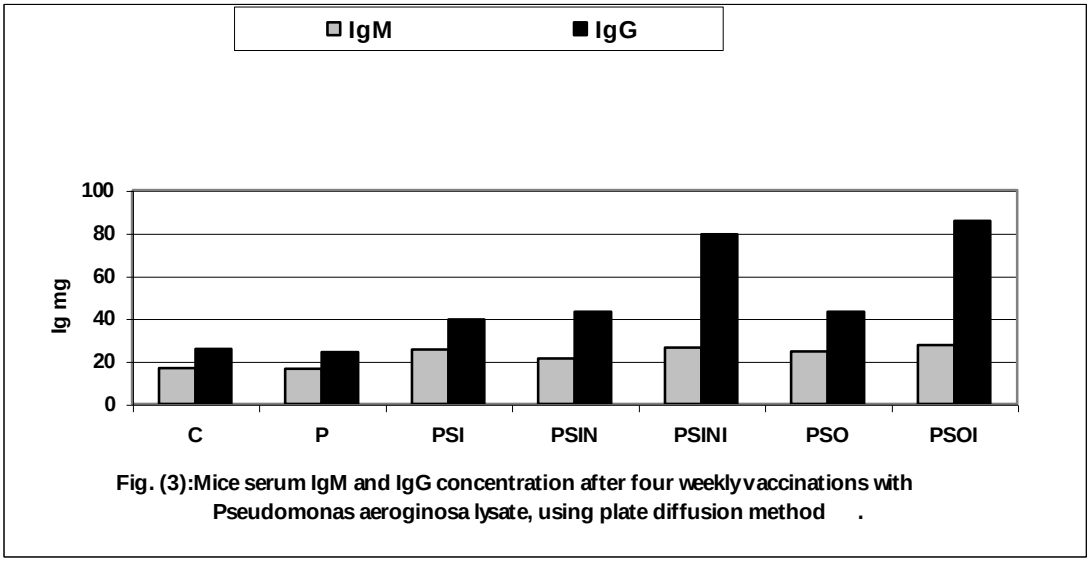

Fig. (3):Mice serum IgM and IgG concentration after four weekly vaccinations with Pseudomonas aeroginosa lysate, using plate diffusion method .

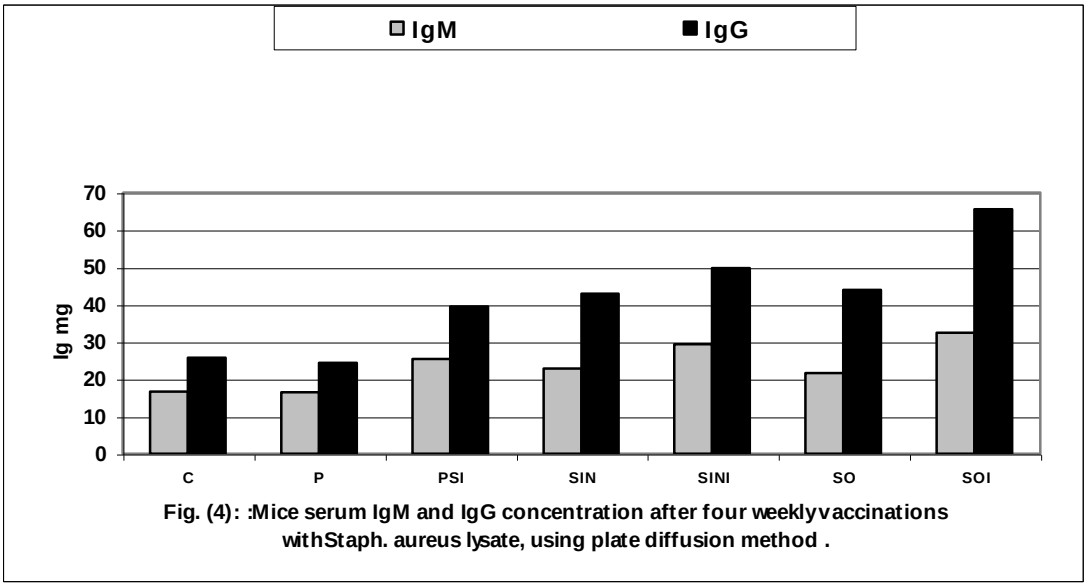

Fig. (4): :Mice serum IgM and IgG concentration after four weekly vaccinations withStaph. aureus lysate, using plate diffusion method .

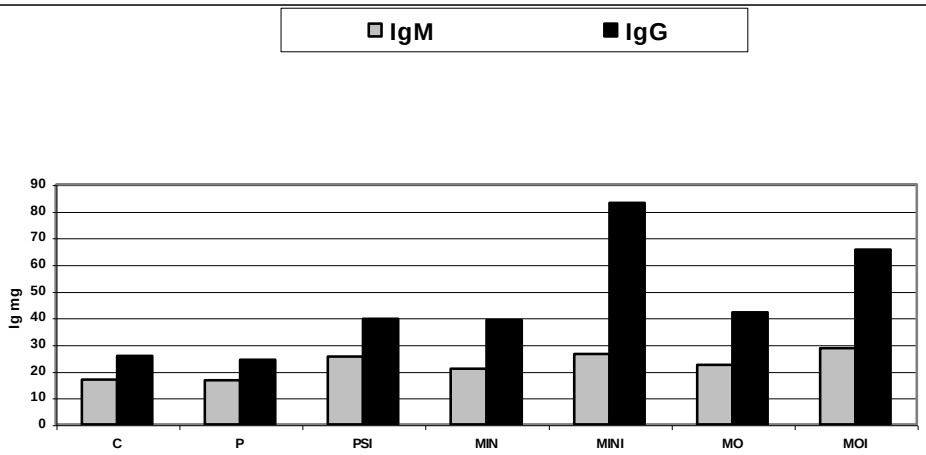

Fig. (5): :Mice serum IgM and IgG concentration after four weekly vaccinations with mixed bacterial lysate, using plate diffusion method .

Discussion

Bacterial vaccines are those composed of live attenuated, killed bacteria or bacterial products. This study has approached the immunoprophylaxis effect of bacterial vaccines using the whole cell bacterial lysates. The use of oral and intranasal bacterial vaccines stimulate the local and central effector sites of the mucosa of intestine and respiratory tract. The microrganisms can elicit an enhanced Ig A response according to the concept of a common mucosal immune system axis (Ruedl *et al*, 1994).

In the present work, Freund's adjuvant has improved the immunoprotective effect elicited by intranasal and oral bacterial lysates vaccination in intraperitoneally bacterial challenged animals where intraperitoneal peritonitis/ sepsis model have shown enhanced survival rates. Bennett- Guerrero *et al* (2000) showed that liposomal core LPS- active immunization of mice provided protection against a lethal challenge with *Esch. coli* 018 LPS. Mader *et al* (1997) have showed that bacterial cell wall complex and antigenic determinants could stimulate the release of tumour necrosis factor- alpha and prostaglandin E2. Rutishauser *et al* (1998) and Grevers *et al* (2000) suggested that bacterial lysate low molecular weight immunomodulators play a major role in the protection against bacterial infections. Other bacterial components that could play a role as immunoprophylactics were ribosomal extracts (Gawlik and Danek, 1999). Our study illustrated the immunoprotective effect of *Esch. coli* bacterial lysates, both on oral and intranasal vaccinations. On the contrary, *Pseudomonas aeroginosa* lysate-vaccinated animal groups showed a suppressed immune response and increased lethality in bacterially challenged groups. This could be explained by the finding of Cotran *et al* (1999) who mentioned that *Pseudomonas* bacteria secrete a leucotoxin that kills neutrophils. O'Brien *et al* (2001) have mentioned that attempts to develop vaccine that could enhance neutrophil phagocytosis by stimulating production of opsonizing antibodies to *Staph. aureus* have met with limited success because of low immunogenicity of the exopolysaccharide capsule surrounding *Staph. aureus. Staphylococcus aureus* can also adhere to and penetrate epithelial tissue. They proved that *Staph*

aureus lysates emulsified in Freund's incomplete adjuvant markedly stimulated the opsonizing antibodies, that were more effective when *Staph. aureus* lysate was incorporated in microspheres. Our study showed similar results where the protective effect of *Staph. aureus* lysate in LD50 bacterially challenged animals was enhanced by Freund's adjuvant. This effect was observed in oral and intranasal vaccinated animals and it was associated with peripheral blood changes and reticuloendothelial system changes, in addition to elevation of serum Ig G and Ig M.

Bonenfant *et al* (2001) have explained the immunoprotective effect of intranasal immunization. They showed that mucosal adjuvants can significantly enhance the immunogenicities of intranasally administered antigens. Cholera toxin and heat-labile enterotoxin are strong mucosal adjuvants with a variety of antigens. These two adjuvants were tested with *Toxoplasma gondii* SAG1 protein in intranasal vaccinated mice . Their study showed that gamma interferon and interleukin- 2 (IL-2) production by splenocytes and (IL-2) production by mesenteric lymph nodes cells were observed in vitro after antigen restimulation, underlying Th-1 like response. Effective protection against pathogens required both mucosal and systemic immune responses. Mucosal adjuvants can significantly enhance the immunogenicities of intranasally administered antigens. Our study could prove this immunostimulant effect of mucosal adjuvants. The animal groups treated intranasally or orally with Freund's adjuvant reported higher survival rates against bacterial challenge. Kuenen et al, 1994 have mentioned that the protective effect of bacterial lysates was accompanied by priming for specific Ig G responsiveness (probably at the T cell level) and a significant Ig A serum antibody levels. Our study have proved that repeated intranasal and oral vaccinations induced strong systemic immunoglobulin G and immunoglobulin M (Ig G & Ig M) response. This response was highly signifycant in bacterially challenged animals. Other studies showed that the stimulated systemic response was associated with stimulated mucosal (IgA) humoral response upon intranasal immunization (Bonenfant *et al,* 2001) and oral immuni-zation with *Salmonella typhimurium* (Harrison *et al,* 1997) and *Helicobacter pylori* lysate (Kim *et al,* 1999) who mentioned that the presence of antibody-secreting cells in intestinal lamina propria lymphocytes was correlated with Ig A level in gut washing fluids. These levels were highly increased on repeated oral booster immunization with *Helicobacter pylori* whole-cell lysate. Ciebiada *et al* (1989) showed that the use of BCG and *Corynbacterium pavum* vaccines whether oral or intranasal in bacterial challenged and non-bacterial challenged animal groups have shown histological changes and morphological differences in the liver , spleen , thymus and lymph nodes depending on the type of the vaccine used . The preparations were characterized by stimulating effect on the reticuloendothelial system. Our study showed that these reticulendothelial system changes were assocaited with haematological changes in the peripheral blood, peritoneal fluid and the bone marrow lymphocytic percentage. These changes were directy reflected on survival rates in the bacterially challenged animal groups.

This study concludes that bacterial lysates and mixed bacterial lysates have an immunomodulatory / immunostimulatory effect. This effect varies according to the type of the bacterial lysate used. Freund's adjuvent enhances the immunostimulatory effect of intranasal and oral applied bacterial lysates.

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التأثير الواقى للأستخدام الفمى والأنفى للمتحللات البكتيرية

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** قسم الحيوان كلية العلوم للبنات بجامعة الازهر.

تمت دراسة التأثيرات المفيدة للتطعيم بالمتحللات البكتيرية وذلك عند أستخدام الجرعة نصف القاتلة لميكروب السودوموناس أيروجينوز هذا وقد تم أستخدام طريقتين لإدخال المتحللات البكتيرية وهما الأنف والفم وذلك فى وجود وعدم وجود المساعد المناعى فروندز . هذا وقد تم حقن الجرعة نصف القاتلة لميكروب السودوموناس أيروجينوزا عن طريق الحقن بالغشاء البريتونى للفئران البيضاء .هذا وقد اشتملت الدراسة على مجموعات الحيوانات الاتية : المساعد المناعى فروندز – المحلول – المتحلل البكتيرى لميكروبات الأشيريشيا كولاى والسودوموناس أيروجينوزا والستافيلوكوكاس أورياس ومخلوط للمتحللات البكتيرية . هذا وقد تم أيضا أستخدام المحلول والمتحللات البكتيرية المختلفة مذابة فى المساعد المناعى فروندز . وقد كانت نتيجة التعامل بالجرعة نصف القاتلة عن طريق الانف بإستخدام المحلول ومتحلل الإيشيرشيا كولاى وتحلل البكتيريا العنقوديه ستافيلو كوكاى والسودوموناس ايروجينوزا ومخلوط المتحللات البكتيرية هى 50% و 37.5 % و 100% وصفر % و 12.5 % على التوالى وعند استخدامها مع المساعد المناعى (فروندز) كانت النتائج 25% و 25% و 12.5 % و 62.5% وصفر % و صفر % على التوالى . وعند استخدام نفس التطعيمات عن طريق الفم كانت النتائج على التوالى 50% و 25% و 62.5 % و صفر % و 37.5 % وبإستخدام المساعد المناعى فروندز كانت على التوالى 12.5% و 12.5% و 12.5% و 37.5% و صفر % و 25% .هذا وقد أثبتت الدراسة أن أستخدام المساعد المناعى قد رفع نسبة المقاومة . هذا وقد تمت دراسة الاوزان الكلية (و) وأوزان الكبد (ك) و الطحال(ط) و غدة الثيموس (ث) وتم أحتساب النسبة ك +ط+ث/ و كذلك تم قياس عد الدم الابيض الكلى والنوعى فى الدم وفى السائل البريتونى والنسبة المئوية للخلايا الليمفاوية فى نخاع العظم هذا و قد تم قياس الجلوبيولين المناعى (أم و جى) بإستخدام طريقة أطباق الانتشار . هذا وقد تمت القياسات منسوبة ألى المجموعة الحاكمة مما أثبت أمكانية أستخدام المتحللات البكتيرية فى التطعيم وذلك كمحفز مناعى وذلك بالأستخدام الفمى أو الأنفى .