

Protective Humoral Immunity Induced by Lipopolysaccharide Incorporated Liposome in Mice Against *Shigella flexneri* Infection

Haider Hussain Naser, Shahlaa M. Salih and Abdulwahid B. Al-Shaibani
Department of Biotechnology, College of Science, Al-Nahrain University, Baghdad-Iraq.

Abstract

This study was conducted to determine the role of lipopolysaccharide-liposome conjugate (LPS-LIP) as a potential vaccine against shigellosis in mice by determining the IgG titer. One hundred stool samples were collected from patients with diarrhea from different hospitals in Baghdad city during the period December 2011-May 2012. Thirty isolates were suspected to be *Shigella*. Four isolates of *Shigella flexneri* were obtained after performing some biochemical tests and Api System. Antibiotic sensitivity test was carried out and results revealed that all isolates were resist to some antibiotics used in this study. One isolate was selected for LPS extraction by phenol hot water extraction method. Chemical characterization of the extracted LPS revealed that the carbohydrate content was 2.34 mg/ml, while the protein concentration was 0.52µg/ml. Partial purification of the extracted LPS was carried out by using gel- filtration chromatography on Sephacryl S-200 and results showed that three peaks were obtained and protein, carbohydrate concentration were estimated for each peak. The second peak observed to have the highest carbohydrate content was (25%). and the lowest contaminated protein was (0.001%). The partial purified LPS was analyzed by Sodium Dodecyl Sulphate Gel Electrophoresis (SDS-PAGE) and results revealed that two bands with molecular weights 100 and 150 KDa were present. Band with MW 100 kDa represented LPS. IgG titer was estimated by Elisa technique. A significant increase in IgG titer was recorded in mice treated with conjugate after infection with *S. flexneri*.

Introduction

Shigellosis is an acute invasive enteric infection caused by bacteria belonging to the genus *Shigella*; it is clinically manifested by diarrhea that is frequently bloody. Shigellosis is endemic in many developing countries and also occurs in epidemics causing considerable morbidity and mortality. Among the four species of *Shigella*, *S. dysenteriae* type 1 is especially important due to its toxin so it causes the most severe disease and may occur in large regional epidemics [1]. *S. boydii* and *S. sonnei* are associated with mild short illness whereas infections caused by *S. flexneri* are more severe and last longer. The predominant serogroup of *Shigella* is associated with the level of socioeconomic development.

S. flexneri is the main serogroup found in developing countries while *S. sonnei* being the next most common [2].

Few studies provide data on the global morbidity and mortality caused by infection with *Shigella* spp.; such estimates are needed, however, to plan strategies of prevention and treatment [3]. It is estimated to cause at least 80 million cases of bloody diarrhea and 700,000 deaths each year. Ninety-nine percent

of infections caused by *Shigella* occur in developing countries, and the majority of cases approximately (~70%), and of deaths approximately (~60%), occur among children less than five years of age. Probably less than one percent of cases are treated in hospital [1].

Many attempts have been made at synthesizing a vaccine which incorporates *shigella* lipopolysaccharide (LPS) antigenic determinants, through the covalent attachment to carrier molecules, such as phospholipid bilayered vesicles (liposomes)[4]. Liposomes have received a considerable amount of attention as carriers for the delivery of a wide variety of biologically active substances to cells and tissues *in vitro* and *in vivo*, and they have been employed as immunological adjuvants for the enhancement or modulation of immune responses, especially CMI, to various antigens [5, 6].

Materials and Methods

Isolation and Identification

Clinical samples

One hundred stool samples were collected from patients with diarrhea from different hospitals in Baghdad. These specimens comprised bloody and watery diarrhea where

Shigella spp. are expected to be found. Different biochemical tests applied after streaking stool samples on selective media as in the Table (1).

Cell Preparation

- Bacterial cells were grown in flask containing 25 ml of brain heart infusion broth at 37°C for 18 hours.
- The fresh cultures were used to inoculated 3.5 L of brain heart infusion broth in 500 ml conical flasks (each of them containing 200 ml broth).
- The inoculated flasks were incubated at 37°C for 24 hrs with shaking at 150 rpm.
- After incubation, cultures were centrifuged at (3000 rpm for 15 minutes).and the pellet was washed twice with phosphate buffer.

LPS extraction

LPS was extracted according to method demonstrated by [7].

Ten grams of bacterial cells were suspended in 250 ml of water; the suspension was heated to 70°C, and an equal volume of a 90% aqueous phenol solution at the same temperature was added. This mixture was stirred for 30 min at 70°C, left in refrigerator over night, and centrifuged at 3000 rpm for 10 min to obtain 3 layers. Aqueous layer was collected and dialyzed (8000-14000 kDa) for 3 days against D.W. then the dialysate was concentrated in a rotary evaporator at 70°C.

Partial Purification of LPS by Gel

Filtration

According to Morrison and Leive [8] 5 ml of crude LPS was applied gently to the column Sephacryl S-200 was prepared as recommended by Pharmacia Fine Chemicals Company. And a quantity of Sephacryl S-200 was suspended in 0.1 M phosphate buffer (pH 7), degassed, and packed in a glass column (1.5x63 cm), and equilibrated with 0.1M phosphate buffer (pH7), and flow rate was approximately 75 ml/hr. Five milliliters fractions were collected, and absorption was read at 280 nm for protein determination [9]. The carbohydrate concentration was estimated according to the method described by Dubois *et al* [10]. The protein concentration was determined according to Bradford, [11] and detected by Sodium Dodecyl Sulphate-PolyAcryl amid Gel Electrophoresis [12].

Preparation of liposome incorporated LPS (LPS – LIP conjugate) [13]

Five ml of chloroform was added to phospholipids vesicles which were prepared as mentioned by (Sigma) at (Cholesterol, 9 µmol/package L-α-Phosphatidylcholine (egg yolk), 63 µmol/package Stearylamine, 18 µmol/package) and evaporated by rotary evaporation at 45° for 1hr. The dried lipid mixture was resuspended by vigorously vortexing in 4 ml Tris-buffer (6µm. pH 8), and this treatment was repeated five times over the next hour to obtain multilamellar vesicles. One ml of LPS solution at a concentration 100 µg/ml was mixed with 2 ml of lipid suspension at a ratio (100 µmol/package) The mixture was heated to 45°C for 5 min, sonicated (50% Hz) for 1 min, and vortexed for 1 min. This treatment was repeated 3 times.

Experimental Design

Twenty five swiss Albino male mice were grouped as he following:

- Group1: Mice injected with 100 µl normal saline
- Group2: mice infected with (100 µl) 1×10^9 *S. flexneri*
- Group3: Intramuscularly mice injected with 100 µl of LPS with complete free adjuvant and infected with *S. flexneri*
- Group4: Intramuscularly mice injected with 100 µl of liposome incorporated LPS and infected with *S. flexneri*
- Group5: Intramuscularly mice injected with 100 µl of LPS mixed with 100 µl of liposome and infected with *S. flexneri*.

Enzyme immunoassay for quantitative mouse IgG titer

The procedure was done according to the instruction of General Bioscience Company.

➤ Assay Procedure

- All reagent and microplate were brought to the room temperature and mixed for a few minutes prior to use. Fifty µl of diluted samples (at the concentration 10µg/ml) was added to each well with gently mixing, the plate was covered with a cardboard to prevent evaporation and put in incubator for 2 hrs at room temperature (25°C).

- Four hundred μl of blocking reagent was added to each well and incubated for 2 hrs at room temperature.
- The plate was washed 3 times using a microtiter plate washer and 1:50 dilution was performed, then one hundred μl was added to each well and the plate was incubated at room temperature for 2 hrs.
- The plate was washed 3 times using a microtiter plate washer.
- One hundred μl of anti-mouse HRP conjugate was added to each well. The plate was incubated for 1 hr at room temperature and washed 3 times.
- One hundred μl of TMP One Solution HRP substrate was added to each well and incubated at room temperature for 10 min.
- The reaction was stopped by addition 1N of sulfuric acid and the absorbance was read at 450nm or 405 nm.

➤ Calculation of the results

The cut-off value was determined by adding 0.01 to the mean absorbance for the negative control readings (Cal X) Cut-off value = Cal X + 0.01

Statistical Analysis

The values of the investigated parameters were given in terms of mean \pm standard error,

and differences between means were assessed by analysis of variance (ANOVA), least significant difference (LSD) and Duncan test, using the computer program IBM SPSS Statistics 19. The difference was considered significant when the probability value (P) \geq 0.05 [14] which were calculated according to the following equation:

$$\text{Treatment efficiency} = \frac{A-B}{B} \times 100$$

A=treated group. B=control group.

Results

Sampling and Isolation

Clinical samples

One hundred stool samples were collected from patients with diarrhea from different hospitals in Baghdad city included Al-Alwia children hospital, Al-Yarmouk hospital, Al-Kathmia hospital and Central child hospital. Types of clinical samples are mentioned in Table (1). Thirty stool samples were suspected to be *shigella*. Results revealed that only 8 isolates were positive for *shigella*.

Table (1)
The clinical samples suspected being *Shigella*.

Type of sample	Number of samples suspected to be <i>shigella</i>	Positive <i>shigella</i>
Bloody diarrhea	14	4
Green diarrhea with mucous	6	3
Brown diarrhea	10	1
total	30	8

Specimens that cannot be cultured within two hours after collection should be placed in transport medium and refrigerated immediately. Unlike some organisms, *Shigella* will die, even in transport media, if they are not refrigerated [1].

Biochemical tests

All expected *Shigella* isolates were Gram negative bacilli, in triple sugar iron agar they gave red (alkaline) slant so they do not

ferment lactose nor sucrose, and yellow (acid) which means they ferment glucose with no production of gas (CO_2) or H_2S . On MacConkey agar they produced colorless colonies with fair to good growth which means they are lactose non-fermenter except one of the isolates gave positive reaction after 48 h of incubation. They are non-motile, Oxidase-negative, Urease-negative, Catalase-positive. Three of the isolates gave positive reaction for indole production except one isolate. Also all

isolates were positive for methyl red test, negative for Voges-Proskauer and citrate

utilization tests.[15,16]. Table (2) shows biochemical tests applied on isolated *shigella*.

Table (2)
Results of biochemical tests applied on isolated organisms.

Isolation No.	TSI				IMViC				Lactose	Urease	Oxidase	Catalase
	Slant	Butt	H ₂ S	Gas	I	M	VP	C				
1	K	A	-	-	-	+	-	-	-	-	-	+
2	K	A	-	-	+	+	-	-	-	-	-	+
3	K	A	-	-	-	+	-	-	-	-	-	+
4	K	A	-	-	-	+	-	-	-	-	-	+
5	K	A	-	-	+	+	-	-	-	-	-	+
6	K	A	-	-	+	+	-	-	-	-	-	+
7	K	A	-	-	-	+	-	-	-	-	-	+
8	k	A	-	-	-	+	-	-	+	-	-	+

K: alkaline; A: acid; +: positive; -: negative.

Microscopic examination of the stool is helpful in making the correct diagnosis for enteric disease caused by *Shigella*. Individuals with disease caused by this pathogen characteristically shed erythrocytes and leukocytes in their stool. Armed with this knowledge, the clinician can instigate rational therapy 24 to 48 h before the results of culture[17].

Chemical Characterization

Chemical characterizations of the crude endotoxin extracted from *S. flexneri* were performed by estimating the carbohydrate contents according to Dubois *et al* [10]) depending on the standard curve of glucose, and estimating the protein contents according to Bradford [11]. **Depending** on the standard curve of bovine serum albumin. Accordingly, the carbohydrate content was 2.34 mg/ml, while the protein concentration was very low (0.52 µg/ml). In agreement with such findings, extraction of Gram negative endotoxin revealed that the aqueous phase contains low proportions of protein associated endotoxin [18]. In addition Fischer [19], and Helander *et al* [20] studied the chemical characterization of endotoxin separated from different Gram negative bacteria

(*Pseudomonas* spp., *Escherichia coli* and *Salmonella* spp.), and their results demonstrated that the main constituents of endotoxin are phosphate group, fatty acids and different forms of sugars (glucose, manose, galactose and glucose-amine).

Partial Purification

Fifty collected fractions were first assessed for the determination of protein by reading the absorbance of each fraction at 280 nm as suggested by [9] After that, each fraction was processed by a method of phenol-sulphuric acid [10] to determine carbohydrate content, and then the absorbance was read at a wave length of 490 nm. The relationship between absorbency and fraction number of each constituent (protein and carbohydrate) was illustrated in Fig.(1).

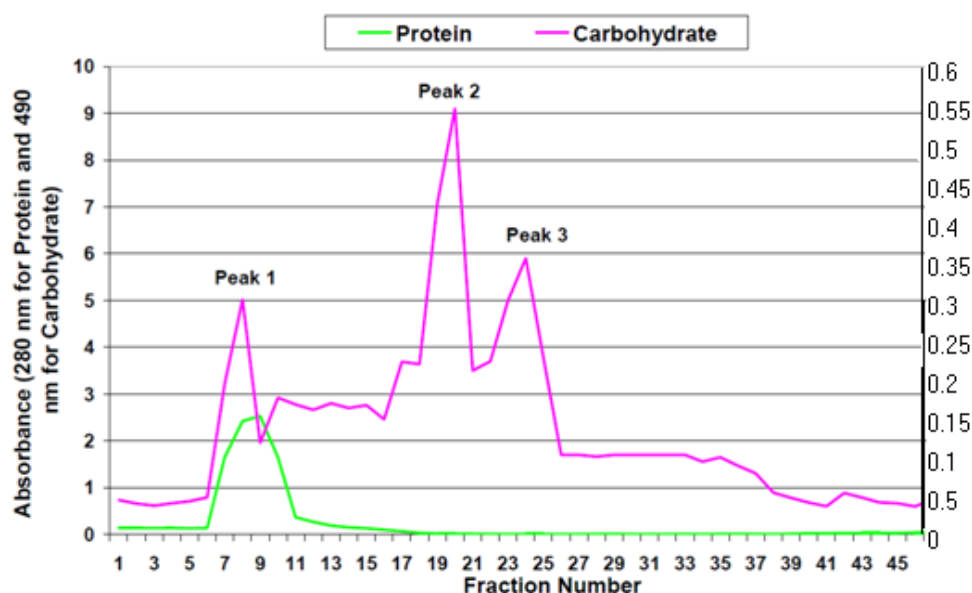


Fig.(1) Gel-filtration chromatography for LPS partial purification from *S. flexneriby* using Sephacryl S200, 75×2cm column equilibrated and eluted with 0.025 M PBS pH 7.2 at a flow rate of 75 ml/hour.

The figure demonstrates that at 490 nm three peaks were observed for carbohydrate. The first and third peaks were minor while the second peak was major. At 280 nm (protein), there was one peak separated at the position of carbohydrate peak one. Such finding suggests that there was a small amount of protein bound to the LPS and it was difficult to separate it from the LPS. Similar findings have been reported by [21, 22, 23,24]. Chemical analysis

of the partially purified endotoxin in the three observed peaks was carried out, and involved determination of carbohydrate, protein and nucleic acid contents. The results indicated that the percentage of carbohydrate was 14.0, 29.0 and 12.0% for peaks 1, 2 and 3, respectively. The percentage of carbohydrate in peak 2 was even higher than that of the crude LPS (29.0 vs. 18.1%) as shown in Table (3).

Table (3)
Carbohydrate and protein contents of crude and partially purified lipopolysaccharide.

Lipopolysaccharide		Carbohydrate (%)	Protein (%)
Crude		18.1	5.2
PartiallyPurified	Peak 1	14.0	4.0
	Peak 2	29.0	0.001
	Peak 3	12.0	0.001

Most studies are in favour of that the percentage yield of carbohydrates following endotoxin purification may vary widely. [2^o] obtained 12-18% carbohydrate from partially purified endotoxin, while Wilkinson and Galibraith[2¹] reported a less percentage range, which was 16.2-24.8%. In addition, 33.3% was obtained by Vinhet *al* [27]. These differences can be attributed to the types of bacterial species from which LPS was

extracted, method of extraction and purification process.

The present finding (carbohydrate yield of 29%) differs from that recorded by Al-azzawi [2¹] who demonstrated that the carbohydrate percentage in the partially purified LPS of local *P. aeruginosa* isolate was 15%, and further contradicting findings was also recorded by Horton *et al* [2^o], in which 12-18% range was observed. However, an agreement was also reported with

further studies. Chester and Meadow [2[^]] demonstrated a yield of 16-24%, and the same finding was reported by Wilkinson and Galbraith [2⁷]. In which the carbohydrate percentage in purified LPS was 16.2-24.8%. In Table (3-6), results showed that the protein percentage in partially purified LPS was 4% in peak 1 and 0.001% in peak 2 and 3.

It was also observed that the carbohydrate percentage in the partial purified LPS (29% in peak 2) was higher than that of the crude LPS

(18.1%), and a similar observation was made for the protein (4.0 and 0.001 vs. 5.2%). Both observations suggest the efficiency of the applied method of purification by gel filtration. The partial purified LPS was analyzed by Sodium Dodecyl Sulphate Gel Electrophoresis (SDS-PAGE) and results revealed that two bands with molecular weights 100 and 150 KDa were present. Band with MW 100 kDa represent LPS (Fig.(2)).

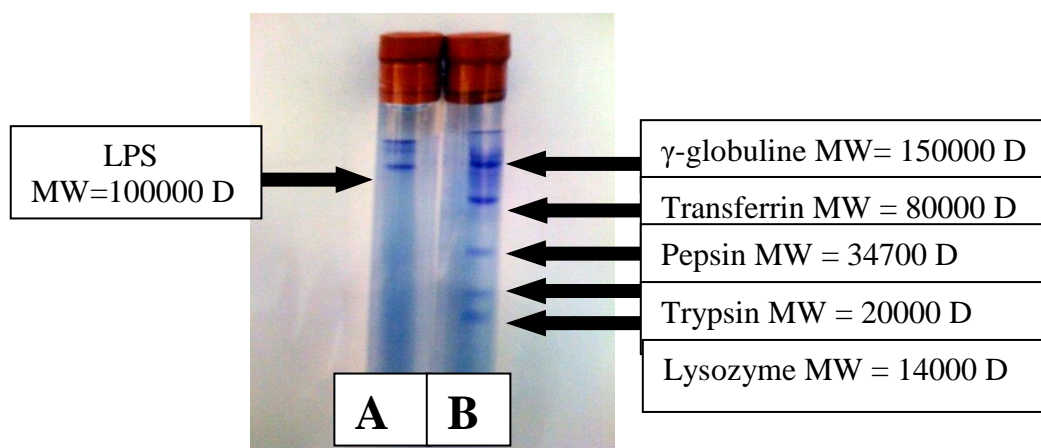


Fig. (2) Electrophoresis by use Sodium Dodecyl Sulphate PolyAcryl amid, tube A act as LPS sample and, tube B act as marker tube (Lammili, 1970).

Serum IgG level

As shown in table (1-4) the mean OD titers of IgG in mice infected with *S. flexneri* was significantly higher as compared with negative control (0.7890 vs. 0.5433) $P \leq 0.05$. The mean OD titer after treatment with conjugate and infected with *S. flexneri*, and mice treated with LPS and infected with *Sh. flexneri* were significantly higher as compared with negative

control (0.9243 and 0.8420 vs. 0.5433). There was no significant difference between mice coinjected with LPS and liposome as compared with negative control (0.6293 vs. 0.5433). There were no significant change in all treated groups and positive control (0.6293 and 0.8420 vs. 0.7890) except mice treated with conjugate and infected with *Sh. Flexneri* (0.9243 vs. 0.7890).

Table (4)
Serum IgG OD titer in mice treated with LPS-liposome conjugate and infected with *Shigella flexneri*.

<i>Mice group</i>	<i>(Mean±SE *)</i>
Negative control	0.5433±0.0180 d
Mice infected with <i>Shigella</i> (Positive control)	0.7890±0.0330 c
LPS-liposome conjugate and infected with <i>Shigella</i>	0.9243±0.1094 a
Mice conjugate with LPS and liposome and infected with <i>Shigella</i>	0.6293±0.0666 d
Mice treated with LPS and infected with <i>Shigella</i>	0.8420±0.1196-5 b

*Different letters: Significant difference ($P \leq 0.05$) between means of column.

Discussions

A liposomal complete core lipopolysaccharide vaccine induces humoral immunity to lipopolysaccharide (LPS), while remaining non-pyrogenic should be beneficial, as high levels of antibodies against LPS are associated with a reduced risk of adverse outcome [29].

According to Cryz *et al.* [30] LPS isolated from several strains of *P. aeruginosa* was derived either from the phenol or water phase, and was found to be highly immunogenic and protective in mice with doses as low as 0.001 µg; the level of protection correlated with anti-LPS antibody titers.

Dissanayake, *et al* [31] found that LPS core specific IgG responses of chickens immunized with different doses of liposome encapsulated LPS and those of non-immunized chickens as detected by ELISA showed significantly high LPS core specific IgG responses.

Conclusions

Lipopolysaccharide-liposome conjugate induce high level of IgG against *Shigella* infection in mice.

References

- [1] WHO, World Health Organization, Guidelines for the control of shigellosis, including epidemics due to *Shigella dysenteriae* type 1. WHO Document Production Services, Geneva, Switzerland, 2005.
- [2] Jafari, F., Hamidian, M., Salmanzadeh-Ahrabi, S., Bolfion, M., Kharaziha, P., Yaghobi, M. and Zali, M.R., Molecular diagnosis and antimicrobial resistance pattern of *Shigella spp.* isolated from patients with acute diarrhea in Tehran, Iran, *J. Gastroenterology and Hepatology from bed to bench*, Vol. 1, No. 1, pp11-17, 2008.
- [3] Kotloff, K.L., Winickoff, J.P., Ivanoff, B., Clemens, J.D., Swerdlow, D.L., Sansonetti, P.J., Adak, G.K. and Levine, M.M., Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies, *WHO Bull*, Vol. 77, No. 8, pp651-666, 1999.
- [4] Masoud, H., LPS-based conjugate vaccines composed of saccharide antigens of smooth-type *Salmonella enteritidis* and rough-type *S.gallinarum* 9R bound to bovine serum albumin, *Scand. J. Infect. Dis.*, Vol. 39, pp315-322, 2007.
- [5] Christensen, D., Korsholm, K.S., Andersen, P. and Agger, E.M., Cationic liposomes as vaccine adjuvants, *J. Expert. Rev. Vaccines*, Vol. 10, pp513-521, 2011.
- [6] Henriksen-Lacey, M., Korsholm, K.S., Andersen, P., Perrie, Y. and Christensen, D., Liposomal vaccine delivery systems, *J. Expert. Opin. Drug. Deliv.*, Vol. 8, pp 505-519, 2011.
- [7] Alexander, B.p., and Boris, F.s., Toxicity and Immunogenicity of *Neisseria meningitidis* Lipopolysaccharide Incorporated into Liposomes, *J. Infection and Immunity*, Vol. 60, pp3897-3903, 1992.
- [8] Morrison, D. C. and Leive, L., Fractions of lipopolysaccharide from *Escherichia coli* O111:B4 prepared by two extraction procedures. *J. Biol. Chem.*, Vol. 250, pp 2911-2919, 1975.
- [9] Bruck, C., Portetelle, D., Glineur, C. and Bollen, A., One-step purification of mouse monoclonal antibodies from ascitic fluid by DEAE affinity gel blue chromatography, *J. Immunol. Methods*, Vol. 53, pp 313-319, 1982.
- [10] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F., Determination of sugars and related substances, *J. Anal. Chem.*, Vol. 28, pp 350-356, 1956.
- [11] Bradford, M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *J. Annu. Biochem.*, Vol. 72, pp 248-254, 1976.
- [12] Laemmli, M. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *J. Nature*, Vol. 227, pp 680-685, 1970.
- [13] Dijkstra, J., Ryan, J. L. and Szoke, F. C., A procedure for the efficient incorporation of wild-type lipopolysaccharide into

- liposomes for use in immunological studies, *J. Immunol. Methods*, Vol. 114, pp 197-205, 1988.
- [14] Norman, G. R. and Streiner, D. L., *Biostatistics: The bar essentials*. Indian J. 2008.
- [15] Rowe, B. and Gross, R.J., The genus *Shigella*. In: Hensyl. W.R.(ed.), *Bergey's manual of determinative bacteriology*, 9th edition, Williams and Wilkins, Maryland, USA, 1994.
- [16] Kumar, D.R., *Diagnostic Microbiology (for DMLT students)*, J.P. Medical publishers (P), LTD, New Delhi, 2007.
- [17] Hirsh, D.C., Davidson, J.N. and Beards, L.R., Microscopic examination of stools from Nonhuman primates as a way for predicting the presence of *Shigella*, *J. Clin. Microbiol.*, Vol. 11, pp 65-67, 1980.
- [18] Kirikae, T., Kirikae, F., Saito, S., Tominaga, K., Tamura, H., Uemura, Y., Yokochi, T. and Nakano, M., Biological characterization of endotoxins released from antibiotic-treated *Pseudomonas aeruginosa* and *Escherichia coli*, *J. Antimicrob. Agents Chemother.*, Vol. 42, pp 1015-1021, 1998.
- [19] Fischer, W., Purification and fractionation of lipopolysaccharide from Gram-negative bacteria by hydrophobic interaction chromatography, *Eur. J. Biochem.*, Vol. 194, pp 655-661, 1990.
- [20] Helander, I.M.; Hurme, R.; Halkara, A. and Moran, A.P., Separation and characterization of two chemically distinct lipopolysaccharide in two *Pectinatus* species, *J. Bacteriol.*, Vol. 174, No. 10, pp 3348-3354, 1992.
- [21] Bruck, C., Portetelle, D., Glineur, C. and Bollen, A., One-step purification of mouse monoclonal antibodies from ascitic fluid by DEAE affinity gel blue chromatography, *J. Immunol. Methods*, Vol. 53, pp 313-319, 1982.
- [22] Al-azzawi, R. H. M., Extraction and partial purification of the local *Pseudomonas aeruginosa* lipopolysaccharide, M.Sc. Thesis, College of Science/University of Baghdad, 1998.
- [23] Johnson, K. G. and Perry, M. B., Improved techniques for the preparation of bacterial lipopolysaccharides, *CAN. J. Microbiol.*, Vol. 22, pp 29-34, 1975.
- [24] Pier, G. B., Sidberry, H. F., Zolyomi, S. and Sadoff, J. C., Isolation and characterization of a high molecular weight polysaccharide from the slime of *Pseudomonas aeruginosa*, *J. Infect. Immun.*, Vol. 22, pp 908-918, 1978.
- [25] Darveau, R. P. and Hancock, R. E. W., Procedure for isolation of bacterial lipopolysaccharides from smooth and rough *Pseudomonas aeruginosa* and *Salmonella typhimurium* strains, *J. Bacteriol.*, Vol. 155, pp 831- 838, 1983.
- [26] Horton, D., Rodemeyer, G. and Haskell, T. H., Analytical characterization of lipopolysaccharide antigens from seven strains of *Pseudomonas aeruginosa*, *J. Carb. Res.*, Vol. 55, pp 35-47, 1977.
- [27] Wilkinson, S. G. and Galbraith, L., Studies of lipopolysaccharides from *Pseudomonas aeruginosa*, *Eur. J. Biochem.*, Vol. 52, pp 331-343, 1975.
- [28] Vinh, T., Adler, B. and Faine, S., Ultrastructure and chemical composition of lipopolysaccharide extracted from *Leptospira interrogans serovar copenhageni* *J. General Microbiol.*, Vol. 123, pp 103-109, 1986.
- [29] Chester, I.R. and Meadow, P. M., Heterogenicity of the lipopolysaccharide from *Pseudomonas aeruginosa*. *Eur. J. Biochem.*, Vol. 58, pp 273-282, 1975.
- [30] Stewart, E.C.; McIntosh, T.J. And Poxton, I.R., The biological activity of a liposomal complete core lipopolysaccharide vaccine. *J. Endotoxin Res.* Vol. 8, No. 1, pp 39-46, 2002.
- [31] Cryz, S. J.; JR; Pitt, T. L.; Furer, E. and Germonier, R., Protection against fatal *Pseudomonas aeruginosa* wound sepsis by immunization with lipopolysaccharide and high-molecular-weight polysaccharide, *J. Infect. Immun.*, Vol. 43, No. 3, pp 795-799, 1984.

- [32] Dissanayake, D.R. Thula G. Wijewardana, T.G. ; Gunawardena, G.A. and. Poxton, I.R., Potential use of a liposome-encapsulated mixture of lipopolysaccharide core types (R1, R2, R3 and R4) of Escherichia coli in controlling colisepticaemia in chickens, J. of Med. Microbio., Vol. 59, pp100–107, 2010.

الخلاصة

أجريت الدراسة الحالية لتحديد دور متعدد السكريد الشحمي المغروز في الجسم الدهني كلقاح ضد امراض الشيكلا في الفئران. جمعت ١٠٠ عينة براز من مرضى مصابين بالإسهال من مستشفيات مختلفة في مدينة بغداد خلال الفترة من كانون الاول ٢٠١١ - مايو ٢٠١٢. تم الحصول على أربع عزلات من *Shigella flexneri* بعد أداء بعض الاختبارات الكيمو الحيوية ونظام API. وبعد اجراء اختبار الحساسية للمضادات الحيوية. أظهرت النتائج أن جميع العزلات مقاومة للمضادات الحيوية المستخدمة في هذه الدراسة. اختيرت احد هذه العزلات لاستخلاص متعدد السكريد الشحمي باستخدام طريقة الفينول-الماء الساخن. اظهرت نتائج الكشف الكيمياوي لمتعدد السكريد الشحمي المستخلص أن محتوى الكربوهيدرات كان ٢,٣٤ ملغ / ملتر، في حين أن تركيز البروتين كان ٠,٥٢ ملغ / ملتر. أجريت تنقية جزئية لمتعدد السكريد الشحمي باستخدام الترشيح الهلامي على هلام الفصل SephacrylS-200 اظهرت النتائج الحصول على ثلاث قمم وبعد تقدير تركيز البروتين والكربوهيدرات لكل قمة. اعطت القمة الثانية أعلى محتوى من الكربوهيدرات (٢٥%). وأقل تركيز بروتيني (٠,٠٠١%). تم التعرف على نقاوة متعدد السكريد المنقى باستخدام الترحيل الكهربائي اذ اظهرت النتائج الحصول على حزمتين ذات وزن جزيئي ١٠٠ و ١٥٠ كيلو دالتون. قيم دور متعدد السكريد الشحمي المغروز في الجسم الدهني من خلال تحديد النسب الكلية للكلوبيولينات المناعية نوع G. سجلت زيادة نوعه في مستوى الـ IgG في الفئران التي عولجت مع متعدد السكريد الشحمي المغروز في الجسم الدهني واصيبت بـ الشيكلا.