

## Response Of Mice Tissues To Intranasal Bacterial Lysate Vaccination: Immunohistopathological Study

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### Abstract

Effective protection against pathogenic bacteria requires both mucosal and systemic immune responses. Intranasal administration of antigens induces these responses. The protective effect of intranasal immunization with different formalin-inactivated bacterial lysates in albino mice was evaluated. This study used formalin inactivated lysate of each of the following *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexeneri*, *Staphylococcus aureus*, *Bacillus subtilis* and mixed bacterial lysates. The lysate was administered intranasally (5µl/ nostril) once daily for seven consecutive days. The results of this study recorded some changes in peripheral blood total and differential leucocytic counts, peritoneal fluid and bone marrow lymphocytic percentages. Spleen and thymus weight changes were reported under the effect of *Salmonella typhi lysate*, *Shigella flexeneri lysate* and *Staph. aureus lysate*. The level of immunoglobulin G (Ig G) was assessed in serum, bronchial lavage and nasal bed harvest. The levels of Ig G were significantly elevated in the three determinants, suggesting an efficient immunostimulatory effect of bacterial lysates. Some of these levels were exceeding 2-3 folds of that of the control group. Histopathological studies recorded changes in some reticuloendothelial system organs including the liver, spleen and thymus gland, besides, some changes were also observed in the lung and bronchi under the effect of intranasal vaccination. This study supports the immunoprotective effect of intranasal vaccination, using bacterial lysates.

### Introduction

Effective protection against respiratory tract pathogens requires both mucosal and systemic immune responses. Intranasal administration of antigens induces these responses. Intranasal immunization is more effective than oral immunization, as it generates an earlier and stronger mucosal immune response (Hirabayashi *et al.*, 1990). 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 to use even in young or pregnant animals (Bey *et al.*, 1997). Havlasova *et al.* (2002), have detected more than 80 different immunorelevant antigens came from whole cell bacterial lysate of *Francisella tularensis*. Such antigens are responsible for immune-modulator,

immunostimulant activities of whole cell bacterial lysates.

Bacterial vaccines can be applied effectively through the intranasal route (Orr *et al.*, 1993 and Ambrosino, 1996). Bonenfant *et al.* (2001) mentioned that the ability for increasing systemic and mucosal responses may be of great importance for the development of an efficient vaccine. Furthermore, the intranasal route requires less antigen than the oral route because there is much less proteolytic activity in the nasal cavity. This route effectively promotes the production of both systemic and mucosal immune responses to the antigen (Wu and Russell, 1997). Di-Tommaso *et al.* (1996) and Partidos *et al.* (1999), showed that bacterial enterotoxins act as appropriate mucosal adjuvants

following oral and intranasal immunization with various antigens that trigger local and systemic immune responses. These adjuvants have also been used with bacterial antigen and protect against infection with *Helicobacter pylori* after oral vaccination (Kim *et al.*, 1999) and intragastric vaccination (Marchetti *et al.*, 1998).

Gawlik and Danek (1999), mentioned that bacterial lysates act as immunomodulators- immunoprophylactics in chronic respiratory diseases. Rutishauser *et al.* (1998) and Grevers *et al.* (2000) used polyvalent bacterial lysates in patients with recurrent respiratory tract infections where an oral immunomodulator 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. Jakobsen *et al.* (1999) showed that the intranasal immunization with *Streptococcus pneumoniae* polysaccharide conjugate vaccine could protect mice against invasive pneumococcal infections. The use of nasal delivery of an acellular *Bordetella pertussis* vaccine, could effectively protect against *Bordetella pertussis* infection through the activation of Th1 and Th2 cells. Greenbaum *et al.* (2004) showed that the levels of mucosal Ig A and serum Ig A and Ig G were elevated in response to intranasal administration of inactivated anti- influenza vaccine.

This study aimed to evaluate the immunoprotective effect of daily intranasal lysate vaccination for seven consecutive days using lysates of each of the following: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri*, *Staphylococcus aureus* and mixed bacterial lysates.

## Material And Methods

### Bacteria:

1. *Escherichia coli* (CAIM- 1357).
2. *Pseudomonas aeruginosa* (clinically isolated).
3. *Staphylococcus aureus* (CAIM- 1352).
4. *Salmonella typhi*.

5. *Shigella sp.* (NMRO).
6. *Bacillus subtilis* (CAIM-1007).

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

### Animals:

Eighty 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 the following groups, control, placebo and intranasal vaccinated.

### Intranasal vaccinated group:

Bacterial lysate vaccinated group which received *Esch. coli* lysate, *Ps. aeruginosa* lysate, *Staph. aureus* lysate and mixed bacterial lysates (Corthesy- Theulaz *et al.*, 1998 and Bonenfant *et al.*, 2001). Each animal received 5 µl of the vaccine in each nostril, once daily that was repeated for seven consecutive days (Ruedl *et al.*, 1994 and Bennet- Guerrero *et al.*, 2000).

### Bacterial lysate preparation :

A 24 hours bacterial growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus subtilis* that had viable counts  $2.72 \times 10^8$ ,  $7.56 \times 10^8$ ,  $13.91 \times 10^8$ ,  $7.33 \times 10^8$  and  $2.91 \times 10^8$  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 (Okamoto *et al.*, 2004). 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).

### Nasal bed harvest :

The nasal-associated lymphoid tissue were harvested under sterile conditions

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using an automatic cell harvester, high speed homogenizer (Variable GKH-GT MOTOR control- Glass- Col. USA). One gram of the dissected nasal bed was harvested in 1 ml of phosphate buffer saline (PBS), then centrifuged aseptically at 4000 rpm for 15 mins. to separate the supernatant that was kept frozen until assayed for estimating the level of Ig G (Bonenfant *et al.*, 2001).

### Bronchial lavage :

Lung washes were obtained by performing bronchial lavage where repeated flushing and aspiration of 1 ml of phosphate buffer saline (PBS) was done under sterile conditions. The wash was centrifuged at 4000 rpm for 15 mins. to remove any cell debris. The supernatant was aspirated and kept frozen until assayed for estimating the level of Ig G (Williamson *et al.*, 2000 and Bonenfant *et al.*, 2001).

### 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- immundiff-usion 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 leucocytic count and differential leucocytic count. Direct smears were withdrawn from the peritoneal fluid for peritoneal fluid total and differential leucocytic counts. Bone marrow smears that were obtained from the femur bone were spreaded for bone marrow lymphocytic count. Leishman

stain was used for the different leucocytic 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 and the (L+S+Th)/T ratio was determined.

### Histopathological examination :

The liver, spleen, thymus gland, lung and tracheae were collected soon after slaughter and fixed in 10% formalin, blocked in paraffin wax, cut in sections 5  $\mu$ m and stained with haematoxyline and Eosin (H&E) for microscopic examination.

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

The results demonstrated in table (1) cleared that bacterial lysate daily vaccinated animal groups showed increased total leucocytic count except in case of *Salmonella typhi* lysate. This leucocytic response was significant on the use of mixed bacterial lysates at  $P < 0.001$ , that was associated with significant increase of neutrophilic count at  $P < 0.05$ . *Escherichia coli* lysate caused significant increase of neutrophilic count that was associated with a significant drop of lymphocytic count.

Table (2) showed that *Salmonella typhi*, *Shigella flexneri*, *Pseudomonas aeruginosa* and mixed bacterial lysates intranasal vaccination caused a significant drop of peritoneal fluid neutrophilic counts that were associated with a significant lymphocytic response and non- significant drop in monocytic and eosinophilic counts

Table (3) showed that mixed bacterial lysates caused an increase of bone marrow lymphocytic percentage in intranasally vaccinated animals, while *Salmonella typhi* lysate intranasal vaccination induced a significant drop of bone marrow lymphocytic percentage at  $P < 0.001$ .

Table (4) illustrated a splenomegalic effect of *Salmonella typhi* and *Shigella flexeneri* lysates intranasal vaccination. This splenomegalic effect of *Shigella flexeneri* lysate was associated with a significant increase of thymus weight at  $P < 0.01$ , similar thymus response was detected on the use of *Staph. aureus* lysate intranasal vaccination.

Table (5) and Fig. (5) showed that the different bacterial lysates caused a significant increase in the serum, nasal bed harvest and bronchial lavage Ig G levels. This increase was non-significant in the serum of the animals vaccinated with *Pseudomonas aeruginosa* lysate, while those vaccinated with *Esch. coli* lysate, showed a non-significant drop in the level of seum Ig G.

#### Histopathological analysis :

The liver of mice which were vaccinated intranasally with *Esch. coli* and *Ps. aerog. lysate* showed activation of Kupffer cells (Figs. 1- 3&4). That activation of Kupffer cells was associated with mononuclear cellular infiltration in the periportal areas in the animals vaccinated with *Shig. flexeneri* lysate, *Staph. aureus* lysate and mixed bacterial lysates (Fig. 1-6,7&8). Fig. 1-7 showed lymphocytic infiltration associated with mild degenerative changes in the portal area and ruptured endothelial lining of hepatic-portal vein. Those vaccinated with *Salm. typhi* lysate revealed congestion of central veins and portal blood vessels, inflammatory lymphocytic infiltration and some cells showed hepatocellular degeneration (Fig. 1-5).

The spleen of mice treated intranasally with *Ps. aerog. lysate* and *Staph aureus* lysat showed congested sinusoidal spaces filled with erythrocytes and early signs of inflammation with mononuclear and polymorphonuclear leucocytes, stagnation of blood within splenic vessels that infiltrated the red pulp could be detected (Fig. 2- 4&7). Bacterial lysates caused a considerable increase in the white pulp,

specially the outer part of the periarterial lymphocyte sheath (PALS), where abundant lymphoid cells appeared. The red pulp contained aggregations of large nuclei varying considerably in size (Fig. 2- 4,6 &8). The spleen of mice that received *Shig. flexeneri* lysate showed heavy lymphocytic infiltration of mature lymphocytes, plasma cells and macrophages in red pulp (Fig. 2-6). The spleen of mice treated with *Salm. typhi* lysate showed degenerative changes. There was a reduction in the overall cellularity of follicles and number of mature lymphocytes. Megakaryocytes were readily lost and myelopoiesis appeared virtually reduced (Fig. 2- 5).

The thymus obtained from animals vaccinated with *Esch. coli* lysate showed degenerated cells in cortex and medulla of the thymus gland and reduced Hassal's corpuscles (Fig. 3-3), other bacterial lysates caused heavy lymphocytic infiltration (Fig. 3-4,5,6,7&8). The thymus sections obtained from mice vaccinated intranasally with *Ps. aerog. lysate* showed some cortical and medulla cells that appeared faintly stained, also corticomedullary differentiation was indistinct (Fig. 3-4). The thymus gland section obtained from animals received *Shigella flexeneri* lysate showed some vacuolated cells in medulla and cortex, also bizarre cells or their debris could be detected (Fig. 3-6).

Lung tissues obtained from bacterial lysates intranasally vaccinated animals showed increased lymphocytic and cellular infiltrations. This infiltration was markedly heavy around the wall of the bronchi in the animals treated with *Ps. aerog. lysate* (Fig. 4-4). Lung sections obtained from animals vaccinated with *Salm. typhi* lysate intranasally showed mild fluid accumulation inside the alveoli and the small bronchioles and thickened alveolar wall associated with fibrosis (Fig. 4-5). *Shig. flexeneri* lysate intranasal vaccination caused degenerative changes in the lung tissue and highly thickened alveolar and bronchiolar walls (Fig. 4-6).

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**Table (1): peripheral blood total and differential leucocytic counts in bacterial lysates intranasal daily vaccinated animals (7- days).**

ANIMAL GROUP	TOTAL LEUCOCYTIC COUNT / CMM	EOSIOPHILS %	NEUTROPHILS %	LYMPHOCYTES %	MONOCYTES %
NORMAL CONTROL	3900±1791	1.88±2.01	42.4±3.1	54±3.0	1.99±0.86
PLACEBO	4135±1233	1.67±1.94	41.3±2.7	53.8±4.7	1.96±0.21
<i>ESCH. COLI</i> LYSATE	4471±1655	1.0±0.92	53.0±4.1 <sup>***,°</sup>	45.1±3.6 <sup>*°</sup>	1.5±0.76
<i>S. TYPHI</i> LYSATE	4017±1749	1.67±0.34	40.1±2.9	54.7±0.9	2.83±0.18
<i>SHIGELLA FLEXENERI</i> LYSATE	5000±1369	1.17±1.60	44.7±3.3	51.3±2.9	2.67±0.83
<i>PSEUDO. AEROGINOSA</i> LYSATE	4688±2464	1.54±0.76	39.6±3.8	56.5±4.4	2.13±0.34
<i>STAPH. AUREUS</i> LYSATE	5100±1873	0.123±0.34	38.9±4.9	59.2±3.8	2.25±0.97
MIXED BACTERIAL LYSATES	7367±721 <sup>***,°°°</sup>	1.5±1.38	48.5±4.6 <sup>*°</sup>	48±4.4	2.25±2.84

\*, \*\*, \*\*\* significant at P<0.05, 0.01 and 0.001, respectively, when compared with control group.  
 °, °°, °°° Significant at ° P<0.05, 0.01 and 0.001, respectively, when compared with placebo group.

**Table (2): Peritoneal fluid total and differential leukocytic counts in bacterial lysates- intranasal daily vaccinated animals (7- days).**

ANIMAL GROUP	TOTAL LEUCOCYTIC COUNT / CMM	EOSIOPHILS %	NEUTROPHILS %	LYMPHOCYTES %	MONOCYTES %
NORMAL CONTROL	2800±1563	0.75±0.89	48.4±3.38	49±1.77	2±1.17
PLACEBO	2994±945	0.71±0.67	49.3±2.71	48.2±2.54	1.75±1.06
<i>ESCH. COLI</i> LYSATE	2900±2379	0.5±0.76	52±8.52	58.6±3.78 <sup>*°,°°</sup>	1±0.76
<i>S. TYPHI</i> LYSATE	2133±920	0.17±0.38	36.5±2.17 <sup>***,°°°</sup>	62±2.83 <sup>***,°°°</sup>	1.33±0.82
<i>SHIGELLA FLEXENERI</i> LYSATE	1583±655	0.19±0.68	41±1.79 <sup>**°,°°°</sup>	58.5±1.52 <sup>***,°°°</sup>	0.17±0.41
<i>PSEUDO. AEROGINOSA</i> LYSATE	2216±1839	1±0.93	35.5±5.71 <sup>**°,°°°</sup>	62.3±5.42 <sup>***,°°°</sup>	1.5±0.96
<i>STAPH. AUREUS</i> LYSATE	3040±1362	0.17±0.28	42.8±9.25 <sup>°</sup>	55±7.63	0.13±0.53
MIXED BACTERIAL LYSATES	2790±1389	0.24±0.28	39.2±6.72 <sup>*°,°°</sup>	66.9±7.21 <sup>**°,°°</sup>	1±0.93

\*, \*\*, \*\*\* significant at P<0.05, 0.01 and 0.001, respectively, when compared with control group.  
 °, °°, °°° Significant at ° P<0.05, 0.01 and 0.001, respectively, when compared with placebo group.

**Table (3): Peripheral blood, peritoneal fluid and bone marrow lymphocytic percentage in bacterial lysates-intranasal daily vaccinated animals (7- days).**

ANIMAL GROUP	PERIPHERAL BLOOD LYMPHOCYTES %	PERITONEAL FLUID LYMPHOCYTES %	BONE MARROW LYMPHOCYTES %
NORMAL CONTROL	54±3.0	49±1.77	49.9±5.11
PLACEBO	53.8±4.7	48.2±2.54	48.2±5.63
<i>ESCH. COLI</i> LYSATE	45.1±3.6*,°	58.6±3.78*,°	48.1±4.29
<i>S. TYPHI</i> LYSATE	54.7±0.9	62±2.83***,°	20.3±3.67***,°
<i>SHIGELLA FLEXENERI</i> LYSATE	51.3±2.9	58.5±1.52***,°	40.5±3.83
<i>PSEUDO. AEROGINOSA</i> LYSATE	56.5±4.4	62.3±5.42***,°	50.1±3.43
<i>STAPH. AUREUS</i> LYSATE	59.2±3.8	55±7.63	38.5±4.14*
MIXED BACTERIAL LYSATES	48±4.4	66.9±7.21**,°	64.1±8.45*,°

\*, \*\*, \*\*\* significant at P<0.05, 0.01 and 0.001, respectively, when compared with control group.  
 °, °°, °°° significant at P<0.05, 0.01 and 0.001, respectively, when compared with placebo group.

**Table (4): Total body weight (T), liver (L), spleen (S) and thymus (Th) weight and L+ S+ Th/ T ratio in bacterial lysate-intranasal daily vaccinated animals (7- days).**

ANIMAL GROUP	TOTAL BODY WEIGHT (T) IN GMS	LIVER (L) WEIGHT IN GMS	SPLEEN (S) WEIGHT IN GMS	THYMUS (TH) WEIGHT IN GMS	L+S+TH/T RATIO
NORMAL CONTROL	24.2±3.49	1.11±0.25	0.123±0.03	0.041±0.006	0.053±0.007
PLACEBO	24.9±4.26	1.18±0.28	0.125±0.04	0.04±0.002	0.055±0.006
<i>ESCH. COLI</i> LYSATE	27.1±4.99	1.35±0.36	1.162±0.04	0.034±0.004	0.055±0.007
<i>S. TYPHI</i> LYSATE	25.9±0.64	1.12±0.22	0.246±0.04***,°	0.043±0.002	0.054±0.001
<i>SHIGELLA FLEXENERI</i> LYSATE	25.7±1.81	1.19±0.18	0.205±0.02***,°	0.052±0.003***,°	0.056±0.004
<i>PSEUDO. AEROGINOSA</i> LYSATE	24.7±2.16	1.17±0.24	0.150±0.05	0.046±0.006	0.055±0.01
<i>STAPH. AUREUS</i> LYSATE	25.8±3.13	1.13±0.26	0.133±0.05	0.049±0.001*,°	0.051±0.008
MIXED BACTERIAL LYSATES	24.4±2.26	1.10±0.08	0.125±0.02	0.047±0.002	0.050±0.002

\*, \*\*, \*\*\* significant at P<0.05, 0.01 and 0.001, respectively, when compared with control group.  
 °, °°, °°° significant at P<0.05, 0.01 and 0.001, respectively, when compared with placebo group.

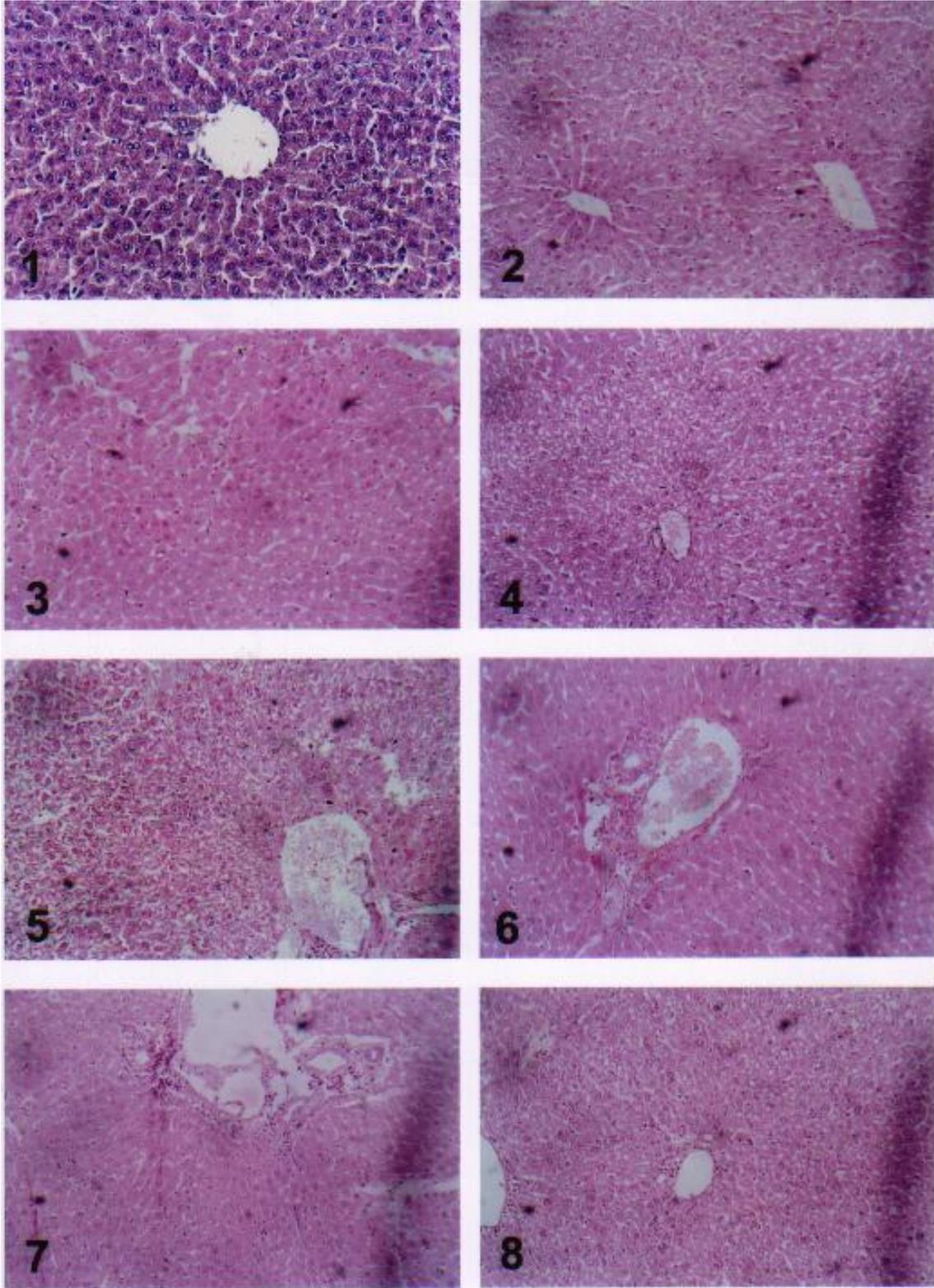
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**Table (5): Immunoglobulin G (IgG) levels using plate diffusion method in the serum, bronchial lavage and nasal harvest in the different bacterial lysates-intranasal treated animals (daily vaccinated for seven days).**

ANIMAL GROUP	IGG MG/L		
	SERUM	BRONCHIAL LAVAGE	NSAL HARVEST
NORMAL CONTROL	27.2±4.6	16.2±3.9	17.3±2.2
PLACEBO	27.3±4.4	16.5±3.1	18.4±1.9
<i>ESCH. COLI</i> LYSATE	19.9±10.4	36.8±4.9***, °°°	33.9±1.9***, °°°
<i>S. TYPHI</i> LYSATE	35.6±3.5*, °	40.9±4.2***, °°°	23.2±3.1*, °
<i>SHIGELLA FLEXENERI</i> LYSATE	39.7±5.1*, °	34.2±2.9***, °°°	37.3±7.4***, °°°
<i>PSEUDO. AEROGINOSA</i> LYSATE	36.3±9.6	39.1±3.8***, °°°	34.1±1.6***, °°°
<i>STAPH. AUREUS</i> LYSATE	36.8±3.8*, °	30±6.4**, °°	37±3.6***, °°°
MIXED BACTERIAL LYSATES	44.4±3.2***, °°°	36.3±3.7***, °°°	25.4±2.7**, °°

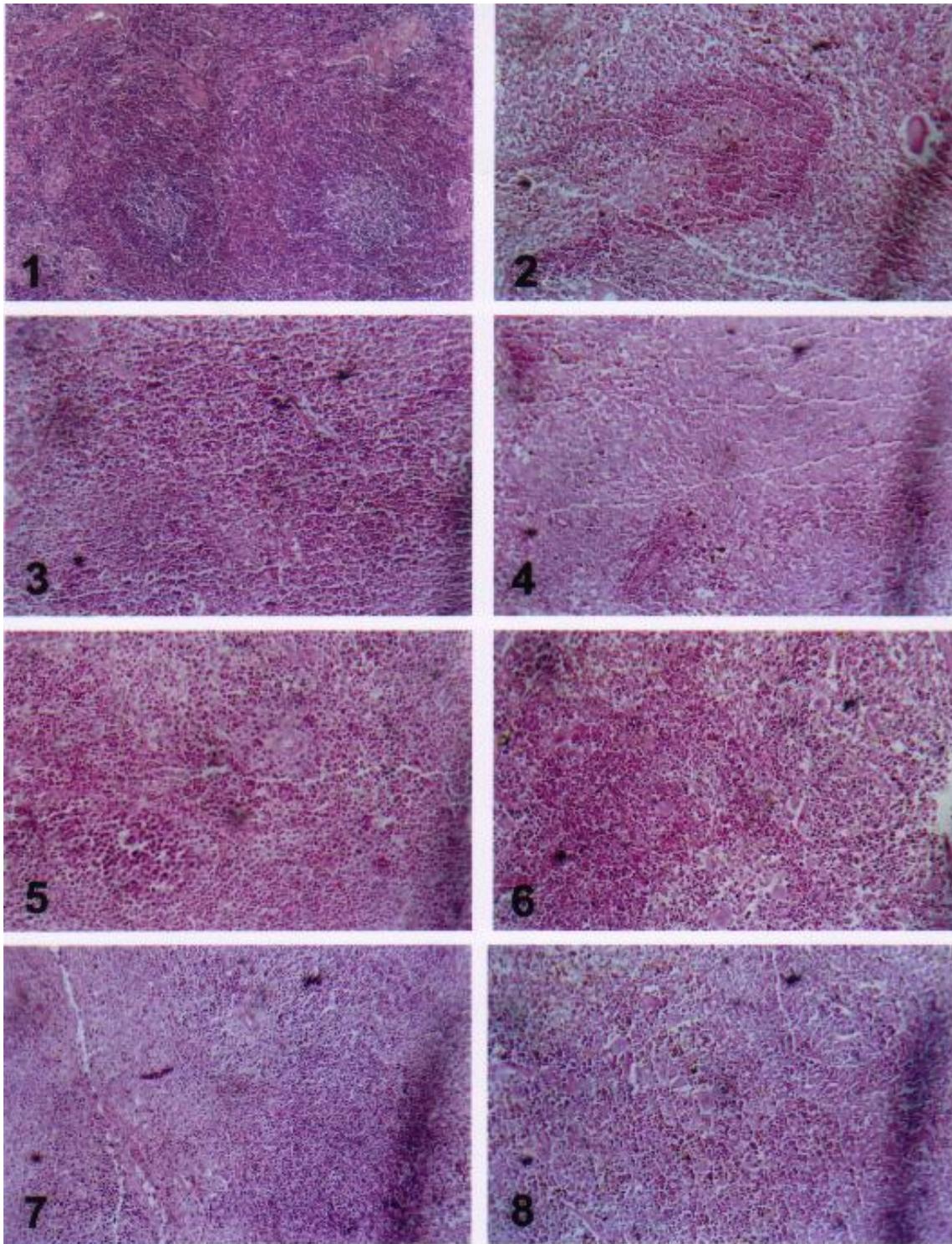
\*, \*\*, \*\*\* significant at P<0.05, 0.01 and 0.001, respectively, when compared with control group.

°, °°, °°° significant at P<0.05, 0.01 and 0.001, respectively, when compared with placebo group.

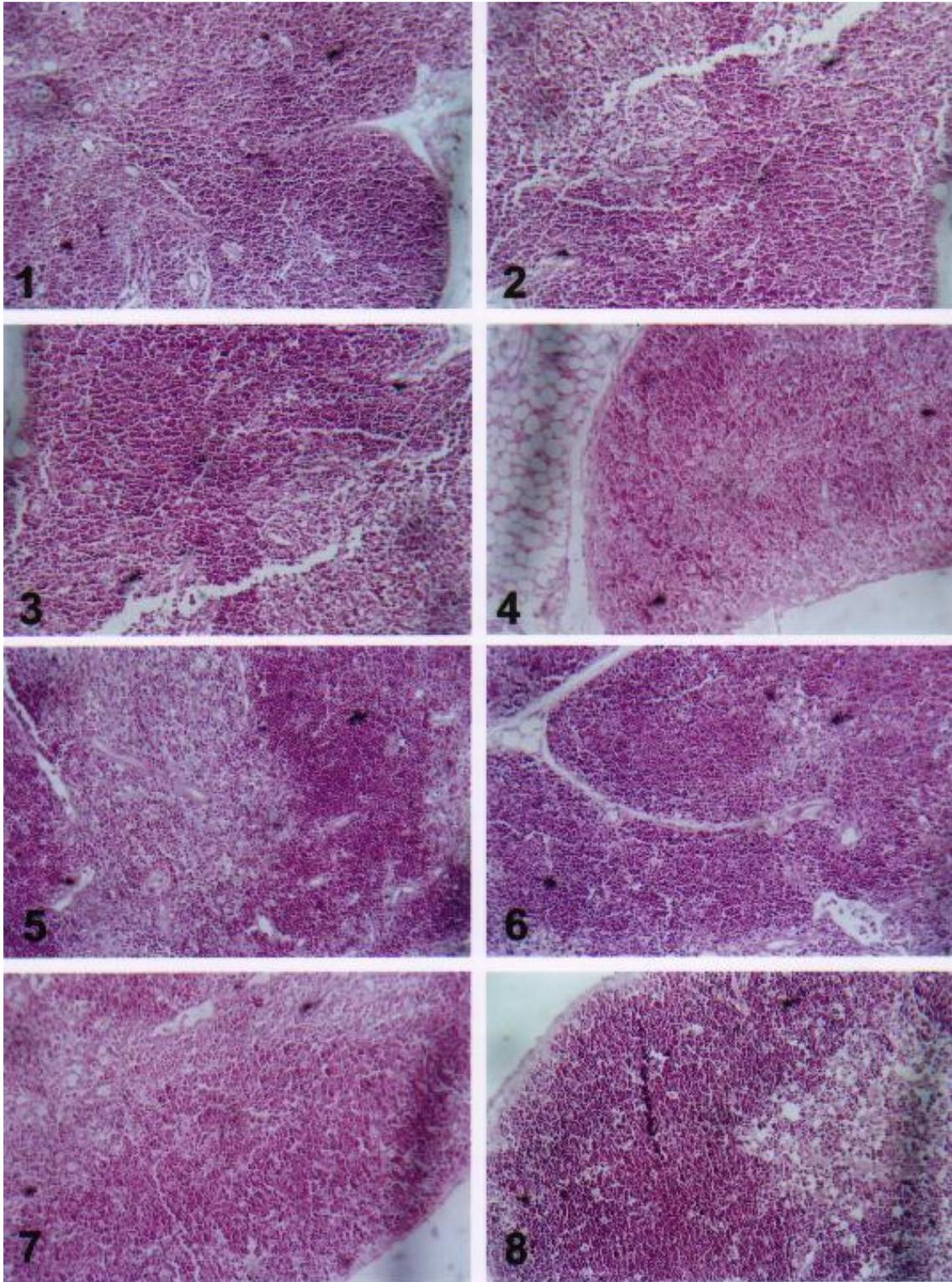


**Fig. (1): Liver sections showing: 1- Normal control 2- Placebo treated 3- Esch coli lysate 4- Ps aeruginosa lysate 5- Salm typhi lysate 6- Shig flexeneri lysate 7- Staph aureus lysate 8- Mixed bacterial lysates (H&E). X200**

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**Fig. (2): Spleen sections showing: 1- Normal control 2- Placebo treated 3- Esch coli lysate 4- Ps aeruginosa lysate 5- Salm typhi lysate 6- Shig flexeneri lysate 7- Staph aureus lysate 8- Mixed bacterial lysates (H&E). X200**



**Fig. (3):** Thymus sections showing: 1- Normal control 2- Placebo treated 3- *Esch coli* lysate 4- *Ps aeruginosa* lysate 5- *Salm typhi* lysate 6- *Shig flexeneri* lysate 7- *Staph aureus* lysate 8- Mixed bacterial lysates (H&E). X200

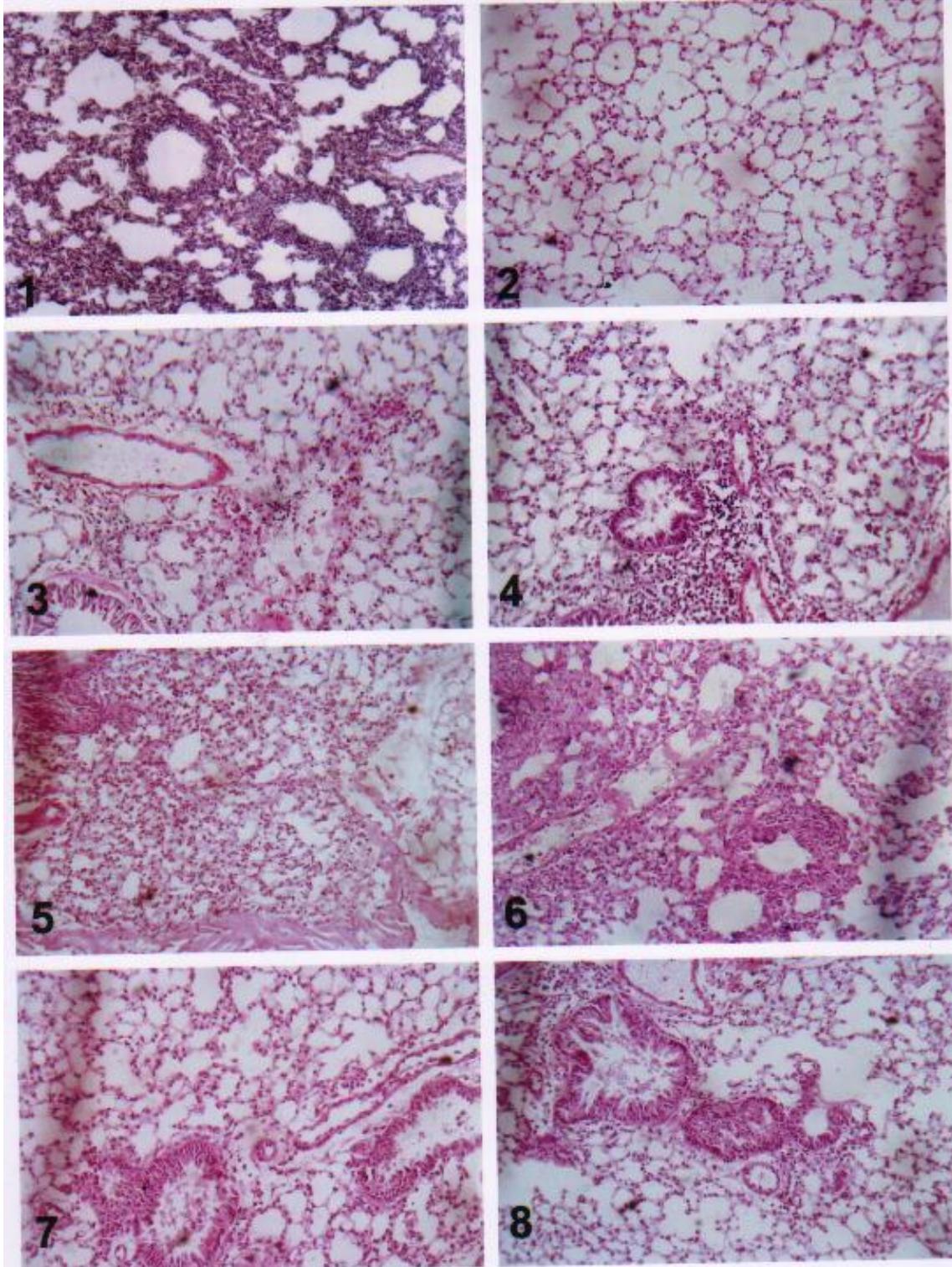
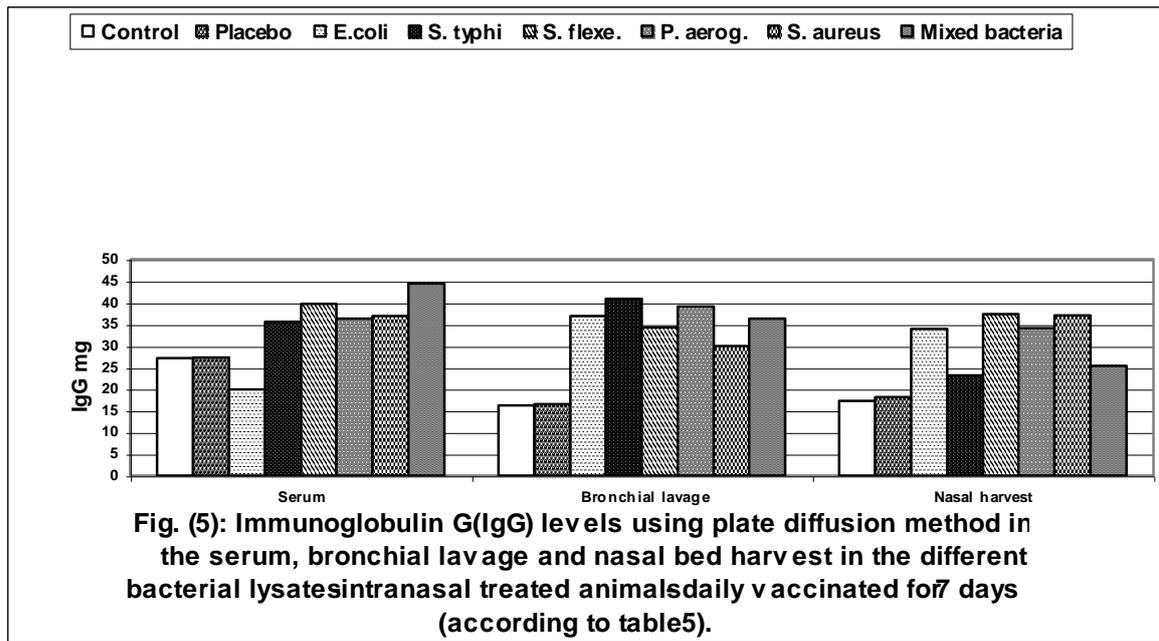


Fig. (4): Lung sections showing: 1- Normal control 2- Placebo treated 3- Esch coli lysate 4- Ps aeruginosa lysate 5- Salm typhi lysate 6- Shig flexeneri lysate 7- Staph aureus lysate 8- Mixed bacterial lysates (H&E). X200



## Discussion

Bacterial vaccines are those composed of killed bacterial products. This study has approached the immunoprophylaxis effect of bacterial vaccines using the whole cell bacterial lysates. Goncharova *et al.* (2002), mentioned that nasal mucosa in the olfactory region is likely to be a promising target for mucosal immunization. The use of oral and intranasal bacterial vaccines stimulate the local and distant effector site such as the intestinal mucosa and the respiratory tract, the microorganism can elicit an enhanced Ig A response according to the concept of a common mucosa-associated immune system (Ruedl *et al.*, 1994). Intranasal immunization delivers the antigen directly to the site of uptake (Kuper *et al.*, 1992). The stimulation of cells from the spleen and mesenteric lymph nodes after nasal immunization points to the existence of a common mucosal immune system (Bonenfant *et al.*, 2001). This may explain the significant increase in peritoneal fluid lymphocytic count recorded in this study that was associated with a significant increase of spleen and thymus weights. On the use of mixed bacterial lysates, these changes were accompanied by significant increase of bone marrow lymphocytic

percentage. Kiyono *et al.* (1992) and Porgador *et al.* (1998) found that cells stimulated in the nasal associated lymphoid tissue (NALT) by the antigen presented by antigen-presenting cells can leave this site for mucosal effector sites. Nasal immunization triggers pulmonary immunity. Antigen administered by the nasal route can reach the trachea area or dendritic cells loaded with the antigen, may have migrated from the NALT to the pulmonary lymph nodes, where they can initiate an immune response (Trolle *et al.*, 1999). Intranasal immunization triggers both mucosal and systemic T and B-cell responses (Wu and Russell, 1997 and Wu *et al.*, 1997). Edinboro *et al.*, (2004) showed that intranasal vaccines could prevent tracheobronchitis in dogs. These reports explain the highly significant increase in the level of Ig G in bronchial lavage and nasal bed harvest in the different intranasal bacterial lysates vaccinated mice, also the significant increase in the serum Ig G. These findings were also supported by Kuenen *et al.*, 1994 who mentioned that the protective effect of bacterial lysates is accompanied by priming for specific Ig G responsiveness. Teloni *et al.* (2004) mentioned that bacterial proteins and oligodeoxynucleotides

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applied intranasally rised antibody classes and subclasses.

This study dealt with the immune changes in response to intranasal vaccination with *Esch. coli lysate*, *Pseudomonas aeruginosa lysate*, *Salmonella typhi lysate*, *Shigella flexeneri lysate*, *Staph. aureus lysate* and mixed bacterial lysates. Ryan *et al.* (1999) mentioned that *Esch. coli* heat labile toxin act as effective mucosal adjuvant for nasal delivery of acellular pertussis vaccine. Bennet-Guerrero *et al.*, (2000) found that active immunization of mice with complete core LPS of *Esch. coli*, *Pseudomonas aeruginosa* and *Bacteroids fragilis* mixed together to form a cocktail, provided protection against a lethal challenge with *Esch. coli*. The vaccine was non toxic, non pyrogenic and immunogenic against a wide variety of pathogens found clinically. The inactivated bacterial lysates used in this study, in the given dose, proved to be non- toxic and immunostimulant. Harrison *et al.* (1997) used *Salmonella typhimurium* vaccine in the murine typhoid model. On the other hand, this strain was used by Corthesy-Theulas *et al.*, (1998) for protecting mice from *Helicobacter pylori* infection by nasal immunization. Furlong and David, (1996) mentioned that shigella liposaccharides complexes can be used as an effective immunogen. These proteosomes preparations induce an effective mucosal response when delivered either orally or intranasally and cause protection against shigella infections ( Orr *et al.*, 1993). O' Brien *et al.* (2000) studied the protective effect of *Staph aureus lysate*. Both Rutishauser *et al.*, (1998) and Ruah *et al.* (2001) studied the immunoprotective effect of mixed bacterial lysates against recurrent respiratory tract infections.

In this study, histological changes of liver, thymus, spleen, lung and bronchi showed morphological differences depending on the type of vaccine used. Preparations were characterized by stimulating effect on reticuloendothelial system, which was much pronounced after giving mixed bacterial lysates. Haschek and Rouseaux, (1998) mentioned that the morphological response can be considered as involution of lymphoid organs were the

thymus being very susceptible to toxic injury and also hyperplasia occurred. Bacterial endotoxins can induce mild hepatic inflammation and varying degrees of hepatocellular cholestasis. The later effect is attributed to the effects of proinflammatory cytokines, released by Kupffer cells and endothelial cells in response to circulating endotoxin (Cotran *et al.*, 1999). Trauner *et al.* (1999) illustrated that intrahepatic cholestasis ( inflammation-induced cholestasis) is induced by inflammatory cytokines in response to various infectious stimuli causing liver oedema, ballooning and cellular infiltration. These cytokine effects are reversible and bile secreting function is restored upon disappearance of the inflammatory injury. In this study, liver inflammatory response to bacterial lysates was clear when *Salmonella typhi lysate*, *Pseudomonas aeruginosa lysate*, *Shigella flexeneri lysate* and *Staph. aureus lysate* were used. Sakiri *et al.*, (1998) stated that shigella toxins caused tissue damage and vascular lesions in which endothelial cells were swollen and detached from underlying basement membranes. They showed also that these vascular changes occur in the presence of proinflammatory cytokines tumor necrosis factor-alpha (TNF-  $\alpha$ ) or interleukin -1 beta (IL- 1  $\beta$ ). Liver sections in this study illustrated liver cell damage changes and vascular lesions and congestion in response to *Shigella flexeneri lysate*. Polotskii *et al.*, (1981) said that shigella lysates caused an increased number of cellular lymphocytic infiltration. This observation was recorded in this study in liver sections, spleen, thymus and lung tissue. The spleen of mice that received *Shig. flexeneri lysate* sections showed red pulp contained aggregations of large nuclei varying considerably in size , heavy lymphocytic infiltration of mature lymphocytes, plasma cells and macrophages in red pulp (Fig. 2-6).The thymus gland section obtained from animals received *Shigella flexeneri lysate* showed some vaculated cells in medulla and cortex, also bizarre cells or their debris could be detected.

Respiratory tract histopathological study revealed increased cellular infiltration

of polymorphonuclear leucocytes (PMNs) and monocytes in response to bacterial lysates, these infiltrates were also detected in bronchoalveolar fluid. Wagner *et al.* (1999) mentioned that bronchoalveolar lavage (BALF)- PMN accumulation in response to intratracheal treatment with LPS was 100%. In contrast, intravenous endotoxemia inhibited pulmonary PMN migration. Fan *et al.* (1998) showed that in a rodent model, animals exposed to antecedent shock exhibited augmented lung inflammation in response to LPS resuscitated shock where enhanced lung neutrophil sequestration and transpulmonary albumin flux in response to intratracheal LPS. They mentioned that primed alveolar macrophages play a major role in enhanced lung leukosequestration and transpulmonary flux in response to LPS following resuscitated shock. Both Mmin *et al.* (1979) and van Daal *et al.* (1992) showed that polyvalent bacterial lysates could protect against respiratory diseases. They showed that immunization with bacterial lysates reduced the intrapulmonary inflammatory reaction to infection, that was assessed by measurement of PMN elastase in BALF.

This study concludes that immunization through the nasal route is an adequate and efficient method of vaccine delivery, this route also offered effective priming. Bacterial lysates play a role as immunostimulant-immunomodulator vaccines. Further studies are needed to establish safe limits of endotoxins in vaccines in different animal models (Mader *et al.*, 1997). Also, more studies should be carried out to assess lung injury by priming for an exaggerated response to a second stimulus, the so-called "two-hit" hypothesis (Fan *et al.*, 1998).

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إستجابة بعض الأنسجة للتطعيم عن طريق الأنف: دراسة مناعية-  
نسيجية

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تم اجراء هذه الدراسة على فئران التجارب البيضاء و التي تم تقسيمها إلى عدد ثمانية مجموعات حيث تعتبر المجموعة الأولى كمجموعة طبيعية ضابطة، أما المجموعات التالية فقد تم تطعيمها عن طريق الأنف و هي كالتالي: المحلول الحامل للمتعللات البكتيرية (بلاسيبو) - المتحلل البكتيري أيشيريشيا كولاي- المتحلل البكتيري سودوموناس أيروجينوزا- - المتحلل البكتيري سالمونيلا تايفي- - المتحلل البكتيري شيجيلا فليكسينيراى- - المتحلل البكتيري ستافيلوكوكاس أوربوس- - المتحلل البكتيري لمخلوط الأنواع البكتيرية السابقة.

أثبتت هذه الدراسة قدرة التطعيم بالمتعللات البكتيرية عن طريق الأنف على إحداث تغيير معنوي فى العد الكلى لكرات الدم البيضاء و العد النوعي لكرات الدم البيضاء و فى النسبة المئوية للخلايا الليمفاوية بنخاع العظم. كذلك تبين حدوث تغيير معنوي بزيادة نسبة الأجسام المضادة G كذلك ظهرت تغيرات معنوية فى أوزان الطحال و الغدة التيموسية و قد صاحب هذه التغيرات حدوث إختلافات و أنشطة خلوية فى أنسجة الكبد و الطحال و الغدة التيموسية و الرئة و الشعبيات الهوائية.

يستخلص من هذه الدراسة أن التغيرات المختلفة المصاحبة للتطعيم بالمتعللات البكتيرية عن طريق الأنف يرشح هذه التطعيمات لأستخدامها كوسيلة مناسبة لتحفيز الجهاز المناعي.