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Effect of Lipopolysaccharide Extracted from *Enterobacter cloacae* on some Pathogenic Bacteria

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ABSTRACT

A 106 different specimen were collected to isolate and diagnose some gram negative and gram-positive bacteria, as it was performed for the period from 15 August to 30 November 2019 from AL-Salam and Al-Khansaa educational hospitals in Mosul. Lipopolysaccharide (LPS) was extracted from *Enterobacter cloacae* by using the Methanol - Chloroform method. extract was diagnosed, using some biochemical tests such as the Molisch test to detect carbohydrate content and Folin reagent in order to determine proteins concentration, lipid solubility in organic solvent and Bio drop technique as well as to detect nucleic acid concentration. The extraction methods showed its efficiency with low protein and nucleic acids concentration in the extract. Lipopolysaccharide demonstrated its efficiency inhibiting microbial growth on all test isolates at concentrations 1100, 1000 $\mu\text{g}\ \text{ml}$ while its lipopolysaccharide showed its inhibition of *Proteus mirabilis* at all concentrations used. LPS showed possibility used as inhibitory, antimicrobial growth.

Keywords: Outer membrane, Inhibiting effect, Extraction, Lipopolysaccharide.

INTRODUCTION

Outer membrane (OM) of Gram negative (G-) bacteria are a round membrane enclosed entities of internal component cell (Jan, 2017). LPS anchored in the (OM) of all (G-) bacteria can pose risk to human health that ranges from irreversible shock to death (Su and Ding, 2015). LPS have many benefits to bacterial cell as it contributes to the structural integrity and protection barrier against harsh environments (Reyes *et al.*, 2015). Also, facilitating biofilm formation, antibiotic resistance and protecting bacteria from threats, LPS consist of three domains that include lipid A, core oligosaccharide and O-antigen (Huszczynski *et al.*, 2020). LPS potent endotoxin causes chronic immune responses associated with inflammation (Salguero *et al.*, 2019). Immune system responses to a low doses LPS lead to rapid clearing of pathogens but a higher doses LPS lead to uncontrolled over production cytokine that lead to endotoxic shock that is often lethal (Williams, 2019). In other word, less immunogenic LPS could serve as therapeutics to antagonize an overreacting innate immune system (Steimle *et al.*, 2016). *Enterobacter cloacae* belonging to order Enterobacteriales, family Enterobacteriaceae gram negative bacteria bacilli shaped (De-Lamaza *et al.*, 2020). Facultative anaerobic it is related with opportunistic infections that is specific in immunocompromised patients (Hong *et al.*, 2018).and nosocomial infections (Jin *et al.*, 2018). Also, these bacteria have a broad-spectrum antibiotic resistance (Annavajhala *et al.*, 2019). The extracted LPS by Methanol- Chloroform method was much easy less costly and safer but lack purity (Kalambhe *et al.*, 2017).

MATERIALS AND METHODS

A 106 specimen were collected that include urine, stool, blood, pus, sputum, nipple swab, vaginalis, nipple discharge and ear swab. The specimen was directly cultured on media then identification was completed depending on gram stain, morphological and biochemical tests using IMVIC to ensure diagnosis used a VITEK 2 technique was utilized. (Koneman *et al.*, 2017; Cappuccino and Welsh, 2020; De-Lamaza *et al.*, 2020).

LPS extraction:

LPS was extracted according to (Mirzae *et al.*, 2011). The dried LPS was dissolved in deionized water and placed in the deep freezer -80°C temperature then lyophilized placed in the lyophilizer (Christ lyophilizer made in Germany).

Biochemical test to detect LPS:

Folin to determine protein concentration, Molisch to detect carbohydrate, Solubility in organic solvent tests used to detect lipid also use Bio drop technique to detect nucleic acids concentration. (U and Sampath, 2014; Awasthi *et al.*, 2013)

Usage of crude of LPS as antibacterial against some pathogenic bacteria:

The antibacterial activity of LPS was extracted from pathogenic *Enterobacter cloacae* done against *Proteus mirabilis* as gram negative bacteria and *Staphylococcus aureus* as gram positive bacteria, using agar well diffusion method according to (Chittepu, 2019). Experimental bacteria were cultured on petri Dish containing 25 ml Muller Hinton agar after comparing bacterial growth with 0.5 McFarland standards tube that equals 1.5×10^8 . A (6mm) cut in agar diameter was performed by a sterile Pasteur pipette and then removed the agar cut by forceps filled 0.1 ml of each well in the different concentration of LPS (1100, 1000, 900, 800, 700) $\mu\text{g} \setminus \text{ml}$ after being cultured with bacteria by swab (three replications were made for each concentration of LPS extract for each bacteria). Also, preparation control sample without of LPS using normal saline to fill in the well. The plates were incubated in 37°C for 24 hours in upright position to keep LPS extract in the well, then the effects LPS on microbial growth were evaluated by measuring the diameters of growth inhibition zones on pathogenic bacteria (Al-Azawy and Abdullah, 2017).

RESULTS

Bacterial isolate:

The following isolates were diagnosed: *Enterobacter cloacae*, *Staphylococcus aureus* and *Proteus mirabilis*.

Biochemical Tests to Detect LPS:**Table 1: Biochemical tests for LPS**

The tests	The results	Concentration
Folin	+	0.0049 µg/ml
Molisch	+	Dark purple range
Bio Drop	DNA ⁻ , RNA ⁺	DNA 0, RNA 598.2 ng/µl
Solubility In Organic Solvent	+	Lipid solubilized in methanol and chloroform

Inhibitory Effect Crude Lipopolysaccharides:

The antimicrobial activity assay of LPS extract of *Enterobacter cloacae* were done against experimental bacterial isolate in different concentrations of (1100, 1000, 900, 800, 700) µg/ml that were evaluated by measuring the diameters of growth inhibition zones on bacterial strain and the results are shown in (Tables 2, 3).

Table 2: Inhibition zone diameter of lipopolysaccharide extract against *Staphylococcus aureus* by agar well diffusion method

LPS concentrations µg/ml	size inhibition zone diameter (mm)			means
1100	16	13	0	9.666667
1000	15	8	0	7.666667
900	12	0	0	4
800	0	0	0	0
700	0	0	0	0
Control Normal Saline	0	0	0	0

**Fig. 1: Inhibition zone diameter for different concentration of LPS extract against *Staphylococcus aureus* by agar diffusion method.****Table 3: Inhibition zone diameter of lipopolysaccharide extract against *Proteus mirabilis***

LPS concentrations µg/ml	size of inhibition zone diameter (mm)			means
1100	20	22	24	22
1000	20	20	19	19.666667
900	19	19	9	15.666667
800	13	13	11	12.333333
700	10	12	12	11.333333
Control Normal Saline	0	0	0	0



Fig. 2: Inhibition zone diameter for different concentration LPS extract against *Proteus mirabilis* by agar diffusion method

DISCUSSION

LPS has shown its efficacy as a growth inhibitor of pathological isolates at concentrations of 1000, 1100 µg/ml, and with varying inhibition ratios at concentrations of at concentrations of 1100, 1000, 900 µg/ml affecting *Staphylococcus aureus* Fig. (1), and LPS has also demonstrated its efficacy in inhibiting *Proteus mirabilis* Fig. (2) at all concentrations (1100, 1000, 900, 800, 700) µg/ml. which lowest inhibitor concentration was only 700 µg/ml for *Proteus mirabilis* among the isolates under study. And this inhibitory effect is compatible with the researcher (AL-Azawy and Abdullah, 2017). LPS effect on gram negative bacteria was more than gram positive bacteria. The LPS mechanisms inhibitory effect on microorganisms its effect on is due to its capability of having anti-adhesive agent that inhabits bacterial adhesion on the surface (Tanek *et al.*, 2018) However, the mechanism of LPS antimicrobial remains indistinct but certain hypotheses were proposed that sustained specific evidence showing loss of membrane integrity: antimicrobial LPS is as a result of the adhesion property of the surface of active agents for the cell membrane integrity that leads to subsequent collapse of nutrition cycle or fatty acid moieties of LPS inserted in to the cell membrane. Instigates a proliferation of membrane size and ultra-structural changes, other hypothesis assumes that lps able to form pores and disrupt the plasma membrane (Ines and Dhouha, 2015). Also, LPS can increase cell membrane sponginess, occurs due to disarrangement of plasma membrane phospholipids (Sotirova *et al.*, 2009), increasing permeability of plasma membrane cells as interact lipids with fatty acids content in the cell membrane leads to inhibition of the membrane –restricted enzyme and efflux of intracellular cytoplasmic components (Cameotra and Makkar, 2004). LPS interacting with membrane protein leads to increase initiating conformational changes of lipids and proteins (Ortiz *et al.*, 2006; Sanchez *et al.*, 2006; Sotirova *et al.*, 2008).

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تأثير متعدد السكريات الدهني المستخلص من *Enterobacter cloacae* على بعض البكتيريا المرضية

الملخص

جمعت 106 عينة مختلفة شملت عينات لعزل وتشخيص بعض البكتيريا المرضية السالبة والموجبة لصبغة كرام والتي تم اجراءها في الفترة من 15 آب - 30 تشرين الثاني لسنة 2019 من مستشفيات السلام والخنساء التعليمية في مدينة الموصل تم اجراء استخلاص متعدد السكريات الدهني لبكتيريا *Enterobacter cloacae* باستخدام طريقة الكلوروفورم - ميثانول ثم تشخيص مستخلص متعدد السكريات الدهني المستخلص باستخدام بعض الاختبارات الكيموحيوية مثل كاشف فولن لتقدير تركيز البروتينات في المستخلص وكاشف مولش للكشف عن السكريات واختبار الذويانية في المذبيبات العضوية للكشف عن الدهون واستخدام تقنية القطرة الحيوية للكشف عن تركيز الاحماض النووية في المستخلص الخام و اثبتت هذه الاختبارات كفاءة عملية الاستخلاص من خلال النسب الضئيلة للبروتينات والاحماض النووية في المستخلص واطهر متعدد السكريات الدهني كفاءته في تثبيط النمو الجرثومي على جميع عزلات الاختبار بتركيز 1000 و 1100 مايكروغرام / سم³ من المستخلص بينما اظهر متعدد السكريات الدهني تثبيطه *Proteus mirabilis* في جميع التراكيز المستخدمة. واطهر كفاءة في امكانية استخدامه كمثبط ومضاد للنمو.

الكلمات الدالة: الغشاء الخارجي، التأثير التثبيطي، الاستخلاص، متعدد السكريات الدهني.