

Immunological effect of a crude soluble extract (CSE) from *Listeria monocytogenes* on the Listerial infection in mice

Nagham M. Al- Gburi

Veterinary medicine College / Baghdad University- Iraq

E-mail: drvet2011@yahoo.com

ABSTRACT

In order to determine the effect of a crude saline soluble extract(CSE) from *L.monocytogenes* on stimulate immune response and protect immunized mice against the course of listeric infection,sixteen mice were immunized twice with CSE with two weeks interval at a dose of 0.5ml subcutaneously ,both humoral and cellular response were monitored using radial immunodiffusion plate kit(IgG RID) and skin test.

At twenty seven days post first immunized the mice were challenged with 0.5/ml subcutaneously of CFU 1×10^8 / ml of virulent *L.monocytogenes* ,while six mice was inoculated with 0.5 ml of sterile phosphate buffered saline as control. Then all immunized and control mice were sacrificed at fifteen days post challenge. The results showed, immunization has increased humoral and cellular immune responses in mice as judged by increas the IgG titer and hyper sensitivity reaction. No *L. monocytogenes* was recovered from internal organs; two mice were died in fifth day after immunization and challenged with total 80% protection.Mild histopathological changes were observed which characterized by lymphoid hyperplasia in spleen and lung, lymphocytes infiltration, small granuloma formation in liver. Control mice died within 3-10days postchallenge. High Bacterial isolation levels were obtained from internal organs. The pathological changes revealed multiple extensive lesions characterized of necrosis of hepatocytes ,multiple granulomatous lesion,and congestion in internal organs.the data investigat that the CSE induced humoral and cellular response and protect the mice from infection .

الملخص باللغة العربية

من اجل معرفة التأثير المناعي للمستخلص الذائب من جرثومه *Listeria monocytogenes* على التحفيز المناعي وحمايه الفئران ضد الاصابه بهذه الجرثومه ,تم تمنيع ستة عشر فارا بمستخلص الذائب بجرعه مقدارها 0.5مل تحت الجلد ولمرتين متتاليتين بينهما اسبوعين. وقد قيست الاستجابه المناعه الخلطيه والخلويه باستخدام اطلاق الانتشار المناعي والقصص الجلدي . وبعد 27 يوما من التمنيع الاول اجري فحص التحدي وذلك بحقن الفئران المنعنه تحت الجلد بـ 0.5مل من عالق جرثومه *Listeria monocytogenes* الضاربه الحاوي على 1×10^8 CFU /مل ,بينما حقنت ستة فئران بـ 0.5مل من المحلول دارئ القوسفات الملحي. قتلت الفئران بعدمرور خمسة عشر يوما من حقنها بجرعه التحدي . اوضحت النتائج بان الفئران المنعنه اظهرت استجابه خلطيه وخلويه بارتفاع مستوى معيار IgG وزياده فرط الحساسيه وهلاك اثنين فقط من الفئران المنعنه ونسبه حمايه 80%. كذلك عدم عزل الجرثومه من الاعضاءالداخليه للفئران المنعنه مع تغيرات مرضيه طفيفه تمثلت بفرط تنسج اللغفي في الطحال والكبد والرئه , ووجود اورام حبيبيه صغيره في الكبد وارتشاح الخلايا اللغفيه . اما حيوانات السيطرة هلكت جميعها خلال 3-10ايام وتم عزل الجرثومه من الاعضاءالداخليه مع تغيرات مرضيه شديده تميزت بتفخرات الخلايا الكبديه , احتقان ونزوفات , واورام حبيبيه متعدده في الاعضاء الداخليه . نستنتج من الدراسه ان المستخلص حفز استجابه مناعيه خلويه وخلطيه واعطى حمايه ضد الاصابه بجرثومه *Listeria monocytogenes* .

into CD4⁺ cells which produces INF Kama in addition to present of cytotoxic CD4⁺ cells for the same purpose and that is explain the development of hyperplasia which indicated to cellular response after infected with the challenged dose ,These findings are agreement with previous studies indicated development hyperplasia in lymphoid tissues of immunized animals after challenge dose (21-23).

The presence of granulomas, which can explained by transmission a small number of *L.monocytogenes* to the liver, and because of severe immune response led to the restriction and eliminate them by granulomas. (24) investigated that the growth of granulomas corponding with severe immune response as delayed hypersensitivity accelerates the growth of granulomas.

The inclusion of histopathological changes in control animal which injected with infected dose during 3-7 days can be indicated the transition the bacteria across the blood to organs and causing typical physical response to bacterial infection and causing congestion of blood vessels ,heamorrhges ,odema and other lesions resulting isolation of bacteria from the organs and this results agreement with(25-28).

It is generally accepted that the development of cell-mediated immunity is crucial for resistance against *L.monocytogenes* (29).in contrast,the role of circulating antibodies in the protection from Listeriosis has been neglected after it was shown by (30) that anti-listerial antisera transferred no resistance to recipients. However,in this experiment antilisterial antibodies protect mice from the lethal effect of *L.monocytogenes* which agreement with (5) which found the role of antibodies protect mice from infected dose of *L.monocytogenes*.

CONCLUSION

The data reported in the present study have resulted in very useful biological activity of a water -soluble extract of *L.monocytogenes* which markedly increased mice resistance toward the challenged dose of *L.monocytogenes*,resulting in decreased mortality of the mice and also protection pregnant mice. this immunostimulating activity ,which can be useful in the development of vaccine against this important food pathogen.

REFERENCES

1. Vázquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Domínguez-Bernal G, Goebel W, González-Zorn B, Wehland J, and Kreft J.(2001). *Listeria* pathogenesis and molecular virulence determinants, Clin. Microbiol. Rev. 14: (3) 584-640.
2. Lecuit M.(2005). Understanding how *Listeria monocytogenes* targets and crosses host barriers, Clin. Microbiol. Infec. 11:(6) 430-436.
3. Murray EG.(1953).The story of *Listeria*.Trans.Roy.Soc.Can.47:15-21.
4. Stanley NF. (1949). Studies on *Listeria monocytogenes*. I. Isolation of a monocytosis-producing agent (MPA). Aust. J. exp. Biol. med. Sci. 27: 123-131.
5. Hany O. (1997).Chemical & Biological studies on Lipids of *Listeria* species..Thesis Submitted for the Fulfilment of the Degree of Doctor of Philosophy. Uni. Karachi.
6. Tadayon RA, Caroll KK. and Murray RGE. (1970).Purification and properties of a biologically active factor in lipid extracts of *Listeria monocytogenes*. Can. J. Microbiol. 16: 535.
7. Galsworthy SB. Gurofsky SM. and Murray RGE. (1977). Purification of a monocytosis-producing activity from *Listeria monocytogenes*. Infect. Immun. 15: 500- 509.
8. Mitsuyama M., Takeya K., Nomoto K. & Shimotori S. (1978) Three phases of phagocyte contribution to resistance against *Listeria monocytogenes*. J. gen. Microbiol. 106: 165-175.
9. OtokunferTV, Shum DT and Galsworthy SB. (1979) . immunological properties partially purified material with monocytosis producing activity from *listeria monocytogenes*. Can. J. Microbiol.25: 706-712.
10. Al-Gburi NM. (2011). Study the antilisterial &immune stimulating effects of crud *Listeria* Lipids on *Listeria monocytogenes*.AL-AnbarJ.vet.Sci.4(2):76-80.
11. Luna LG.(1968).Manual of Histologic Stainig Methods of the Armed forces Institute of Pathology.3rd ed.Mcgrow-Hill BookCompany.New York.
12. Benaceraft B.(1978).Ahypothesis of relate the Iregion specific Ir gen to macrophages and B-lymphocytes. J.immunol.120 : 1809-1812.
13. North J. (1973).Importance of thymus-derived lymphocytes in cell-mediated immunity to infection. Cell. Immunol. 7:166.

Control mice:

The main lesion in liver characterized by multiple extensive lesion characterized by necrosis of hepatocytes with neutrophils, in other section macrophages aggregation together and congested of blood vessels and sinusoid and proliferation of kuffer cells and aptosis of hepatocytes(fig 4). Lesions in spleen there is sever congestion and Hemorrhage of red pulp, capsular area with neutrophils infiltration and depletion of white pulp, multiple granulomatous lesion and proliferation of megakaryocyte (fig5). the lung show thickening inter alveolar septum due to mesenchymal proliferation and congestion of capillaries blood vessels and mononuclear infiltration, also Hemorrhage in other sector were reported and emphysema and collapse well seen, other section there is hyperplasia of tunica media of consephector blood vessels ,also red blood cells was seen in the lumina of alveoli.

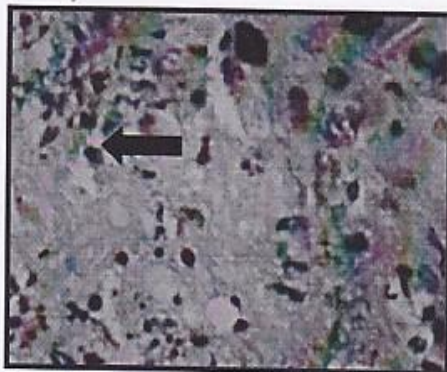


Fig (4): liver of control mice show diffuse necrotic area and infiltration with inflammatory cells (H&E x40)

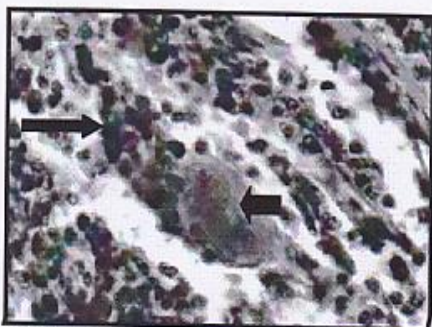


Fig (5): spleen of control mice show congestion of red pulp with neutrophils infiltration and proliferation of megakaryocyte (H & E x40)

DISCUSSION

In the present study the CSE have been found to stimulate both the humoral and cell-mediated immune response in the experimental animals and give 80% protection even the two pregnant mice. The augmentation of the humoral response by CSE ,as evident by increase in the number of respective antibody titers in mice Indicated that the CSE have the ability to stimulated the immune response, involved in the antibody synthesis (12).in view of the pivotal role played by macrophages in coordinating the processing and presentation of antigen to T-and B- cells, the elicitation of the humoral response to the antigen reveals that SE may enhance the effect by facilitating these processes.

Increase in the delayed type hypersensitivity reaction in mice response to SE revealed the stimulatory effect of on T-lymphocytes and accessory cell types required for the expression of the reaction. The roles of thymus-derived lymphocytes (13,14)and of activated macrophages (15-18) in resistance of infection of *L.monocytogenes* has been convincingly demonstrated.

In some experiments, SE showed presence of *in vivo*, adjuvant activity which was presumably masked by the immunosuppressive activity as reported previously by (10).SE may lack the components which cause the *in vivo* suppressive activity. The findings outlined above have demonstrated that the saline extract possesses immunostimulant activity .(Kim, *et al* al) showed that the crud SE of delipidiated cells suppressed the antibody response of mice to sheep erythrocytes, provided that the extract is administered before antigen. Similarly prepared extract in our experiment showed immunostimulating activity which is in accordance with the work of (5,7,20). In the present work immunostimulating activity of SE was observed when the extract was administered a twice dose.

The Results of cellular and humoral response corresponded with histopathological changes as well as with the results of bacterial isolation, the immunized animals show enlargement of the spleen and infiltration of lymphocytes in organs which can be attributed to hyperplasia in lymph tissue. That hyperplasia indicated infiltration of lymphocytes type T in huge numbers as a result of persist stimulation lymphocyte cells type Th0 which sensitized by histocompatibility complex and expression of antigens and then divides and differentiation

Table (2):Bacterial isolation from internal organs of immunized and control mice

Immunized mice sacrificed at fifteen day	No. of mice	Bacterial isolation			
		Liver	Spleen	Lung	Heart
	*1	+	+	+	+
	*2	+	+	+	+
	3	+	-	-	-
	4	-	-	-	-
	5	-	-	-	-
	6	-	-	-	-
	7	-	-	-	-
	8	-	-	-	-
	9	-	-	-	-
	10	-	-	-	-
Control died within 3-10	1	+	+	+	+
	2	+	+	+	+
	3	+	+	+	+
	4	+	+	+	+
	5	+	+	+	-
	6	+	+	+	-

*pregnant died at 5th day of Challenge

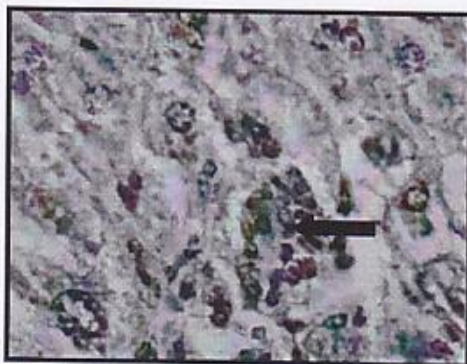


Fig (1) : liver of immunized mice show granulomatous consist of aggregation of macrophages in the paranchyma of liver with profilartion of kuffer cells (H&E ×40)

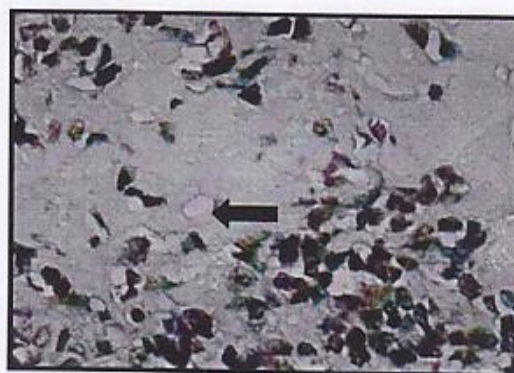


Fig (2): spleen of immunized mice show amyloid like substances deposition around atrophic white pulp (H&E ×40)

In spleen there is hyperplasia around central vein and mononuclear cell infiltration ,also amyloid depostion(fig 2). Also theres infiltration of mononuclear cells including macrophages and plasma cells in red pulb. While in Lung theres lymphocytes aggregation around the bronchules and lung pranchyma and hyperplacia ,a nother section theres lymphoid hyperplacia of lymphoid tissues around peribronchial lymphoid tissue (fig 3).

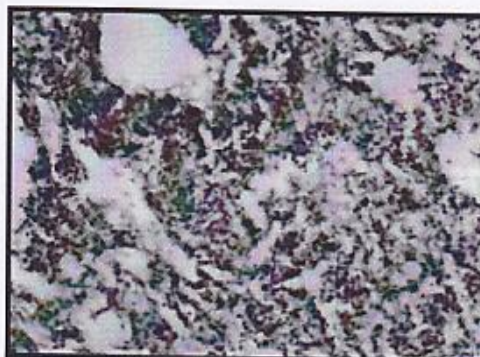


Fig (3):lung of immunized mice show hyperplasia and lymphoid tissue around bronchules and in the lung parenchyma (H&E ×40)

Table(1): Cellular and and humoral response in mice immunized with CSE

Cellular response			Humoral response	
Thickness of food pad				
No.of mice (10mice)	After24 h	After48 h	No.of mice (6mice)	IgG titer
1	2.1	1	1	1509.5
2	1.5	0.9	2	860.9
3	2.3	1.6	3	1249.4
4	1.6	0.8	4	1333.8
5	2.2	1.5	5	1208.0
6	2.1	1.7	6	1249.4
7	1.8	1	-	-
8	1.6	0.9	-	-
9	1.7	1.2	-	-
10	1.9	1.3	-	-
mean±SE	1.9±0.09	1.4±0.2	mean	1235.2
Control(6mice) mean	0	0	Control(3mice) mean	158.1

Effect of CSE on the course of Listerial infection:

The results showed dies only tow mice from immunized which were pregnants through fife days post challenge , the protection percentage was 80%, and isolated *L.monocytogenes* from liver,spleen ,Lung and heart from these mice while not isolated from other immunized mice that sacrificed at fifteen days post challange . all control mice were dies within 3-10 days post challenge and *L.monocytogenes* was isolated from liver,spleen Lung and heart .(Table2).

**Histopathological changes:
Immunized mice:**

The histopathological changes in Liver characterized by proliferation of kuffer cells ,small granulomatous lesion of the liver paranchyma, lymphocytic aggregation area in bile duct and central vein In another section theres focus aggregation of mononunclear cells specially(macrophages) in liver paranchyma with cuffing cells from lymphocytes and monocytes around the blood vesseles of portal area and central vein. (Fig 1).

INTRODUCTION

Listeria monocytogenes a Gram-positive pathogenic bacterium that has adapted to various environments, from soils and food products to the intestinal tract and intracellular compartments of diverse animal species and humans (1). In order to infect mammalian host and to cause the most severe pathological changes, *L.monocytogenes* is able to cross the intestinal, blood-brain and maternal fetal barriers(2).

As several bacteria possess an array of components which participate in the host immune system .Like wise , Listeric infections in several animals species and man are characterized by the presence of large circulating mononuclear cells. Although this phenomenon is not unusual in infections, the large numbers of such cells produced in response to *L.monocytogenes* infection is unique(3). (4,5) demonstrated that a chloroform-soluble extract of *Listeria* contained the agent which caused this response and named the extract monocyto-sis-producing agent (MPA), this agent can be found in saline extract (SE) of heat-killed ,dilapidated cells of *L.monocytogenes* (6,7). The SE is no-toxic, water soluble contain of protein, carbohydrates and phosphorus. CSE promotes a significant elevation in the level of circulating monocytes ,this characteristic is present only in both live and killed virulent strain of *L.monocytogenes* but not in another *Listeria* Spp. Monocytes is essential for the development of cellular and humoral incompetence and there is an intimate inter play between the mononuclear phagocytes and T-lymphocytes and the mononuclear phagocytes play critical roles in the defense against Listeric infection(8).

In relation to the role of MPA in immune responses studies were undertaken to survey the immunological properties of crude SE and their fractions partially purified preparation from *L.monocytogenes* which is enriched in MPA and the immunostimulation of saline extract have been reported (9,5).

MATERIALS AND METHODS

The crude SE was obtained from delipidated of heat killed *L.monocytogenes* according to (7,10) Briefly SE was prepared as follows: the residue from the delipidated cells was mixed with 1M NaCl in flask glass beads and agitation for 18hr. at 4c, then centrifugation at

20,000xg for 20 min. and the supernatant present the saline extract. Total protein was measured by Biuret method .

Sixteen mice was immunized with a twice dose of CSE (0.5 ml/ S.C) with two weeks intervals, to detect humoral immune response after ten days from second dose, six mice and three mice as control (not immunized) were sacrificed and blood collected from heart in sterile test tubes and allowed to clot for two hours at 4c, tubes were centrifuged for 10 minutes at 4000 xg , and the serum was separated, the titer of IgG were determined by radial immune-diffusion plate kit (IgG RID/ Bussero-MilanITALY) .

Delayed type hypersensitivity (DTH) test was performed by inject the remaining ten immunized mice with 0.05ml of CSE on right foot pad, and inject 0.05ml of sterile phosphate buffered saline on the left foot pad as control. The thickness of foot pads were measured after 24 & 48 hours. after that immunized mice (four of them become pregnant in late stage) during experiment and six mice used as control were challenged with 0.5 ml S.c virulent *L.monocytogenes* (CFU 10^8 / ml). At fifteen days post challenge immunized and control mice sacrificed to isolate *L.monocytogenes* from internal organs ,and pieces from internal organs were taken in 10% formalin for fixation ,then after ,processing routinely in histokinette, cut at 5µm thickness and stained with hematoxyline and eosin and examined under light microscope(11) and study the histopathological changes.

RESULTS

The saline extract was obtained from delipidated of heat killed *L.monocytogenes*. Total protein was measured by Biuret's method. The protein in CSE was 90mg/ml.

Effect of CSE on Humeral and Cellular response:

The result showed increased level of IgG titer (1235.2) as compared with control mice (158.1). On other hand the CSE stimulate of delayed hypersensitivity reactions in immunized mice by increase the thickness of foot pad after skin injection with mean 1.9 ± 0.09 , 1.4 ± 0.2 after 24, 48 hr. respectively while no changes in thickning of foot pad in control mice. (Table 1).

14. North J.(1973). Cellular mediators of anti-*Listeria* immunity as an enlarged population of short-lived, replicating T-cells. Kinetics of their production. J. exp. Med. 138: 34
15. McGregor DD. and Logie PS. (1973).The mediator of cellular immunity. VI. The effect of the antimitotic drug vinblastine on the mediator of cellular resistance to infection. J. exp. Med. 137:660.
16. Stanly ER, Cifonne M., Heard PM. and Defendi V. (1976). Factors regulating macrophage production and growth: identity of colony-stimulating factor and macrophage growth factor. J. exp. Med. 143: 631.
17. Klimper GR. and Henney CS.(1978).BCG-induced suppressor cells. I. Demonstration of a macrophage-like suppressor cell that inhibits cytotoxic T-cell generation in vitro. J. Immunol. 120: 563.
18. Yoshikai Y,Miake S.,Matsumoto K. and Takeyaka .(1980).Relation ship between non-specific activity of macrophages and immune responses to *Listeria monocytogenes* . Immunol 40: 295-301.
19. KimJJ, Sinclair NR ,Singhal Sk and Carroll,KK.(1976).Immunosuppression activity of extract from *Listeriamonocytogenes* .Int.Arch.Allerg.Appl.Immunol.50: 641-650.
20. Galworthy SB.(1982).immunomodulation by surface components of *Listeria monocytogenes*: areview.Clin.Invest.Med.7:223-227.
21. Remer KA,Jungi TW,Fatzer R,Tauber MG ,and Leib SL.(2001).Nitric oxide is protective in Listeric meningoencephalitis of rats. Infect.Immun.96: 4086-4093.
22. Pfister H, Remer KA, Fatzer R,Christen S, Leib SL, Jungi TW.(2002). inducible nitric oxide synthase and nitrotyrosine in Listeric encephal-it is: A cross studay in ruminats.Vet.Pathol.39: 190-199.
23. Nazar MS.(2006).Study on immunopathological changes caused by *Listeria monocytogenes* in Mice and Lambs.Ph.D.thesis. Pathology/Vet. Med.Baghdad Universty.
24. D'orazio SE,Halme DG,Ploegh HL,and Starnbach MN.(2003).Class Ia MHC-deficient BALB/c mice generate CD⁺T cell-mediated protective immunity against *Listeria monocytogenes* infection .J.Immunol.17:291-298.
25. Marco AJ,Parts N,Ramose J,Briones V,Balnoc M,Dominguez L,and Domingo M. (1992). Amicrobiological , histopathological ,and immunological study of the intragastric inoculation of *Listeria monocytogenes* in mice J.comp.Path.107: 1-9.
26. Marco AJ,Altimira J,Prats N,Lopez S, Dominguez L, Domingo M,and Briones V.(1997).Pentration of *Listeria monocytogenes* in mice infected by the oral rout. Microbiol.Pathogenesis.23:255-263.
27. Justin JD,Ingo B,and Werner G.(2000).Interaction of *Listeria monocytogenes* with the intestinal epithelium .FEMS Microbiology Letters.190:323-428.
28. Hamrich TS,Horton JR,Spears PA,Havell EA, and Smoak IW.(2003).Influnence of pregnancy on the pathogenesis of Listeriosis in mice.infect.Immun.71:5202-5207.
- 29.Njoku-Obi AM,and Osebold JM.(1962). Study on Mechanism of Immunity of Listeriosis.I-interaction of pertoneal exudate cells from sheep with *Listeria monocytogenes* in vitro.J.Immunol.89:87-94.
30. Mackaness GB.(1962). Cellular resistance to infection .J.Exp.Med.116:381-406.