*Histopathological and Lymphoproliferative effects for some experimental nanovaccines prepared from some bacteria

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Abstract
Chitosan used as carrier nanoparticles (CNP), where used at low-molecular-weight chitosan (LWCS) and loading antigens on Chitosan nanoparticles. After LD50 of Lipopolysaccharide (LPS) antigen were determined (239 µg/ml of LPS of *S. typhi*, 183 µg/ml of LPS of *V. cholerae* and 160.5 µg/ml of LPS of *B. melitensis*), and mixed these LPS to prepare Triple LPS antigen. The experimental study included 45 Albino Swiss male rats which divided into nine groups, 5 rats in each group. The results showed occurring pathological and inflammatory changes in spleen and thymus gland in rats vaccinated with LPS alone such as depletion of white pulp, proliferation of red pulp and sever congestion with hemosidrosis, while in case of rats vaccinated with LPS and chitosan showed occurring immunological and inflammatory changes such as proliferation of white pulp and normal red pulp, and increment in the size and numbers of the lymph node, also increment in the size of the germinal centers.

Keywords: chitosan nanoparticles, vaccine, Lipopolysaccharide.

Microbiology classification : QR 75-99.5

Introduction:
Vaccination are very important and have saved literally millions of lives; The smallpox vaccine first used in 1789 eliminated smallpox from the face of the earth; Measles and polio are rarely if ever seen because of worldwide vaccination(26), and drastically reduced the incidence of diphtheria, tetanus, whooping cough, mumps and rubella (27).Vaccination is the administration of a vaccine (immunogen) to stimulate a protective immune response that will prevent disease in the vaccinated person if contact with the corresponding infectious agent occurs subsequently(13).

Lipopolysaccharide (LPS) is the main outer membrane component of gram negative bacteria which constitutes about 75% of the surface (30) and 5-10% of the total

***The Research is a part of on M.Sc. thesis in the case of the First researcher**

dry weight of gram negative bacteria, It is not found in Gram positive bacteria(32). LPS activates the immune system leading to release of endogenous proinflammatory cytokines such as TNF, IL-1 and IL-6 (4, 10). Polymers are macromolecules composed of repeating structural units of monomers connected by covalent chemical bonds and this process is known as polymerization (23). Polymeric nanoparticles are colloidal carriers that vary in size from 10 to 1000 nm(20). These biodegradable polymers can be either natural or synthetic. The former generally provide a relatively quick drug release, while the latter enable extended drug release over periods from days to several weeks (6, 17).

Chitosan is a versatile natural polymer, it is modified natural, biodegradable, biocompatible, non toxic, as well as linear nitrogenous polysaccharides, a basic polysaccharide homo-polymer(15). Chitosan is produced commercially by deacetylation of chitin, naturally occurring polysaccharides which is the structural element in the exoskeleton of crustaceans, it acts as a copolymer of varying amounts of N-acetyl glucosamine and N-glucosamine repeated units(31).

Salmonella, a genus of the Gram-negative family Enterobacteriaceae comprises a large, closely related group of often medically important bacteria(34 , 7). Infection with *S. enterica* causes typhoid fever, a serious, systemic infection for which new and improved vaccines are required (36).

Vibrio cholerae, a member of the family Vibrionaceae, is a facultatively anaerobic, Gram-negative, non-spore-forming curved rod (28). Cholera which is a major public-health problem in developing countries, caused by infection of the intestine with toxigenic *Vibrio cholerae* (19) .

Brucellae spp. are Gram-negative, facultative, intracellular coccobacilli or short rods bacteria that can infect many species of animals and man (8). *Brucella* belongs to the (alpha)2 subdivision of the proteobacteria. The *Brucella* spp. are obligate parasites of animals and humans. *Brucella melitensis* typically infects goats. The disease in humans, brucellosis (undulant fever, Malta fever)(29).

Materials and methods:

Collection of Samples :

Samples were collected from patients who attended to Maternity and Children Teaching Hospital, AL-Diwaniya Teaching Hospital, Central Health Laboratory and other different regions in AL-Diwanyia province according to (22), during the period from October 2012 to the January 2013.

Laboratory animals:

Seventy-five Albino Swiss male rats (*Rattus norvegicus*) were supplied by the College of Veterinary Medicine in AL- Qadisiyah University. Their ages at the start of the experiments were 6-8 weeks. They were divided into groups; each group contain 5 rats was kept in a separate plastic cage.

Isolation and Identification of bacterial species: according to(18).

Biochemical Tests according to (11).

Preparation and Extraction of LPS:

Preparation of bacterial cultures for extraction LPS according to (33) and Extracted LPS according to proteinase K digested method(9).

Preparation of Chitosan Nanoparticle:

Two grams of chitosan were dissolved in 100 ml 0.1 M HCl and stirred for 30 min. Then, H_2O_2 was added in one of five concentrations (1%, 1.5%, 2% and 2.5%). The mixture was heated and stirred at 60°C for 2 h and then vacuum filtered. The upper residue was neutralized with distilled water, baked, and weighed. Ethanol was added to the lower solution, which was left for 24 h to precipitate, after which it was filtered, dried, and weighed. This gave low-molecular-weight water-soluble chitosans denoted by C1, C1.5, C2, and C2.5. After H_2O_2 treatment, the molecular weight of the chitosan decreased and continued to decrease as more H_2O_2 was added. This was due to the degradation of the chitosan molecular chain by H_2O_2.

Then, 0.5 g of LWCS was dissolved in 1L of 2% acetic acid and stirred for 30 min. Then, 100 ml of each solution was added to 40 ml of TPP (0.05, 0.1, 0.2g/L), stirred for 2 h at ambient temperature and then centrifuged at high speed. The isolated nanochitosan was rinsed with distilled water, freeze-dried and analysed. These samples were denoted N-Cn (N-C1, N-C1.5, N-C2 and N-C2.5), respectively(16).

Detection the optimum concentration of chitosan nanoparticles :

To detection the optimum concentration of chitosan , gradient concentrations of chitosan 2.5%, 5%, 10%, 15% and tripolyphosphate TPP 0.2, 0.4, 0.5, 1.0 g/L were worked, then measured the absorbency at 595 nm wavelength (12).

Loading antigen on Chitosan Nanoparticles :

Chitosan nanoparticles were re-dispersed in 25 ml of distilled water at concentration of 5 mg/ml under continuous ultrasonication to disaggregate the chitosan nanoparticles. The loading procedure was performed by incubating different concentrations of antigen with chitosan nanooparticles under mild agitation at room temperature for 15 min. One milliliter of antigen loaded chitosan nanoparticles suspension was centrifuged at 13,200 rpm for 20 min and the amount of antigen in the supernatant was measured by spectrophotometer at 570 nm . The supernatant of blank chitosan nanoparticles was adopted as the blank to correct the absorbency reading value of the antigen-loaded chitosan nanoparticles. The corrected optical density (OD) value was then used to calculate the concentration of antigen in the supernatant (21).

Median lethal dose (LD50) of LPS:

To determine lethal dose (LD50), various doses prepared of LPS (100, 150, 200, 250, 300µg/ml), 1ml from each concentration was injected (5 rats in each group) by intraperitonieum (i.p.) and the 50% lethal dose LD50 was determined by counting deaths during 5 days. Control group was injected by 1ml from phosphate buffer (PBS). A count of live and dead rats were used for the determination of median lethal dose(2).

Proportional distance = 50% -the percentage of dead rats at concentrations lower than 50% the percentage of dead rats in the highest concentrations of 50% − the percentage of dead rats at concentrations lower than 50%

Proportional distance for 50% = Proportional distance × (The highest concentration of 50% − The lower concentration of 50%)

LD50% = Proportional distance for 50% + The lower concentration of 50%
Safety test: according to(35).
Sterility test: according to(14).

Challenge Test:
Forty-five Albino Swiss male rats were used in challenge test, then divided this animals into nine groups, each group contain 5 rats. First group injected with 0.25ml of LD50% LPS of *S. typhi* in intraperitonium, then injected with Boosting dose of the same antigen in intraperitonium, after two week from first dose, After two week from boosting dose injected with challenge dose (100LD50% LPS) in intraperitonium. Second group injected with 0.25ml of LD50% LPS of *S. typhi* with chitosan nanoparticles(LWCS) low molecular weight chitosan in intraperitonium, then injected with Boosting dose of the same antigen in intraperitonium , after two week from first dose, After two week from boosting dose injected with challenge dose (100LD50% LPS +LWCS) in intraperitonium. Third group injected with 0.25ml of LD50% LPS of *V.cholerae* in intraperitonium, then injected with Boosting dose of the same antigen in intraperitonium, after two week from first dose, After two week from boosting dose injected with challenge dose (100LD50% LPS) in intraperitonium. Forth group injected with 0.25ml of LD50% LPS of *V. cholerae* with chitosan nanoparticales(LWCS) low molecular weight chitosan in intraperitonium, then injected with Boosting dose of the same antigen in intraperitonium , after two week from first dose, After two week from boosting dose injected with challenge dose (100LD50% LPS +LWCS) in intraperitonium. Fifth group injected with 0.25ml of LD50% LPS of *B. melitensis* in intraperitonium, then injected with Boosting dose of the same antigen in intraperitonium, after two week from first dose, After two week from boosting dose injected with challenge dose (100LD50% LPS) in intraperitonium. Sixth group injected with 0.25ml of LD50% LPS of *B. melitensis* with chitosan nanoparticales(LWCS) low molecular weight chitosan in intraperitonium, then injected with Boosting dose of the same antigen in intraperitonium, after two week from first dose, After two week from boosting dose injected with challenge dose (100LD50% LPS +LWCS) in intraperitonium. Seventh group injected with 0.25ml of LD50% Triple LPS of *S. typhi, V. cholerae* and *B.melitensis* (mixture) in intraperitonium, then injected with Boosting dose of the same antigen in intraperitonium, after two week from first dose, After two week from boosting dose injected with challenge dose (100LD50% LPS) in intraperitonium. Eighth group injected with 0.25ml of LD50% Triple LPS of *S. typhi, V. cholerae* and *B.melitensis*(mixture)with chitosan nanoparticles(LWCS) low molecular weight chitosan in intraperitonium, then injected with Boosting dose of the same antigen

in intraperitonium , after two week from first dose, After two week from boosting dose injected with challenge dose (100LD50% LPS +LWCS) in intraperitonium. Ninth group control group injected with 0.25ml of phosphate buffer saline(PBS) (pH=7.2) in intraperitonium.

Histopathological Study: according to (3).

Statistical Analysis :

They data were statistically analyzed using the statistical package SPSS version 10.0 for windows. The investigated parameters were presented in as mean ± standard error (S.E.), and differences between means were assessed by ANOVA, followed by LSD or Duncan test. The difference was considered significant when the probability (P) value was ≤ 0.05 (24).

Results :

Isolation and Identification of bacterial species :

The isolate of *S. typhi* showed that 16 (19.2%) samples were positive. While, the isolation and identification of *Vibrio* isolates showed that only 9 (16.1%) samples were positive. Whereas *Brucella melitensis* isolates showed that 13 (16.6%) samples were positive.

Detection the optimum concentration of Chitosan nanoparticles :

The results showed that the higher absorbency at the concentration of Chitosan is 2.5% with 0.2 of tripolyphosphate (TPP) which equal to 0.609 nm wavelength, which its representing the optimum concentration of Chitosan nanoparticles, while the other concentrations of Chitosan and tripolyphosphate were lower absorbency than the optimum concentration.

Table (1): The optimum concentration of Chitosan nanoparticles.

Conc.CNP / conc.TPP	Absorbency at 570 nm		
	0.05	0.1	0.2
C 1 %	0.075	0.109	0.382
C 1.5 %	0.096	0.150	0.493
C 2 %	0.145	0.209	0.512
C 2.5 %	0.184	0.230	0.609

Loading antigen on Chitosan Nanoparticles :

The results of loading antigen on Chitosan Nanoparticles showed that the absorbency increasing when antigen loaded on Chitosan, except the LPS of *S. typhi*, while it was decreasing in the case antigen alone. The higher absorbency when the triple vaccine loaded on Chitosan(1.031nm) and the lower absorbency when the LPS of *V. cholerae* (0.347nm),Table (2).

Table (2): The absorbency of loading antigen on Chitosan Nanoparticles.

Sample	Absorbency at 595 nm
CNs	**0.617**
LPS (*S. typhi*)	**0.600**
LPS(*S. typhi*) + CNs	**0.543**
LPS(*V. cholerae*)	**0.347**
LPS(*V. cholerae*) + CNs	**0.923**
LPS(*B. melitensis*)	**0.435**
LPS(*B. melitensis*) + CNs	**1.015**
LPS(Triple Vaccine)	**0.886**
LPS(Triple Vaccine)+CNs	**1.031**

Determination lethal dose (LD50%) of antigens :

LD50% of the LPS for *S. typhi* :

When injected five serial concentrations of LPS into the peritoneum of rats, found that the lethal dose of rats are about 239 µg /ml, as indicated in the table (3):

Proportional distance = 0.78

Proportional distance for 50% = 39

LD50% = 239 µg / 1ml

Table (3): Results of lethal dose (LD50%) of the LPS for *S. typhi* :

Concentration Of LPS µg / 1ml	No. of Rats			The cumulative number of rats		Total cumulative numbers	Percentage of dead rats
	Treated	Dead	Live	Dead	Live		
300	5	4	1	9	1	10	90%
250	5	2	3	5	4	9	55.55%
200	5	2	3	3	7	10	30%
150	5	1	4	1	11	12	8.3%
100	5	0	5	0	16	16	0%

LD50% of the LPS for *V. cholerae* :

When injected five serial concentrations of LPS into the peritoneum of rats, found that the lethal dose of rats are about 183 µg / ml, as indicated in the table (4):
Proportional distance = 0.66
Proportional distance for 50% = 33
LD50% = 183 µg / 1ml

Table (4): Results of lethal dose (LD50%) of the LPS for *V. cholerae* :

Concentration Of LPS µg / 1ml	No. of Rats			The cumulative number of rats		Total cumulative numbers	Percentage of dead rats
	Treated	Dead	Live	Dead	Live		
300	5	5	0	14	0	14	100%
250	5	3	2	9	2	11	81.8%
200	5	3	2	6	4	10	60%
150	5	2	3	3	7	10	30%
100	5	1	4	1	11	12	8.3%

LD50% of the LPS for *B. melitensis* :

When injected five serial concentrations of LPS into the peritoneum of rats, found that the lethal dose of rats are about 160.5 µg /ml, as indicated in the table (5):
Proportional distance = 0.21
Proportional distance for 50% = 10.5
LD50%= 160.5 µg / 1ml

Table (5): Results of lethal dose (LD50%) of the LPS for *B. melitensis* :

Concentration Of LPS µg / 1ml	No. of Rats			The cumulative number of rats		Total cumulative numbers	Percentage of dead rats
	Treated	Dead	Live	Dead	Live		
300	5	5	0	16	0	16	100%
250	5	4	1	8	1	9	88.8%
200	5	3	2	7	3	10	70%
150	5	3	2	4	5	9	44.4%
100	5	1	4	1	9	10	10%

LD50% of the Chitosan nanoparticles :

When injected three serial concentrations of chitosan nanoparticle into the peritoneum of rats, found that the lethal dose of rats are about 87.5 µg / ml, as indicated in the table (6):

Proportional distance = 0.75
Proportional distance for 50% = 37.5
LD50% = 87.5 µg / 1ml

Table (6): Results of lethal dose (LD50%) of chitosan nanoparticle :

Concentration Of chitosan nanoparticle µg / 1ml	No. of Rats			The cumulative number of rats		Total cumulative numbers	Percentage of dead rats
	Treated	Dead	Live	Dead	Live		
150	3	3	0	5	0	5	100%
100	3	2	1	2	1	3	66.66%
50	3	0	3	0	4	4	0%

Histopathological & Lymphoproliferative effects :

Salmonella typhi **:** the histopathological changes in spleen and thymus gland recorded a clear changes as follow :

i . LPS antigen :

a. Spleen : Deposition of brown pigment (hemosidren) , depletion of white pulp, hemorrhage of red pulp, sever congestion in the lymph node and enlargement in the size of the germinal centers of the lymph node, figure (1).
b. Thymus: wide cortex and medulla areas in the thymus which contain congestion of blood vessels of thrombi, also there is enlargement of the lymph node which contain thymocytes and dentritic cells, figure(2).

ii . LPS antigen + Chitosan :

a. Spleen : proliferation of white pulp which contain normal arterioles and surrounding of normal red pulp, there is no congestion in lymph node and

enlargement in the size of the lymph node, increment in the size of the germinal center and marginal zone, as shown in figure (3).

b. Thymus : Depletion of medulla and proliferation of cortex , with presence of congestion and thrombi in the medulla of thymus, also increment in the numbers of the lymph nodes, as shown in figure (4).

Vibrio cholerae **:** the histopathological changes in spleen and thymus gland recorded a clear changes as follow :

i . LPS antigen :

a. Spleen : there is congestion of blood vessels , also there is depletion of white pulp which surrounding by hemorrhage of red pulp, also increment in the numbers of the lymph nodes, as shown in figure (5).
b. Thymus:There is depletion of lymphoid follicles, deposition of brown pigment(hemosidren) this case called hemosidrosis also there is increment in the macrophages - leaden hemosidren and there is congestion of blood vessels in the medulla of thymus, as shown in figure (6).

ii . LPS antigen + Chitosan :

a. Spleen : remarkable proliferation of white pulp which contain normal arterioles and surrounding by normal red pulp was recorded, also presence congestion in the lymph node and increment in the numbers of the lymph nodes, as shown in figure (7).
b. Thymus : There is normal cortex and presence of mild congestion in the medulla of thymus, presence of normal trabiculae, also there is extension of the lymph node, as shown in figure (8).

Brucella melitensis **:** the histopathological changes in spleen and thymus gland recorded a clear changes as follow :

i . LPS antigen :

a. Spleen : There is wide white pulp with normal arterioles surrounding with proliferating red pulp and there is no congestion in lymphoid tissue and there is presence megakariocyte in lymph node and increases in the numbers of the inflammatory phagocytic cells, as shown in figure (9).
b. Thymus: Deplesion of lymphoid follicles, with congestion in the lymphoid tissue of thymus, as shown in figure (10).

ii . LPS antigen + Chitosan :

a. **S**pleen : There is normal wide white pulp surrounding by proliferating red pulp with mild congestion in the lymph node, also extension of the germinal centers and increment in the numbers of the lymph nodes, as shown in figure (11).
b. Thymus : Normal cortex and medulla of thymus, with congestion in the lymphoid tissue of thymus, as shown in figure (12).

Triple bacteria: the histopathological changes in spleen and thymus gland as follow:

i . Triple LPS antigen :

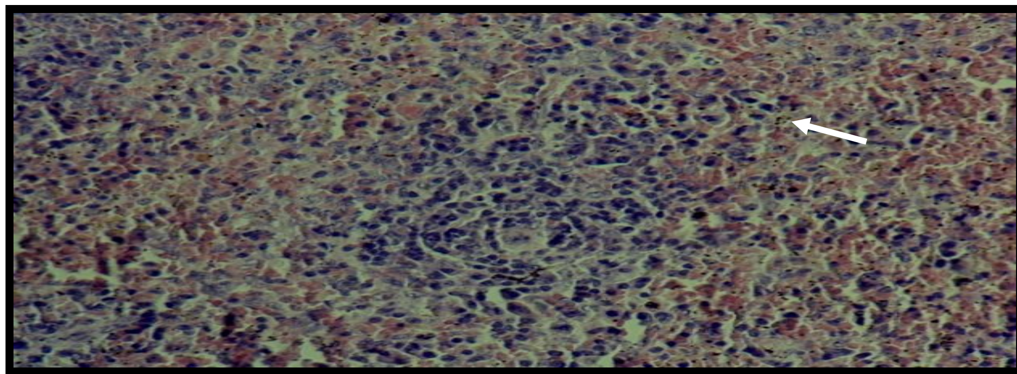
a. Spleen : Sever depletion of white pulp and proliferation of red pulp , congestion of blood vessels in the lymphoid tissue and deposition of brown pigment (hemosidren) and increases in the numbers of the inflammatory phagocytic cells with increment in the numbers of the lymph nodes, as shown in figure (13).

b. Thymus : There is sever congestion and hemorrhage with vaculation in the medulla of thymus, deposition of the golden-brown pigment (hemosidren) in the lymphoid tissue, as shown in figure (14).

ii . Triple LPS antigen + Chitosan :

a. **S**pleen : There is remarkable enlargement and proliferation of white pulp which contain normal arterioles , normal proliferating red pulp , normal trabiculae, with extension of the active germinal centers and marginal zone and increment size and numbers of the lymph nodes, as shown in figure (15).

b. Thymus : There is normal cortex and medulla with normal lymphoid lobes which separated by trabiculae , also there is mild congestion, as shown in figure (16).



Figure(1) : Spleen of rat, received LPS of *S. typhi* : Deposition of brown pigment (hemosidren)(white arrows), depletion of white pulp (red arrow), hemorrhage of red pulp in the lymph node, and enlargement in the size of the germinal center (yellow arrows). (50X

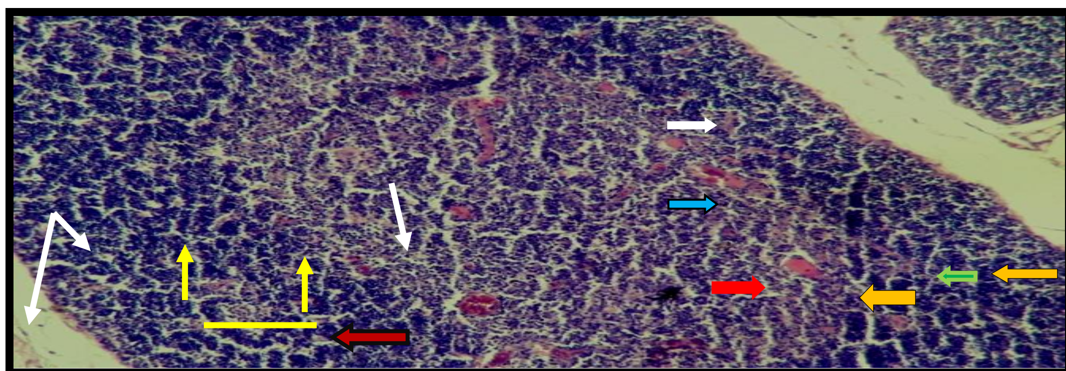


Figure (2): Thymus of rat, received LPS of *S. typhi* : wide cortex (white arrow)and medulla(red arrow) areas in the thymus which contain congestion of blood vessels of thrombi(yellow arrows), also there is enlargement of the lymph node which contain thymocytes(blue arrow) and dentritic cells(green arrow) (20X H&E).

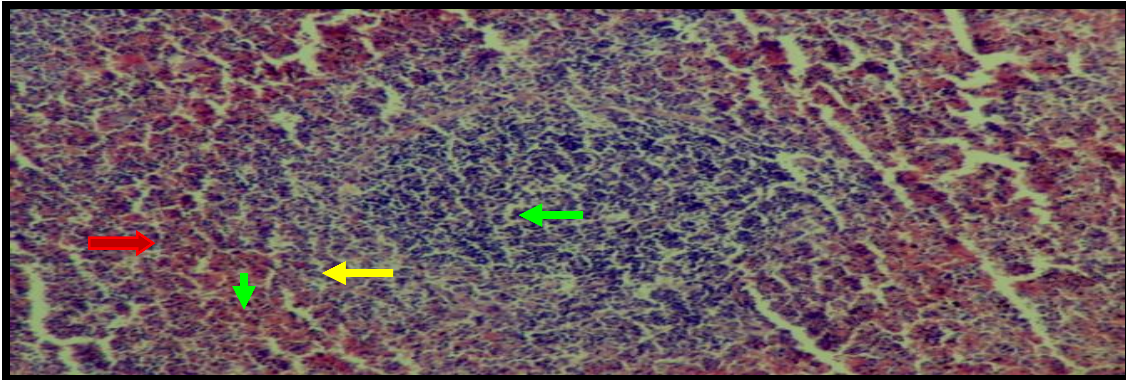


Figure (3): Spleen of rat, received LPS of *S. typhi* with Chitosan: proliferation of white pulp(red arrow)which surrounding by normal red pulp, there is no congestion in lymph node, and increment in the size of the germinal center (yellow arrows) and marginal zone (green arrows) in lymph node. (200X H&E).

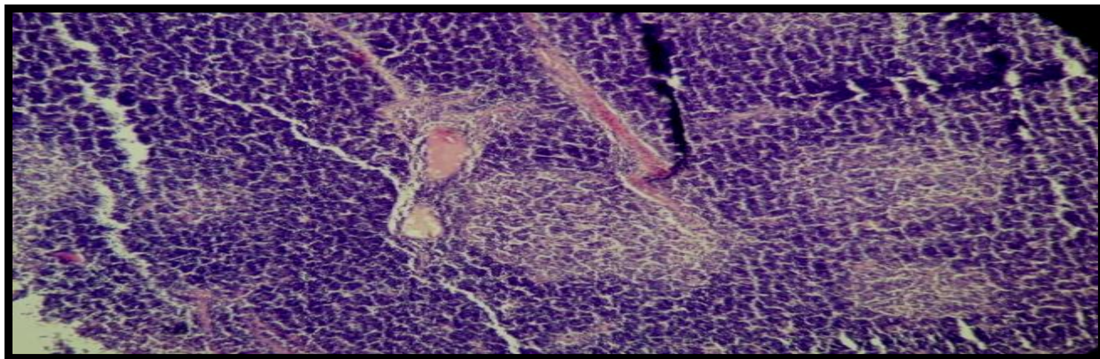
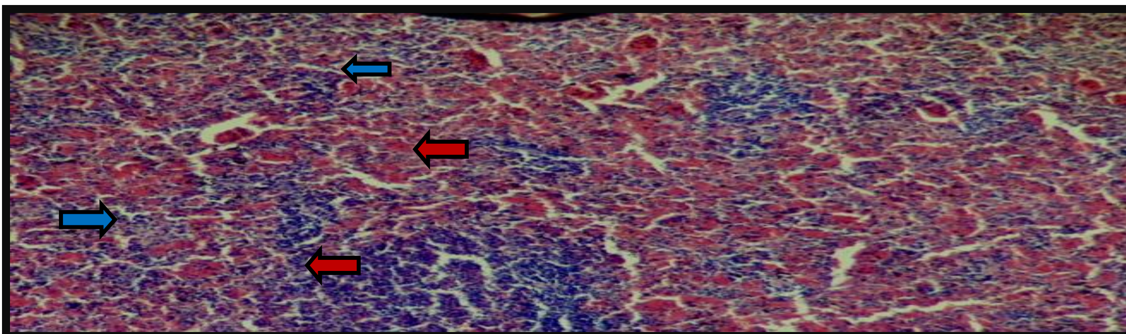


Figure (4): Thymus of rat, received LPS of *S. typhi* with Chitosan: Depletion of medulla (red arrows) and proliferation of cortex , with presence of congestion and thrombi in the medulla of thymus(yellow arrows), also increment in the numbers of the lymph nodes(green arrows). (50X H&E)



Figure(5): Spleen of rat, received LPS of *V. cholerae*: presence of megakaryocytes in lymphoid tissue (blue arrows), also there is depletion of white pulp (red arrows) which surrounding by proliferation of red pulp, increment in the numbers of the lymph nodes. (50X H&E).

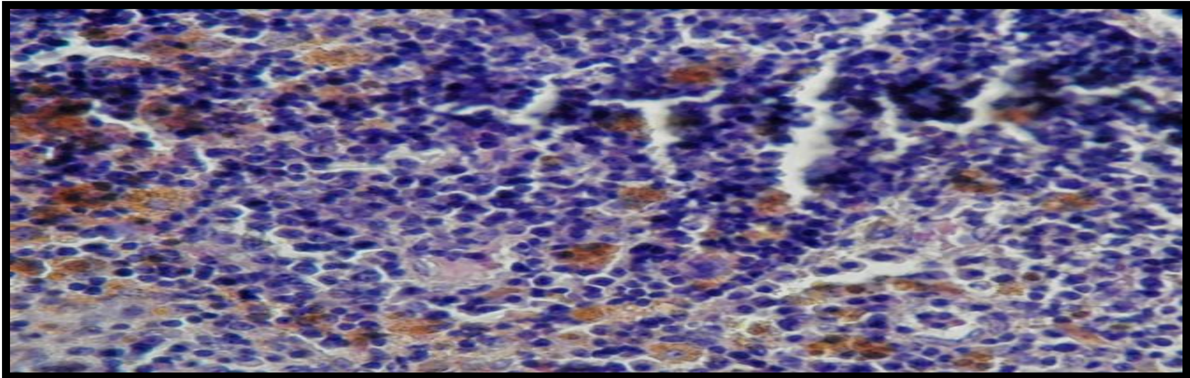


Figure (6): Thymus of rat, received LPS of *V. cholerae*: Deposition of brown pigment (hemosidren), this case called hemosidrosis, also there is macrophages-leaden hemosidren (white arrows) in the medulla of thymus. (200X H&E).

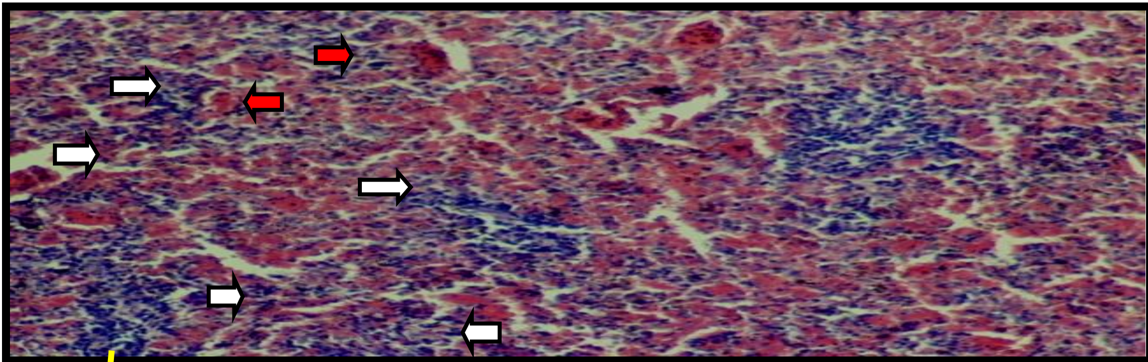


Figure (7): Spleen of rat, received LPS of *V. cholerae* with Chitosan: Wide and proliferation of white pulp which contain normal arterioles and surrounding by normal red pulp, presence congestion in the lymph node(red arrows) and increment in the numbers of the lymph nodes(white arrows)(50X H&E).

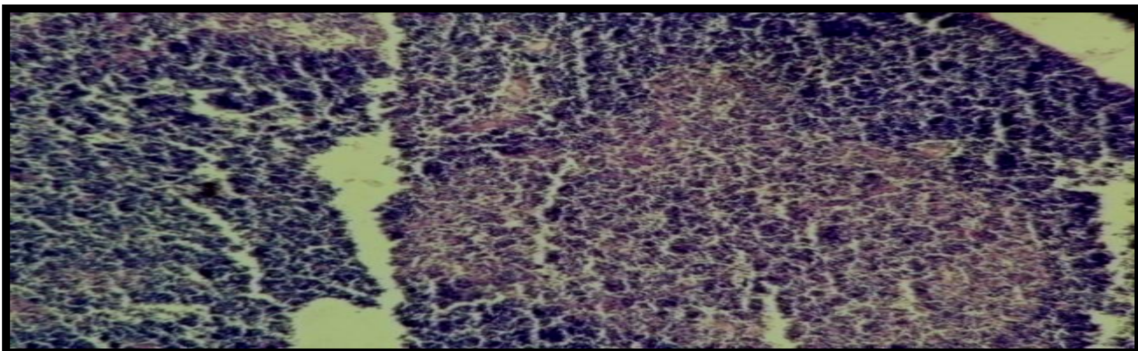


Figure (8): Thymus of rat, received LPS of *V. cholerae* with Chitosan: Presence of mild congestion (red arrows) in the medulla of thymus, and presence of normal trabiculae, also there is extension of the lymph node(yellow arrows). (50X H&E).

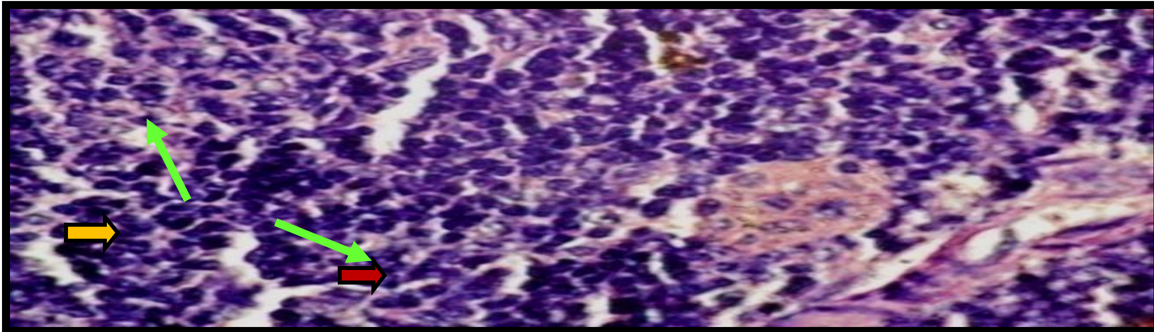


Figure (9): Spleen of rat, received LPS of *B. melitensis*: Higher magnification, note wide white pulp (yellow arrow) and there is no congestion in lymphoid tissue and there is presence megakaryocyte in lymphoid tissue(red arrow),increases in the numbers of the inflammatory phagocyticcells(greenarrows)(200XH&E)

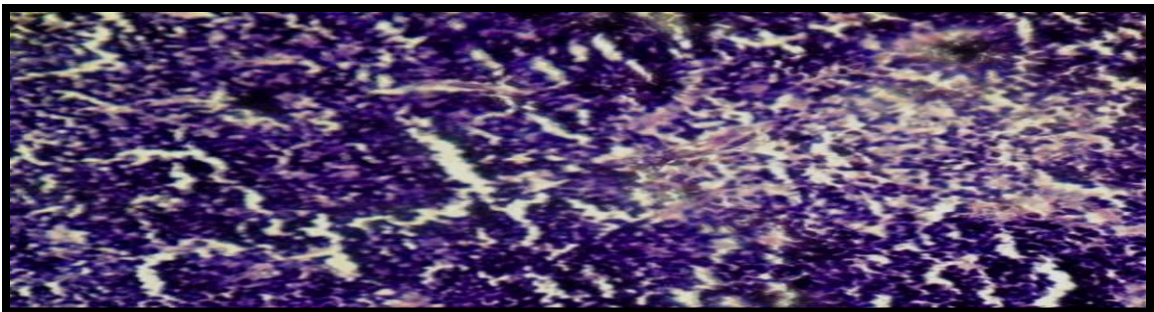


Figure (10): Thymus of rat, received LPS of *B. melitensis*: Deplesion of lymphoid follicles (red arrow) in medulla of thymus, with congestion (yellow arrow) in the lymphoid tissue of thymus. (200X H&E).

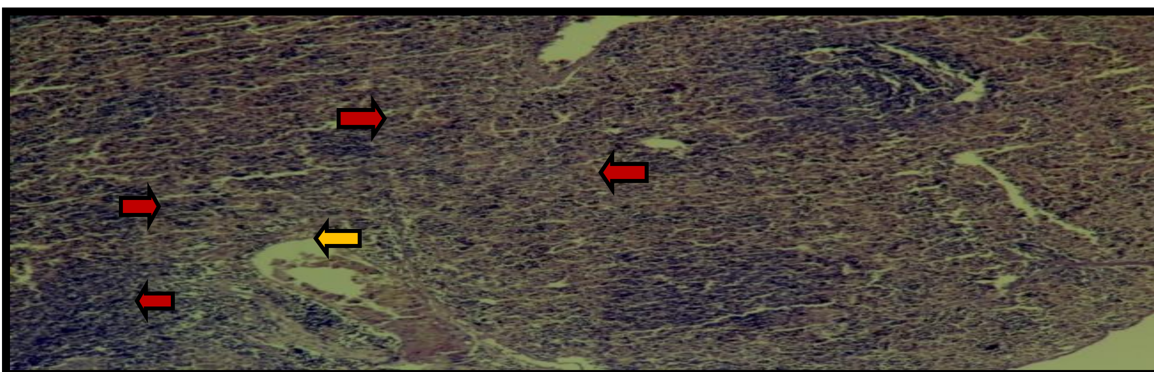


Figure (11): Spleen of rat, received LPS of *B. melitensis* with Chitosan: normal wide white pulp surrounding by proliferating red pulp with mild congestion in the lymphoid tissue (yellow arrow) with increment in the numbers of the lymph nodes (red arrow). (20X H&E).

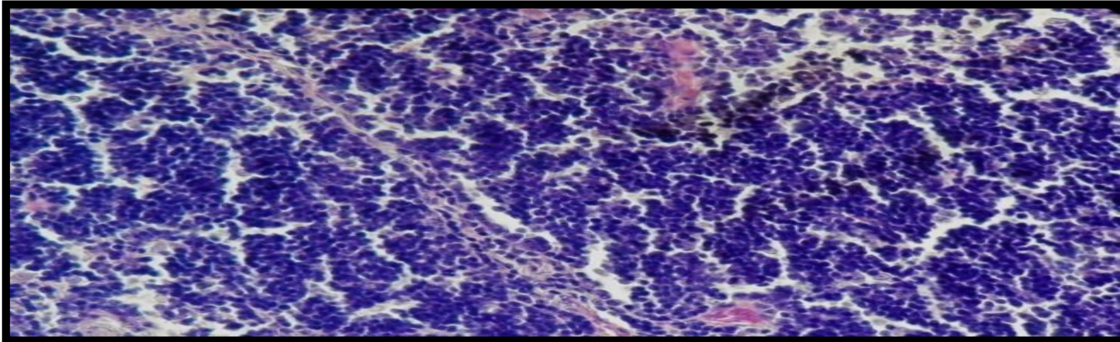
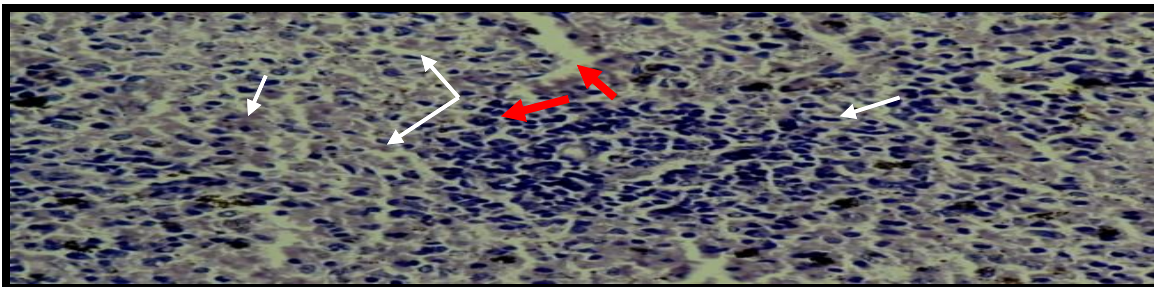
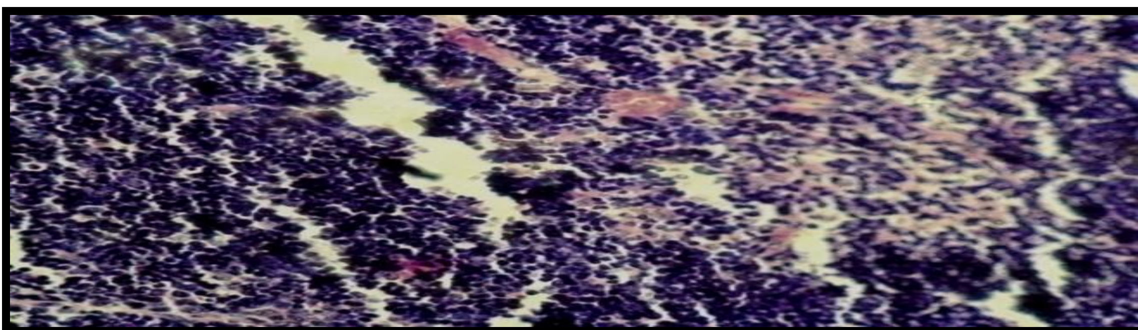


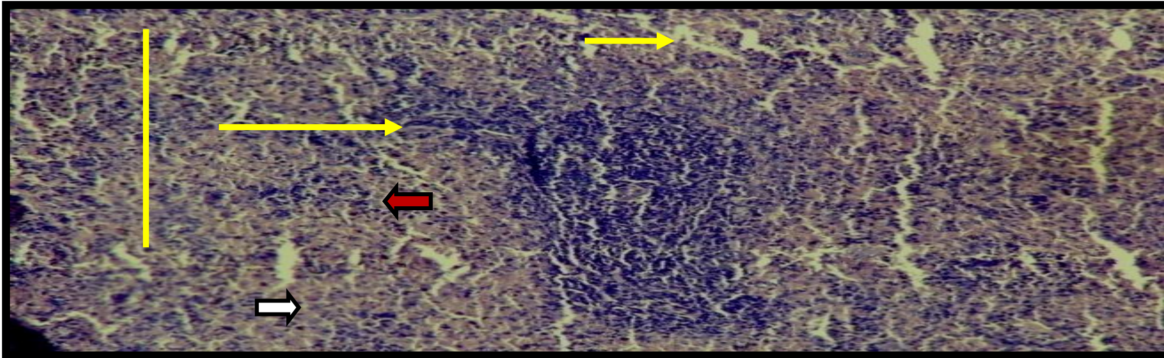
Figure (12): Thymus of rat, received LPS of *B. melitensis* with Chitosan: Normal cortex and medulla of thymus with presence of trabiculae (red arrow) with congestion(green arrow) in the lymphoid tissue of thymus. (50X H&E).



Figure(13): Spleen of rat,received Triple LPS antigen:Higher magnification Sever depletion of white pulp and proliferation of red pulp and deposition of brown pigment (hemosidren) (thin arrows) increases in the numbers of the inflammatory phagocytic cells(red arrows). (200X H&E).



Figure(14): Thymus of rat, received Triple LPS antigen: Sever congestion (red arrow) and hemorrhage lymphoid tissue of thymus, deposition of the golden-brown pigment (hemosidren) in the lymphoid tissue (yellow arrows). (200X H&E).



Figure(15): Spleen of rat, received Triple LPS antigen with Chitosan: proliferation of white pulp (red arrow) which contain normal arteriole (white arrow) , normal proliferating red pulp, extension of the germinal centers and marginal zone and increment size of the lymph nodes(yellow arrows). (50X H&E).

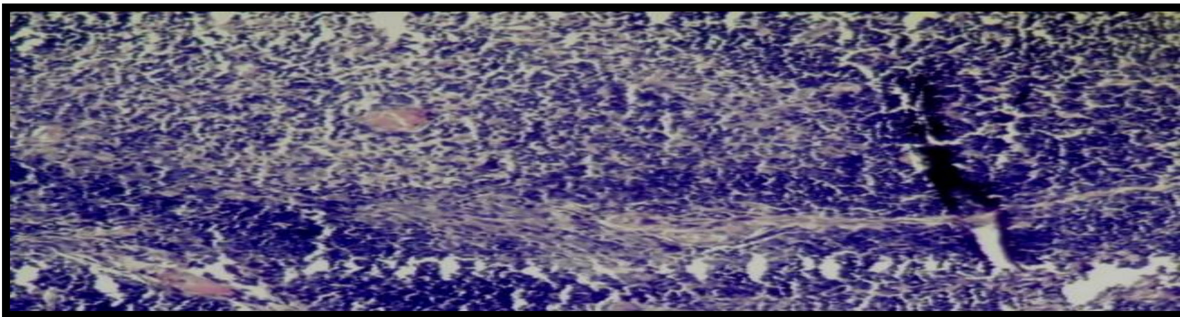


Figure (16): Thymus of rat, received Triple LPS antigen with Chitosan: normal cortex and medulla with normal lymphoid follicles which separated by trabiculae (yellow arrow) , also there is mild congestion (red arrow). (200X H&E).

Discussion :

Histopathological & Lymphoproliferative effects :

In this study, histopathological changes in rats vaccinated with LPS alone showed occurring pathological and inflammatory changes in spleen and thymus gland such as depletion of white pulp, proliferation of red pulp and sever congestion with hemosidrosis, while in case of rats vaccinated with LPS and chitosan showed occurring immunological and inflammatory changes such as proliferation of white pulp and normal red pulp, and increment in the size and numbers of the lymph node, also increment in the size of the germinal centers. Rats vaccinated with Triple LPS showed increases in the numbers of the inflammatory phagocytic cells in spleen. LPS induces Th-1 cells to attracting leukocytes chemically, including macrophages then occur inflammation. These results agreed with researcher who mentioned that The spleen of LPS-stimulated rats showed that more active to depletion of white pulp in spleen and acute inflammatory cells (PMN) (25).

The sectional of thymus gland treated with LPS of *S. typhi* and Triple LPS and the sectional of thymus gland treated with LPS + Chitosan of *S. typhi, V. cholerae*, *B. melitensis* and Triple LPS showed wide cortex and medulla areas in the thymus which contain congestion of blood vessels of thrombi, also there is enlargement of the lymph node which contain thymocytes with increment in the numbers of the lymph nodes , these related to that The antibodies response to LPS and Chitosan is considered thymus independent because immunized rats with LPS and immunized rats with LPS + adjuvant are able to mount an antibodies response similar in magnitude to that of conventional thymus-bearing rats (5). Rats vaccinated with LPS of *B. melitensis* and vaccinated with LPS + Chitosan of *V. cholerae*, *B. melitensis* and Triple LPS showed Wide and proliferation of white pulp and increases in the numbers of the inflammatory phagocytic cells with increment in the numbers of the lymph nodes in spleen. This occur due to the ability of LPS antigens with chitosan nanoparticles that induce Th-1 cells to induce inflammatory phagocytic cells chemically, including macrophages, lymphocytes and neutrophils that leading to enlargement and increment of numbers of the lymph nodes in spleen. These results agreed with researcher who mentioned that showed widening of the white pulp and enlargement of the lymph nodes (1).

Conclusions and Recommendations :

LPS perform a good production against typhoid, cholera and Malta fever infections by stimulating humoral and cellular immune response. Possibility production of triple vaccine by mixture the LPS of each bacterial species. Chitosan had a strong potential to increase both cellular and humoral immune responses and elicited a balanced Th1/Th2 response. The using of Chitosan nanoparticles with LPS more safe and have better stimulation of the immune system than immunization by LPS alone. Following up studies are required to design safe and cost effective vaccines for many deadly diseases specially typhoid, cholera and Malta fever which are now endemic in our country.

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*التأثيرات المرضية النسجية واللمفاوية لبعض لقاحات النانو التجريبية المحضرة من بعض الأنواع البكتيرية

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الخلاصة :

الجيتوسان استخدم كجزيئات نانوية ناقلة (CNP)، حيث استخدم الجيتوسان ذو الوزن الجزيئي الواطئ (LWCS) وتم تحميل المستضدات على جزيئات الجيتوسان النانوية . بعدها تم تحديد قيمة الجرعة نصف القاتلة LD50 لمستضد متعدد السكريد ألشحمي (LPS) والتي كانت (239 ميكروغرام /مل من LPS لبكتريا السالمونلا التيفية و 183 ميكروغرام /مل من LPSلبكتريا ضمات الكوليرا و 160.5ميكروغرام /مل من LPS لبكتريا الحمى المالطية)، وخلط هذه المستضدات لتحضير مستضد LPS الثلاثي. وشملت الدراسة التجريبية 45 من ذكور الجرذان البيضاء السويسرية والتي قسمت إلى تسع مجموعات، 5جرذان في كل مجموعة .أظهرت النتائج حدوث تغيرات مرضية والتهابات في الطحال والغدة الصعترية في الجرذان التي لقحت بمستضد LPS لوحدة مثل حدوث نقصان في اللب الأبيض، وتضاعف اللب الأحمر وحدوث احتقان حاد مع هيموسيديرين، بينما في حالة الجرذان الملقحة بمستضد LPS مع الجيتوسان أظهرت النتائج حدوث تغيرات مناعية والتهابية مثل تضاعف اللب الأبيض ولب أحمر طبيعي ، وازدياد في حجم وأعداد العقدة الليمفاوية، وأيضا ازدياد في حجم المراكز الجرثومية.

الكلمات المفتاحية: جزيئات الجيتوسان النانوية، اللقاح، متعدد السكريد ألشحمي .

Microbiology classification : QR 75-99.5

***The Research is apart of on M.Sc. thesis in the case of the First researcher**