Efficacy of Chitosan Immune Response Against *Listeria Monocytogenes* Infection in Mice

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Abstract

The present research aimed to study the effect of dietary chitosan supplementation against murine experimentally infection by *Listeria monocytogenes*.

forty mice were divided equally into 4 groups. The 1st and 2nd groups fed on diet supplement with chitosan (1mg/kg diet) and (1.5mg /kg diet) for (4) weeks respectively, While 3rd and 4th groups considered as control positive and negative groups. At (4) weeks the first three groups were inoculated intraperitoneally i/P with (0.2) ml (1×10^9) CFU/ml, while the 4th group (control negative) inoculated with (0.2) sterile normal saline.

At (7) days post infection, the result revealed diet one of mice in each control positive and treated group at (24hrs.) post infection with heavy bacterial isolation from brain, spleen and liver of infected positive group and mild to absent bacterial isolation in the 1st and 2nd group respectively.

Grossly presence of severe congestion in the internal organs with necrotic foci seen on the splenic surface of infected positive control while the characteristic feature in the treated infected group was hepatosplenomegaly.

Sever pathological changes were noticed in the infected positive control group characterized by suppurative inflammation with necrosis accompanied with lymphoid depletion and amyloid like substance deposition while the main lesion in treated infected groups showed granulomatous lesion, lymphoid hyperplasia and mononuclear cells infiltration with heavy bacterial isolation from brain, spleen and liver of infected positive group and mild to absent bacterial isolation in the first and second group respectively, We concluded that chitosan stimulated and improve the immune responses in mice against *Listeria monocytogenes* infection.

Key word: *chitosan, Listeria monocytogenes, immunized, mice, pathology.*

Introduction

Listeria monocytogenes is regular Gram-positive motile from, rod with rounded ends, its cells found as single units or short chains or may be arranged in V, L and Y forms or in palisades [(1)]. *Listeria monocytogenes* does not produce spores and capsules are not formed [(2)]. Spread in nature where, exists largely in decaying vegetation, soil, animal feces, feed and water as make it one of the major pollutants of food and play essential role in transmitted of infection between humans and animals [(3)] also infection by *Listeria monocytogenes* can be haematogenous spread directly from the mother to fetus [(4, 5)]

Chitosan is a modified natural carbohydrate polymer derived from chitin, it have many medical uses because their ability to reduce bleeding also help deliver drugs through the skin also in limiting of fat absorption[(6)], also has been bio adhesive property for that used as a safe excipient formulations of drug, it has been used in dentistry because adhere ability to hard and soft tissues also uses in orthopedics, ophthalmology and in surgical procedures, it adheres to epithelial tissues and mucus coat present on tissues surface also has a antifungal or antibacterial, antineoplastic and anticholestermic action[(7)].

Material and Method

Chitosan was obtained from university of Al-

Bahasra, collage of veterinary medicine. Commercial assorted pellets were grinded by food grinder and weighed(1) gm and (1.5)gm of Chitosan was added to each kilogram of grinded pellets mixed well and converted into paste which passed through meat grinder to mould the paste into the original pellets from, left exposed to dry in room temperature [8]. The *Listeria monocytogenes* isolate was obtained from the unit of Zoonotic diseases in the College of Veterinary Medicine, the isolate confirmed by some biochemical tests and gram stain according to [9].

A total number(n=40) male white Swiss BALB/C mice which obtain from the (National Center of Researches and Drugs Monitor in Baghdad); then divided into fourth groups. The 1st group (n=5) mice were fed on diet supplement with chitosan (1mg/kg diet) and (1.5mg /kg diet) for (4) weeks respectively, While 3rd and 4th groups considered as control positive and negative groups. At (4) weeks the first three groups were inoculated intraperitoneally i/P with (0.2) ml (1×10^9) CFU/ml, while the 4th group (control negative) inoculated with (0.2) sterile normal saline, histopathological examination of internal organs(liver, spleen and brain) were taken from both control and infected groups about (1cm3) was taken and fixed in 10% formalin saline for histopathological section which was done according to[10].

Result and Discussion

1) Gross pathological changes:

The main gross feature in control group was severe congestion in the visceral organs specially in the liver, spleen and kidney with presences necrotic foci at the edge of spleen, while treated groups show hepato-splenomegaly was the characteristic gross lesion in the treated groups.

2) Bacterial isolate and clinical signs:

No clear clinical signs noticed on experimental animals specially the treated groups were appeared healthy and well feeding. The result showed heavy bacterial isolation mainly from brain, spleen and liver of control positive groups, while mild growth to absences in other treated groups. Also the isolate was confirmed again on blood agar then we made smear from isolate and stained with grams stain.

3) Histopathological examination:

The characteristic lesion in hepatic tissue of **control positive** show aggregation of PMNCs cells in liver paranchyma (suppurative foci) mainly in portal area accompanied with atrophy of some hepatic cords together with sinusoidal dilation and cellular infiltration in their lumen, The splenic tissue showed destructive changed with variable degree of lymphoid depletion in the white pulp, other section showed formation of multiple cystic cavities containing cellular debris together with focal amyloid like substances deposition, The brain tissue expresses sever neuronal degeneration and apoptosis accompanied with nuclear pyknosis and appearance of hypertrophic swelling astrocytes (gamistocyte), another section showed irregular cystic cavities with neuronal vaculation.

While the characteristic lesion in the liver of **treated 1st group (fed on diet with 1gm\kg of chitosan)** were development of early small granulomatous lesion seen in dilated sinusoids together with proliferation of kupffer cells (figure:1), the microscopic examination in the spleen revealed mild white pulp hyperplasia with proliferation of megakaryocyte (figure:2), together with slight vacuolar changes in some neurons also the results showed moderate gliosis (figure:3).

The pathological lesion in liver of **treated 2nd group (fed on diet with 1.5gm\kg of chitosan)** characterized by focal mononuclear cells (MNCs) aggregation mainly around central vein (figure:4) while presence of follicular hyperplasia in the white pulp was the main lesion observed in splenic tissue (figure:5), while the main brain lesion in the treated infected mice characterized by focal aggregation of MNCs in brain tissue, associated with no clear lesion in the neurons seen mainly in the brain section.

The present study shown sever pathological lesion in the internal organs (liver, spleen and kidney) of the **control positive groups** these results indicate that exposed to highly virulent microorganisms overcome the innate immune system and disseminates to internal organs induce tissue damage, these observation were in consistent with [11] who explained that virulent *Listeria monocytogenes* was one of intracellular bacteria disseminated via blood stream to internal organs and induce nonspecific inflammatory reaction by production listeriolysin O which destroyed the endothelial cells of blood vessels to induce necrosis and suppurative inflammation [12]. In addition, survival and proliferation of microorganisms in the hepatic and splenic cells will

lead to the formation of infection foci that result the infiltration of alarge number of WBCs and activate neutrophil phagocytic cells to work on other resist the invading germs [13]. We also recorded depletion of white pulp of spleen of control positive group these observations may indicate that *Listeria monocytogenes* induced reduction in acquired immune response via depletion of lymphocytic cells [14], Neuronal necrosis and microcavites formation may due to excess of nitric oxide generation literal infection which is important for intracellular signaling of new transmission both inducible and constitute nitric oxide synthase (NoS) are expressed in brain cells include neural lesion, further more inflammatory cells include neutrophils, macrophages express both (NoS and iNoS) may play an important role in elimination *Listeria monocytogenes* [15]. Also the present study explain that feeding infected mice showed mild to moderate pathological lesion in the spleen, liver and brain tissue post challenge with *Listeria monocytogenes* and these lesion characterized by appearance of granulomatous lesion mainly in liver tissue this evidence was agreement with [16] Where noted that the granulomatous reaction was considered the strongest body defense against virulent microorganism's infection, furthermore there are numerous response indicate that chitosan improve the immune response [17]. Our results showed lymphoid hyperplasia in splenic tissue mainly in mice feeding with (1.5gm\kg) chitosan this indicate that chitosan elicited both humeral and cell mediated immunity and activated immune cells to secret cytokines that play essential role in initiated mature granuloma in the liver and this evidence was in agreement with [18] Who demonstrated that feeding of chitosan increase OX62+ percentage and DCs which up regulate the major histocompatibility complex class-II Ags. without expression changing of co-stimulatory (CD80 or CD86) molecules and Ag presenting cells produced TNFα and IL-12 and activation T-lymphocytes, lymphoid tissue hyperplasia in animals fed diet supplement with chitosan may due to chitosan stimulated proliferation of lymphocytic cells.

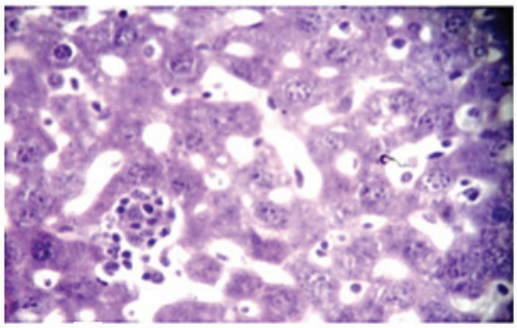


Figure 1 :histopathological section in the liver of 1st group showed small granulomatus lesion with dilated of sinusoid and proliferation of kupffer cells (H&E stainX40)

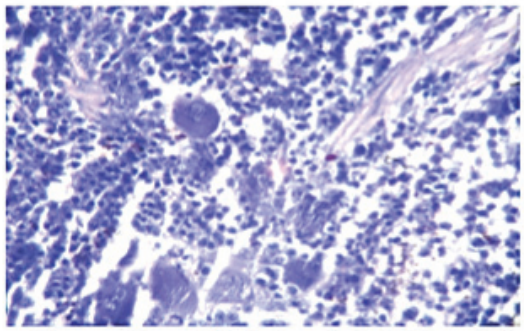
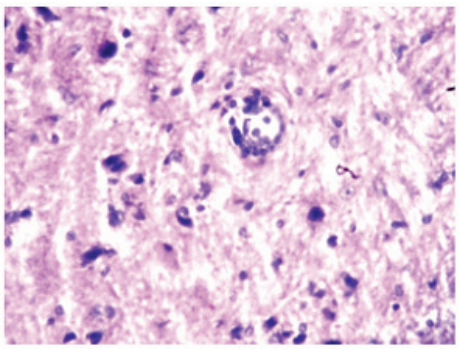
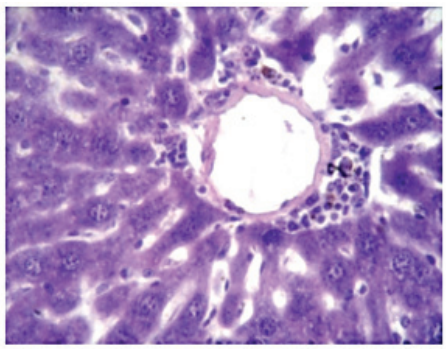


Figure 2 histopathological section in the liver of 1st group showed moderate hyperplasia of megakaryocyte (H&E stain X40).



Figure(3):histopathological section in the liver of 1st group showed focal MNCs aggregation with slight gliosis (H&E stainX10).



Figure(4):histopathological section in the liver Of 2nd group showed MNCs mainaly around c.v wit kupffer cell proliferation (H&E stainX40)

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq**.**

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