Study the antilisterial and immune stimulating effects of crud Listeria Lipids on *Listeria monocytogenes*

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Abstract

In order to known the antilisterial, immune stimulating effects of crud Listeria Lipids (CLE) on *Listeria monocytogene*. crud Lipid Extract (CLE) were prepare, sensitivity test was don of this extract as antilisteria effect and comparing with antibiotics E15, C LR15, RA5, AM10, DO30, PY100 the result showed that the CLE of LM affect growth of LM and the inhibition zone 21.33±0.34 mm. comparing with antibiotics E15, C LR15, RA5, AM10, DO30, PY 10020.3±0.89, 19.66±0.33, 10.66±0.67, 23.33±0.89, 17.66±0.68, 24.66±0.34 respectively. The statical analysis has showed that there was asignifacans differences ($p<0.05$) between the antibiotics groups comparing with CLE and low with RA and highly with PY100. Also in order to known the effect of this crud Lipid on Listeria infection, 10 mice were immunized with CLE I/P (0.05 mg/0.5 ml) a twice dose with antervales period 14 days, after 10 days from second dose Delyed type hyper sensitivity test was done by skin test on foot pad with CLE 0.1 ml, then the thickening of foot pad measuring after 24 and 48h. then after 27 dayes of 1st dose mice were challenged with infected Lm, the result showed that the mean thickening of foot pad was, 1.6±0.048, 1.2±0.056 after 24,48 h. respectively. Result also showed decreased mortality rate 30% of immunized mice, while 100% of control and isolation of LM Liver, spleen and heart from the three immunized mice and all control which are died after 4-10 dayes that's indicated the crud Lipid stimulate immune response that protect the mice.

دراسة التأثير التثبيطي وكعامل محفز مناعي لمستخلص اللبد الخام لجرثومة *Listeria* على جرثومة *L. monocytogenes*

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الخلاصة

من اجل معرفه تأثير مستخلص اللبد الخام المستخلص من جرثومة *Listeria monocytogenes* كمثبط لنمو Listeria وكعامل محفز للمناعة ضد هذه الجراثيم. تم تحضير مستخلص اللبد ومن ثم اجراء فحص الحساسية لمعرفة تأثيره التثبيطي لنمو هذه الجرثومة ومقارنته مع المضادات الحيوية E15, C LR15 ,RA5, AM10, DO30, PY100 أوضحت النتائج فعالية المستخلص التثبيطي لنمو LM وأعطى قطر تثبيطي 21.33±0.34 mm بينما أعطت المضادات الحيوية E15, CLR1 5,RA5, AM10, DO30, PY100 20.3±0.89, 19.66±0.33, 10.66±0.67, 23.33±0.89, 17.66±0.68, 24.66±0.34 وان هناك فرق معنوي $P<0.05$ بين مجاميع المضادات الحيوية مقارنة مع المستخلص وكانت اقلها مع RA5 وأعلاها مع PY100. ولمعرفة تأثير المستخلص على الإصابة بهذه الجراثيم وذلك بتمنيع الفئران وبجرعتين بينهما 14 يوما ومن ثم اجراء الفحص الجلدي بعد 10 ايام من التمنيع الثاني بحقن وسادة القدم باللبد الخام بجرعة 0.1 مل وتم قياس التثخن بعد 24 و48 ساعة. وبعد 27 يوم من التمنيع الأول تم إصابة الحيوانات بجرعة تحدي من جراثيم LM. بينت نتائج الفحص الجلدي 1.6±0.048 و1.2±0.056 بعد 24، 48 ساعة على التوالي. كذلك أوضحت

النتائج ان نسبة هلاك الحيوانات الممنعة 30%، بينما نسبه هلاك حيوانات السيطرة 100% وتم عزل الجراثيم من كبد وطحال وقلب لثلاثة حيوانات الممنعة والسيطرة التي هلكت خلال 4–10 ايام مما يشير إلى ان مستخلص اللبد الخام حفز استجابة مناعية وفرت حماية لفئران.

Introduction

Listeria monocytogenes (L M) is 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) To infect its mammalian host and to cause the most severe pathologies, LM is able to cross the intestinal, blood-brain and maternofetal barriers (2). Several bacteria possess an array of components which participate in the host immune system immunologically active components of streptococcus pyogenes include an ajuvant peptidoglygan activity (3). Whole killed cells of corynbacterium pervum depress cell-mediated immunity and can also act as an adjuvant (4), Like wise, LM contains an array of components (lipid, saline extracts) with considerable biological importance. The LM contains 77.8% dry weight of chloroform soluble lipid, 4.4% protein and 4.8% Carbohydrate, Low toxicity of LM Lipids preparations, leaves a wide safety margin in their application (5). LM crud Lipid produces several responses of considerable biological nature in addition only LM lipid contain monocytosis producing ability (MPA) and A small doses of MPA elevated the level of circulating monocyts. Also Lipid extracts and the active fractions of Lipid are able to induce lymphopenia and granulocytosis (6, 7, 8). Therefore this immunostimulating activity, can be useful in the development of vaccine against this important food pathogen. Jablonska et al., (9) established the influence of Lipids on some infection caused by Gram-positive microorganisms in mice. Described (10) a similar effect of the crude of Listeria Lipids in infections eliciated by Gram-negative organisms. Most antibiotics are inactive against intracellular residing bacteria the use of adjuvants of bacterial origin might be asolutation of these problems. As these adjuvants markedly increase animal resistance to anumber of infectiouse induced by common food (salmonella, E. coli, staphylococci, Listeria) pathogen. So the aim of this study to elvuated the effect of *Listeria mono cytogenes* crud Lipid on LM in vitro by sensitivity test and in vivo by immunization of mice with CLE.

Material and Methods

- **Crud Lipid extraction**: Lipid extraction: we are depending on (11) for Lipid extraction with some modification. LM were grown on Trypticase soya broth contain 0.6% yeast extract and incubated for 7 days at 4c, the culture was checked for purity after incubation period. Cells were killed by autoclaveing at 121c°/15min. wash twice with PBS (pH. 7). The cells mass (5 gram) were freezing over night. To freeze dried cells to which of 114 ml solvents were added in the sequence: chloroform: methanol: water to achieve a final chloroform: methanol: water ratio of 1:2:1 (v:v:v) with glasses beads the mixture were shaken with vortex 2000 rpm/ 15 min. and allowed to stand in room tempreture for 4 h. and then in 4c overnight. The mixture were centrfugated at 6000 rpm/ 30min and taken the lower phase (lipid extract) was dried with oven 50c and harvested by 0.9% NaCl containg 0.3% sodium lauryl sulfate and filtrated with 0.45 milipore.
- **Antilisterial effect of CLE:** Bacterial suspension was prepared and compared to 0.5 turbedity with1×10^8 MacFarland tube, by using cotton swab spread the culture on Nuterient agar by streaking in different direction and then putting the antibiotics discs including (E15, C LR15, RA5, AM10, DO30, PY100), incubated the plates at 37c for 18-24h. (12). The same method done on CLE by dipping the filter discs with lipid extracts and after incubation period the inhibition diameter zone was measured by ruler in mm.

- **Effect of Lipid on immune response:** 10 mice was immunized with CLE I/P (0.05 mg/0.5 ml) a twice dose with antervales period 14 days, after 10 days from second dose Delayed type hyper sensitivity test was done by skin test on foot pad on 7 mice with CLE 0.1 ml another three inject with same solvent used in Lipid suspention (Nacl contain 0.3% sodium lauryl sulfate, then the thickening of foot pad measuring after 24 and 48h. then after 27 days of 1^{st} dose all ten immunized and 5 mice as control were challenged with infected Lm 1×10^{8} CFU/ ml.

Results

- **Antilisterial effect of crud Lipid extract:** Antilisterial activity of crud Lipid extract (CLE) compared with Antibiotics the result showed that the CLE affect growth of LM and the inhibition zone was 21.33± 0.34mm. comparing with antibiotics E15, C LR15, RA5, AM10, DO30, PY10020. (3±0.89, 19.66±0.33, 10.66±0.67, 23.33±0.89, 17.66±0.68, 24.66±0.34) respectively, the statical analysis has showedthat there was asignifacans differences ($p<0.05$) between the antibiotics groups comparing with CLE and low with RA and highly with PY100 (Table 1) (Fig.1, Fig. 2).

Table (1) antilisterial activity of CLE and Antibiotics

Mean value inhibition zone(mm±) SE						
E15	C LR15	RA5	AM10	DO30	PY100	CLE
20.3±0.89 b	19.66±0.33 bc	10.66±0.67 e	23.33±0.89 a	17.66±0.68 d	24.66±0.34 a	21.33± 0.34 b

L.S.D = 1.6.

E15 = Erythromycin, CLR15 = clarithromycin, RA5 = Rifampin, AM 10 = Ampicillin, DO30= Doxycyclin,PY100= Carbencillin

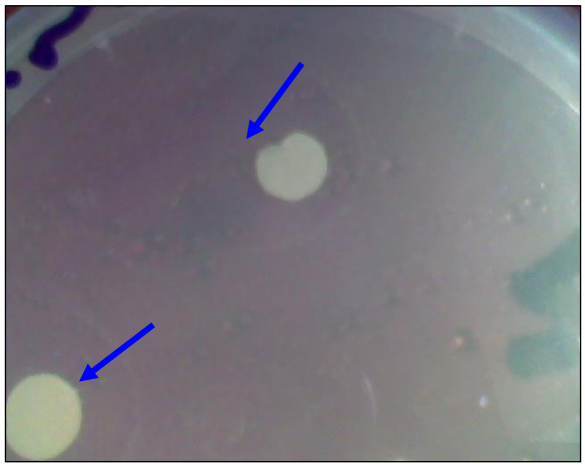

Fig. (1) antilisterial effect of CLE

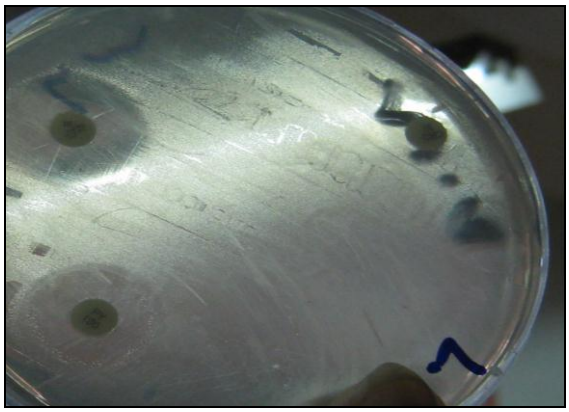

Fig. (2) antilisterial of Antibiotic

- **Effect of crud Lipid extract on immune response:** In skin test the result showed the mean thickninig of foot pad was, 1.6±0.048, 1.2±0.056 after 24,48 h. respectively Table (2).

Table (2) skin test result in mice immunized with CLE

No.of animals	Thickening of food pads	
	After 24 h	After 48 h
1	1.8	1.4
2	1.6	1
3	1.5	1.2
4	1.7	1.3
5	1.6	1.3
6	1.5	1.2
7	1.6	1.2
mean	**1.6**	**1.2**
SE	**0.048**	**0.056**
Control		
8	-	-
9	-	-
10	-	-

Result also showed dies only three from immunized mice with mortality rate 30%, while all control dies and mortality rate was 100%, and isolation of LM Liver, spleen and heart from the three immunized mice and all control which are died after 4-10 dayes of infection.

Discussion

The microbicidal effects of a variety of lipids have been extensively studied in recent years. A number of free fatty acids and their 1-monoglycerides have a broad spectrum of microbicidal activity against enveloped viruses and various bacteria in vitro including pathogens like group B streptococcus (13), herpes simplex virus (14), and *Chlamydia trachomatis* (15). *Neisseria gonorrhoeae* (16) and *C. albicans* (17). The mechanism by which these lipids kill bacteria is not known, but electron microscope studies indicate that they disrupt cell membranes (14, 13), Hence the Lipid of LM has antilisterial effect which is further confirmed by the present work and the results show that Listeria Lipid active in killing LM and may therefore be useful for treatment of infections caused by that pathogen, possibly in conjunction with antibiotic therapy. Vaccines against infectious pathogens remain one of the most effective and efecient means of disease prevention available. However, non-living vaccine preparations often fail to induce the same level of protective immunity when compared to a live vaccine, especially for intracellular pathogens such as *Listeria monocytogenes* or *M. tuberculosis*(18). Julk (19) and Mara et al (20) studied the immunostimulatory effects of isolated Lipids of anumber of Gram-negative and Gram-positive bacteria on experimental Listeric infection in mice and established possible differences in the chemical composition of fatty acids with respect to immunological activity. Animals vaccinated with mycobacterial lipids showed reduced bacterial burdens in the lung and spleen at 4 weeks after infection. In addition, the lungs of lipid-vaccinated animals also had signifcantly less pathology, with granulomatous lesions being smaller and more lymphocytic support an important role for lipid antigens in the immune response to *M. tuberculosis* infection, potentially through the generation of CD1-restricted T cells. Immunogenic lipids thus represent a novel class of antigens that might be included to enhance the protective effects of subunit vaccine formulations (21,22). Jakoniuk et al., (23), Borowski *et al* (10), Jakoniuk *et al* (24) observed that the Lipids of LM markedly increased natural immunity of the animals against bacterial and fungal infection and the mortality rate of mice was decreased and the elimination of bacterial and fungi from the

tissues was accelerated.our result also investigated development acellular immunity by development delyed type hypersensitivity and increased resistance against experimental Listeria infection that stimulating by CLE and decreasd mortality rate, similary, (5), found resistance developed against experimental Listerial infection by crud Lipid, as regarded Lipids of various species of the genus Listeria have asignificant immunostimulatory effect and Lipids isolated from pathogenic LM and administration of this Lipid preparation markedly increased animal resistance toward the challenged dose of LM resulting in adecreased mortality of the mice. A stimulation for the production of large monocytes, and only virulent strain of Lm produce MPA, Hence the MPA of LM has proved to be part of the lipid material of the organisms, monocyt is essential for the development of cellular and humoras immunocopetence. There is an intimate interplay between the mononuclear and the T-lymphoctes play critical roles in the defence against Listeria infection (5,25). In addition, lipid antigens themselves may have the capacity to traffic more freely between intracellular membrane compartments (26) and to partition directly into the cellular membranes of an antigenpresenting cell (27). Thus, lipid antigens administered as vaccines may be more effective than proteins in eliciting T cells with appropriate cytolytic effector functions based in part on their biophysical properties. Our data provide the foundation for further investigation of the practical utility of lipid antigen vaccination for prevention of Listeriosis and other infectious.

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