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The effect of a lipopolysaccharide extracted from Escherichia coli on some innate immunity indices of albino rats

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Abstract

Escherichia coli is wide world distribution enteric bacteria that cause different diseases. Many pathogenic and toxic effects may relate to it is lipopolysaccharide (LPS) which is part of bacterial outer membrane bearing endotoxic activity released after cell lysis. The LPS have bi-action either as potent activators of the immune system or injured tissue producing inflammation, dissemination intravascular coagulation, hypotension which finally end by endotoxic shock followed by death, the LPS also can induce the stimulation of phagocytic cells and increase its ability to kill any invader through its direct effect on activation of the respiratory burst. This manuscript aimed to study the modulatory effect of extracted E. coli LPS on some innate criteria of albino rats for that, either extracted E. coli LPS (ELPS) or standard E.coli O55:B5 LPS (SLPS) was injected intraperitoneally or intravenously by two different doses. Rats show transient leukopenia, lymphocytopenia, granulocytopenia, and midocytopenia (monocytes, eosinophils, and basophils) followed by leukocytosis, lymphocytosis, granulocytosis, and midocytosis at 24 hours, while different responses noted in WBCs according to different routes, doses, or types of LPS used. The existing study concluded that extracted E. coli LPS from (local strain) was able to elevation the phagocytic and respiratory burst of blood leukocytes with different effects of low and high dose, route of injection on total and differential white blood cells. The extracted E. coli LPS (local strain) can be used to produce immunomodulation in rats.

Keyword: *E. coli* LPS. White Blood cells, innate immunity, rat

تاثير حقن متعدد السكريد الشحمي المستخلص من جراثيم الإشريكيا القولونية على بعض معايير الاستجابة المناعة الفطرية والخلطية في الجرذان البيضاء

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الخلاصة:

يمثل متعدد السكريد الشحمي جزءاً مهماً من الغشاء الخارجي لبكتيريا الإشريكيا القولونية اذ يحمل نشاطاً ساماً يتم إطلاقه بعد تحلل الخلية. كما يمتلك متعدد السكريد الشحمي تأثيرا مزدوجا فقد يعمل كمنشط قوي للجهاز المناعي أو يسبب إصابة الانسجة مؤديا الى

الصدمة السمية، هدف البحث الحالي إلى دراسة التأثير المعدل لحقن متعدد السكريد الشحمي المستخلص من البكتيريا الإشريكيا القولونية على بعض المحددات المناعية الفطرية والخلطية في الجرذان البيضاء، ولأجل تحقيق هدف الدراسة حقنت الجرذان اما بمستخلص متعدد السكريد الشحمي او متعدد السكريد الشحمي القياسي في داخل الصفاق أو الوريد. حيث تم جمع عينات الدم والمصل في أوقات مختلفة، وسجل معدل خلايا الدم البيضاء الكلي والتفريقي، مؤشر البلعمة، الانفجار التنفسي والأجسام المضادة لمتعدد السكريد الشحمي. أظهرت الدراسة تنشيط مؤشر البلعمة والانفجار التنفسي بواسطة متعدد السكريد الشحمي في كافة اوقات الدراسة، ولوحظ زيادة في التنشيط بطريقة تعتمد على الجرعة بعد 28 يوماً لكل من مؤشر البلعمة والانفجار التنفسي بعد استخدام جرعتين منشطتين من متعدد السكريد الشحمي. أظهرت الجرذان المعاملة قلة في كل من الكريات البيض العابرة، واللمفاويات، والمحببات، والكريات المتوسطة، وزيادة في كل من الكريات البيضاء، واللمفاويات، والكريات الحبيبية، والخلايا المتوسطة، وذلك خلال 24 ساعة بينما لوحظت استجابة مختلفة لكريات الدم البيضاء مع طريقة الاعطاء والجرعة ونوع متعدد السكريد الشحمي المستخدم. كما لوحظ زيادة في معيار الاجسام المضادة لمتعدد السكريد الشحمي المستخلص ليصل الى 1/ 640 في الجرذان. خلصت الدراسة الى ان استخدام متعدد السكريد الشحمي له تأثير معدل للمناعة الفطرية ويؤدي الى انتاج استجابة خلطيه في الجرذان البيضاء.

الكلمات المفتاحية: متعدد السكريد الشحمي لبكتريا الإشريكيا القولونية، كريات الدم البيضاء، المناعة الفطرية والخلطية، الجرذان.

Introduction:

Escherichia coli is a worldwide distributed bacteria belong to the Enterobacteriaceae family and could be found in food and cause infection in humans and animals (1), *E. coli* outer membrane is composed mainly of lipopolysaccharide (LPS) which bearing endotoxic activity and is released after cell lysis (2).

Immunity to LPS clearly understood in the last 20 years after discovered signaling response of the immune cell; LPS acting as potent activators of the immune system especially innate immunity (3), and produces a large variety of adverse, unfavorable physiological responses leading to injury of tissue and endotoxic shock and lastly, death (4).

The LPS can produce fever and leukopenia or leukocytosis (5) after activation and/or stimulation of macrophage, monocytes (6) dendritic cells, T and B cells, and epithelial cells (7) leading to recruitment of monocyte, neutrophils, eosinophils to the site of infection (8) and stimulation of innate and adaptive arms of immunity by attachment to its receptor CD14, TLR4/MD2 (9) which mediated by inducing proinflammatory cytokine-like tumor necrosis factor α, interferon β, interleukin 6, interleukin 1 (6). The effect of LPS dose was studied by different researchers, high LPS dose to cause death from aseptic shock produced after releasing large quantities of cytokines either pro or anti-inflammatory from inflammatory cells and vascular endothelium, these cytokines leading to the dissemination of intravascular coagulation, hypotension, fever, and shock (10). However, the low dose of LPS induced hyporesponsive (11), so it can be used as an immunomodulator for the production of the immune serum to inhibited growth and killing invading bacteria by complement mediate antibody pathways and prevent its spread (12).

LPS can also stimulate professional phagocytes (neutrophils, monocytes, macrophages) and modulate its activity by increase NADPH oxidase leading to increase oxygen consumption and production of reactive oxygen species (ROSs) as O_2, H_2O_2, OH^-, O; the high oxygen consumption was elevated respiratory burst and have a positive correlation with the activity of phagocytosis of leukocytes which consider as an indicator of innate immune response stimulation (13).

In the present work, we aimed to investigate the modulatory effect of extracted *E. coli* LPS from (local isolate) on some innate and humoral indices in albino rats.

Materials and methods:

Inoculum preparation: twenty mg from both standard *E. coli* O55:B5 LPS(Chem Cruz©) (SLPS) and extracted *E. coli* LPS (ELPS) which was previously extracted (14) was dissolved in 4 ml progeny free distilled water to reach a concentration of 5 mg/ml which was used as standard

concentration and diluted to reach other injectable doses after dilution, then the dose was an adjustment to the animal's weight before injection.

Experiment design: 120 male Wister albino rats were used in this study, animals were housed in cages food and water were introduced, and the experiment was taken into consideration to reduce animals pain according to the International Animal Ethics Committee, rats were divided into 10 groups each contain 12 rats randomly, the group was marked and housed for two weeks before the experiment begins; all animals were provided from Mosul University/college of veterinary medicine.

The group of rats received a different dose of LPS in different routes if injection; the animals were injected (from both SLPS and ELPS) with two different doses of LPS 5 mg/kg (15) and 100 µg/kg (16,17). Animals in the groups (G1-G4) were used for ELPS. However, the groups (G5-G8) were used for SLPS and the last two groups (G9, G10) were used as control. The (G1 and G5) groups received high LPS dose (5 mg/kg) using the intraperitoneal route, (G2 and G6) received low LPS dose (100 µg/kg) using intraperitoneal route; (G3 and G7) groups have received high LPS dose (5 mg/kg) using intravenous route; (G4 and G8) received low LPS dose (100 µg/kg) by intravenous route. Blood from the different groups was collected in heparinized tubes after injection with an interval of 6, 12, 24 hours respectively for the detection of the phagocytic index, respiratory burst, and some other blood parameters.

Respiratory burst: The respiratory burst (RB) was done according to (18). 0.1 ml from heparinized blood was pipetted in an Eppendorf tube and mixed with 0.1 ml working solution of 0.1% nitroblue tetrazolium chloride (NBT) and incubated for 15 minutes at 37°C. Smear was prepared from blood –NBT mixture on a clean microscopic slide, stained with May-Grünwald– Giemsa, examined under 40× objective lens, then using oil immersion lens to count the phagocytic cells contain formazan deposit as a percentage.

phagocytic Index: This test was done according to the method described by (19) by mixing 0.1 ml from both blood and Candida albicans spore suspension(supplied from microbiology lab/college of veterinary medicine / Mosul University) and incubated at 37°C/30 minute, the microscopic slide smear was prepared from blood - Candida mixture, stained with May-Grunwald - Giemsa and examined under 40 × objective lens, then using oil immersion lens. the percentage was calculated according to the following equation:

% phagocytosis = (Number of phagocytic cells containing Candida/ total number of the phagocytic cell) ×100.

Statical analysis

The results were analyzed with IBM SPSS Version 24, all hypothesis was tested by ANOVA test, Univariate analysis of variance and the means variance between groups then further analysis by Duncan test and T-test.

Results:

Disregarding the time and route of administration, the low dose of LPS revealed an unmeaningful increase in the number of respiratory bursts. Intravenous route administration of SLPS showed that the group 8 animals exhibit a significant rise in a respiratory burst (RB) after 6 hours of injection compared to G2, and G4 throughout the whole times of experiment, as well as compared to G6 after 12, and 24 hours, and G8 after 12 hours. Furthermore, G8 rats showed elevation in RB numbers compared to G1, G3, G5, and G7 receiving a high dose of LPS regardless of the time except for 12 hours after injection in G7 animals. After 24 hours of injection of LPS, G6 and G7 exhibit significant development in RB response at $P \leq 0.05$. table 1

Table (1): The Respiratory Burst of rats injected with extracted *E. coli* LPS and standard *E. coli* O55: B5 LPS at different times and routes

Dose	Route of injection	LPS type	Time			Average of dose, route of injection, LPS type	average LPS type Standard Vs extract	average of Route of injection effect Ip vs IV	Average Dose effect
			6 h	12 h	24 h				
High	IP	G1	57.30 ±2.26 def	64.10 ± 4.94 cdef	54.46 ± 18.17 f	58.62 ± 10.41 d	63.64 ± 8.17	65.60 ± 8.50	62.42± 8.92
		G5	68.20 ±2.83 bcd	67.63 ±1.96 bcde	56.43 ±3.88 ef	64.08 ± 6.30 cd			
	IV	G3	53.40 ± 3.60 f	66.60 ±0.00 bcde	64.16 ± 13.03 cdef	61.38 ± 9.09 cd			
		G7	70.63 ± 2.96 bc	72.03 ± 1.80 abc	54.10 ± 5.70 f	65.58 ± 9.26 bcd			
Low	IP	G2	68.06 ± 2.28 bcd	63.53 ± 5.68 cdef	70.53 ± 2.95 bc	67.37 ± 4.58 bc	69.23 ± 8.33 *	67.27 ± 8.86	70.46 ± 6.28 *
		G6	78.23 ± 4.77 ab	69.33 ± 4.83 bc	69.46 ± 4.12 bc	72.34 ± 5.94 ab			
	IV	G4	67.93 ± 5.22 bcd	64.30 ± 3.56 cdef	69.36 ±2.40 bc	67.20 ± 4.06 bc			
		G8	81.90 ±7.10 a	69.43 ± 3.61 bc	73.46 ± 4.24 abc	74.93 ±7.12 a			
	Time average		68.20 ± 9.73 A	67.12 ± 4.27 A	64.00 ± 10.43 A				

- The (G1 and G5) groups received high LPS dose using IP, (G2 and G6) received low LPS dose using IP method; (G3 and G7) groups have received high LPS dose using IV, (G4 and G8) received low LPS dose using IV route.
- The average of LPS type (Standard Vs. Extract), an average of the route of injection effect IP vs. IV, average dose effect, and time-average indicates the mean of all groups that represented Standard LPS, IP, dose, times vs. the mean of all groups that represented Extracted LPS, IP, dose time.
- (*) means there was a significant difference when $P \leq 0.05$.
- All data represented as mean ± standard deviation of Respiratory burst of leukocyte.
- The different letters indicated a significant difference when $P \leq 0.05$.

Leukocytes of G1 animals revealed no variation in the number of RB compared to the leukocytes of G2, G3, and G4 after 6 and 12 hours of injection, while G3 showed a significant decline in RB number in comparison to the leukocytes of G2, G4 at 6

hours and with G5, and G7 after 12 hours of injection. With concern to the average dose, route of administration, and type of given LPS, the leukocytes of G1 animals have a less significant number of RB in comparison to G2, G4, G6, and G8 at $P \leq 0.05$. In addition, G8 leukocytes display a significant rise in RB numbers vary of G1, G2, G3, G5, and G7 leukocytes at $P \leq 0.05$. Concerning the effect of time on RB, the data revealed no variations among all studied groups. While using SLPS in comparison with ELPS showed elevation in RB between the same groups. Also, similar elevation in respiratory bursts is found when using intraperitoneal or intravenous route of injection. Average low LPS dose gives significant respiratory burst elevation rather than high LPS dose. table 1, figure 1.

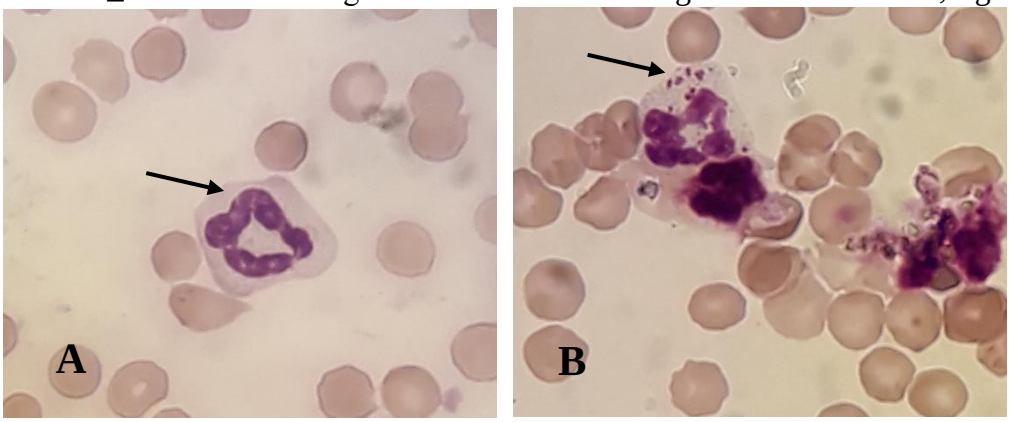

Figure (1): **The Respiratory burst by phagocytic cells after stimulation with Extracted *E. coli* LPS and standard *E. coli* O55:B5 LPS:** Arrows in B indicated formazan particles inside phagolysosomes in the cytoplasm of phagocytic cells, arow in A show normal cytoplasmic white blood cells; magnification power 1000 X

Regarding the phagocytic index in leukocytes revealed that there were no variations concerning the LPS dose and time of injection in most studied groups except in leukocytes of G6 and G8 were shown a slight decrease in phagocytic index values after 24 hours of injection. The highest non-significant elevation in the phagocytic index was recorded in G8 after 12 hours of injection in comparison with G2, G4, G6, and G8 groups with times other than 12 hours. While, the value of phagocytic index showed low significant value after 6 and 12 hours in G3 compared with 24 hours of the same group, as well as compare to G5, G7, and G1 in exception to that recorded at 6 hours. Moreover, the higher dose of LPS revealed a low value of phagocytic index after 6 hours injection in rats of G1, G3, G5, and G7, while the low dose of LPS in G4, G6, and G8 showed a high phagocytic index value after 12 hours of injection. The comparison of average dose, route, and types of LPS exhibit a significantly low effect on the phagocytic index in leukocytes of G1 and G3 comparing with G2, G4, G5, G6, G7, and G8. In addition to that phagocytic index in relation to the time showed no significant variations within studied groups. The analyzed data of LPS types both extracted and standard showed that the injection of SLPS exhibits a high response on the phagocytic index of leukocytes compare to those in rats injected with ELPS in the same animals and groups. Meanwhile, the route of injection revealed no difference occurs when using the intraperitoneal or intravenous routes on the phagocytic index. Moreover, the average dose indicated that low LPS dose gave significant elevation effect on the phagocytic index comparison with high LPS dose used. table 2, figure 2.

Table (2): The phagocytic index in Rats injected with extracted *E. coli* LPS and standard *E. coli* O55: B5 LPS at different times and routes

Dose	Route of injection	LPS type	Time			Average of dose, route of injection, LPS type	average LPS type Standard Vs extract	average of Route of injection effect Ip vs IV	Average Dose effect
			6 h	12 h	24 h				
High	IP	G1	65.43 ± 2.97 d	70.83 ± 3.01 bc	71.90 ± 13.21 bc	69.38 ± 7.56 b	77.96 ± 10.97	84.66 ±9.54	78.94 ± 11.76
		G5	87.06 ± 0.92ab	90.03 ± 4.85 ab	92.30 ± 2.06 a	89.80 ± 3.51 a			
	IV	G3	64.00 ±8.18 d	60.86 ±18.17 d	77.96 ±10.6 bc	67.61 ± 13.70 b			
		G7	87.30 ±9.65 ab	88.86 ±2.77 ab	90.73 ± 1.90 ab	88.96 ± 5.32 a			
Low	IP	G2	91.03 ±3.85 ab	89.60 ± 5.66 ab	85.13 ± 6.48 ab	88.58 ±5.41 a	89.94 ± 1.93 *	83.23 ±10.50	88.96 ±3.02 *
		G6	91.20 ± 3.36a	92.10 ± 2.15 a	89.36 ±4.08 ab	90.88 ± 3.10 a			
	IV	G4	83.41 ± 6.09 ab	90.60 ±4.35 ab	84.80 ±5.99 ab	86.27 ± 5.82 a			
		G8	89.83 ± 0.28 ab	92.66 ± 3.05 a	87.83 ±1.10 ab	90.11 ± 2.66a			
	Time average		82.41 ±11.21 A	84.44 ±11.84 A	85.00 ± 6.91 A				

- The (G1 and G5) groups received high LPS dose using IP, (G2 and G6) received low LPS dose using IP method; (G3 and G7) groups have received high LPS dose using IV, (G4 and G8) received low LPS dose using IV route.
- The average of LPS type (Standard Vs. Extract), an average of the route of injection effect IP vs. IV, average dose effect, and time-average indicates the mean of all groups that represented Standard LPS, IP, dose, times vs. the mean of all groups that represented Extracted LPS, IP, dose time.
- All data represented as mean ± standard deviation of phagocytosis of leukocytes.
- (*) means there was a significant difference when $P \leq 0.05$.
- The different letters indicated a significant difference when $P \leq 0.05$.

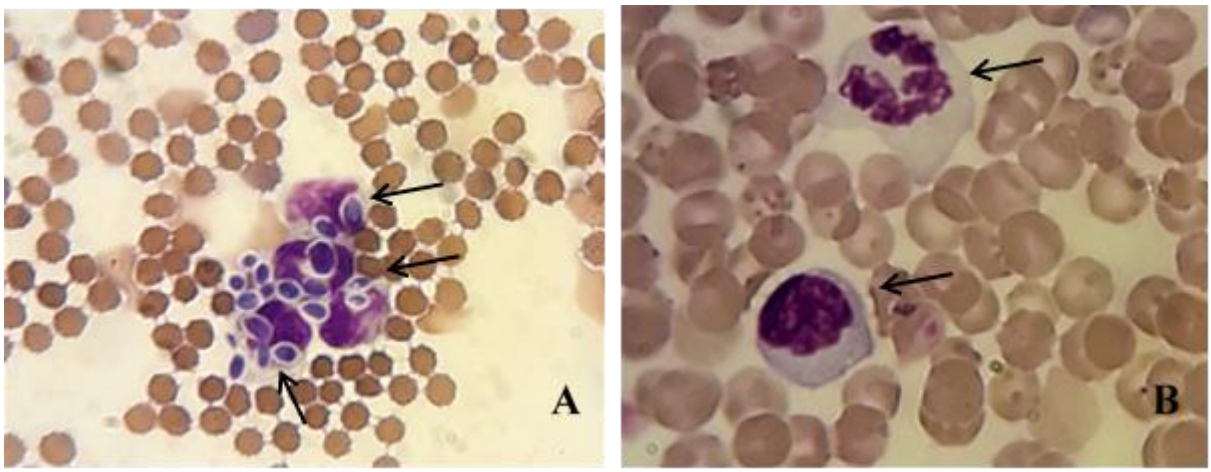

Figure 2: The phagocytosis index by phagocytic cells after stimulation with LPS extracted *E. coli* LPS and standard *E. coli* O55: B5 LPS: arow in A indicate *Candida albicans* spore inside phagocytic cells, arow in B show normal nonphagocytic white blood cells; magnification power 1000 X

Administration of LPS with different doses and routes of injection leads to an increasing number of leukocytes after 12 hours, and 24 hours in G2, G4, G6, and G8 compare to G3 blood samples at 6 hours. Also, the analysis of hematological data revealed that there was no variation between G3 and G7 during all the times except at the time 12 hours there was significantly lower. However, G1 blood samples showed a rise in WBCs count after 12 hours of injection compared to G3 and G7. About the time effect, it was shown that there was a decline in WBCs count during the first 6 hours of injection followed by elevation in WBCs count to reach 13.97×10^9 cell /Liter after 24 hours. At the same time studying the average effect of dose plus route of injection showed an increase in WBCs count in G1 compared to other groups. However, it recorded a decline in WBCs count in G3 blood samples concerning G1, G2, G4, and G6. Using both types of LPS did not show any variation in WBCs count in all studied blood samples, meanwhile, the route of injection exhibits significant elevation through intraperitoneal route than intravenous route and low dose has a significant rise in WBCs count than the high dose in rats. table 3.

Table (3): White blood cells count in response to ELPS and SLPS at different times and routes of injection

Dose	Route of injection	Experimental groups	Time			Average of dose, route of injection, LPS type	Average LPS type Standard vs. Extract	Average of Route of injection effect Ip vs. IV	Average Dose effect
			6 h	12 h	24 h				
High	IP	G5	7.10 ± 4.24 def	11.25 ± 3.32 abcdef	15.00 ±8.01 abcd	11.11 ± 5.19 bed	12.34 ±4.47	13.75 ±3.46 *	10.59 ±4.69
		G1	14.06 ± 3.32 abcd	19.90 ± 3.26 a	13.85 ±5.72 abcd	15.93 ± 4.10 a			
	IV	G7	6.86 ± 1.73 def	8.70 ± 5.37 cdef	9.13 ±0.45 bcdef	8.23 ±2.51 cd			
		G3	3.35 ± 3.06 f	4.90 ± 8.31 ef	12.60 ± 6.01 abcde	7.11 ± 5.79 d			
Low	IP	G6	10.13 ± 2.34 bcdef	14.50 ± 2.10 abcd	17.93 ±0.77 ab	14.18 ± 1.73 ab	11.06 ±3.33	9.65 ±3.27	12.87 ± 2.66 *
		G2	11.43 ± 1.36 abcdef	14.20 ±2.45 abcd	16.10 ±3.95 abc	13.91 ± 2.58 ab			
	IV	G8	9.83 ±8.85 bcdef	10.56 ± 5.84 bcdef	12.76 ±4.86 abcde	11.05 ± 6.51 bcd			
		G4	9.56 ± 2.91 bcdef	13.06 ± 2.60 abcde	14.36 ±4.00 abcd	12.33 ±3.17 abc			
	Time average		8.91 ± 4.60 B	12.18 ± 5.82A	13.97 ± 4.38 A				

- The (G1 and G5) groups received high LPS dose using IP, (G2 and G6) received low LPS dose using IP method; (G3 and G7) groups have received high LPS dose using IV, (G4 and G8) received low LPS dose using IV route.
-The average of LPS type (Standard Vs. Extract), an average of the route of injection effect IP vs. IV, average dose effect, and time-average indicates the mean of all groups that represented Standard LPS, IP, dose, times vs. the mean of all groups that represented Extracted LPS, IP, dose time.
- All data represented as mean × 10^9/L ± standard deviation of White Blood Cells.
- (*) means there was a significant difference when $P \leq 0.05$.
- The different letter indicates a significant difference when $P \leq 0.05$.

A low dose of LPS and the route of injection did not affect lymphocyte count. As well as, the lymphocyte count was raised significantly in G2 and G4 after 24 hours of injection compared to G6 and G8. In addition, a significant elevation in lymphocyte count was recorded in blood samples of G1 after 12h of injection with comparison to G3, G4, G5, G6, G7, and G8. Blood lymphocyte count in G3 showed a low number of lymphocytes recorded at 6 hours compared to 12 and 24 hours of the same group. While G3 does not differ from G7 in all times recorded at $P < 0.05$. Meanwhile, G1 appears significant elevation of lymphocyte number at 6 and 12 hours compared to G5 at the same time. Overall, the analysis of hematological data revealed a significant increase in lymphocyte count of G1 compared to all studied animals except those belonging to G4. Moreover, G7 showed a decreased lymphocyte count in their circulating blood compared to G1, G2, and G4

at $P \leq 0.05$. analysis of time-response data exhibits an initial decrease in lymphocyte count followed by a significant increase after 24h of injection.

The ELPS showed elevation of lymphocyte in blood in comparing with SLPS, meanwhile significant statistical response in lymphocyte count by intraperitoneal route of injection rather than the intravenous route of injection regardless of dose amount used which appeared to no effect in high or low dose on lymphocyte count. table 4

Table (4): Lymphocyte count in response to ELPS and SLPS at different times and routes of injections

Dose	Route of injection	Experimental groups	Time			Average of dose, route of injection, LPS type	Average LPS type Standard vs. Extract	Average of Rote of injection effect Ip vs. IV	Average Dose effect
			6 h	12 h	24 h				
High	IP	G5	2.86 ± 1.70 def	3.25 ± 0.77 bcdef	4.97 ± 3.50 abcde	3.96 ±2.09 cd	5.13 ±2.61 *	5.00 ± 2.31 *	4.00 ± 2.56
		G1	6.70 ± 1.57 ab	7.83 ± 2.41 a	6.30 ±1.5 abc	6.94 ±1.78 a			
	IV	G7	2.36 ± 0.86 def	2.53 ± 1.77 def	3.00 ± 0.65 cdef	2.63 ±1.07 d			
		G3	1.07 ±1.05 f	1.73 ± 3.00 ef	5.33 ± 0.40 abcde	2.71 ±2.55 cd			
Low	IP	G6	3.46 ± 1.24 bcdef	3.60 ± 2.51 bcdef	6.40 ±1.01 abc	4.48 ±2.06 bcd	3.65 ± 2.04	3.76 ± 2.43	4.77 ±2.22
		G2	2.57 ± 0.41 def	4.90 ± 0.40 abcde	7.17 ±1.41 a	4.88 ± 2.13 bc			
	IV	G8	3.57 ±2.86 bcdef	2.30 ± 1.30 def	5.53 ±2.40 abcd	3.80 ± 2.44 cd			
		G4	5.63 ± 2.30 abcdef	4.73 ± 0.20 abcde	7.34 ±2.41 a	5.93 ± 2.05 ab			
	Time average		3.55 ± 2.20 B	3.86 ± 2.46 B	5.77 ± 2.05 A				

- The (G1 and G5) groups received high LPS dose using IP, (G2 and G6) received low LPS dose using IP method; (G3 and G7) groups have received high LPS dose using IV, (G4 and G8) received low LPS dose using IV route.
-The average of LPS type (Standard Vs. Extract), an average of the route of injection effect IP vs. IV, average dose effect, and time-average indicates the mean of all groups that represented Standard LPS, IP, dose, times vs. the mean of all groups that represented Extracted LPS, IP, dose time.
-All data represented as mean × 10^9/L ± standard deviation of lymphocyte cells count.
- (*) means there was a significant difference when $P \leq 0.05$.
- The different letter indicates significant difference when $P \leq 0.05$

Analysis data of other midocytes revealed a variable effect on circulating cells (midocytes) with concern to dose, time, and route of injection in all studied groups such as G2 animals after 24 hours of injection showed a significant increase in the total number of these cells compared to G2, G4, and G6 after 6 hours and G6, G8 after 12 hours of injection. G1

blood samples showed significant elevation in midocytes numbers after 12 hours of injection with comparison to G2, G3, G4, G5, G6, G7, and G8 groups. The study of average dose, route, and type of LPS revealed that animals in G1 were significantly elevated their midocyte number comparison to all groups except that animals in G3. Also, there was an initial reduction in midocyte number followed by a significant increase after 12 hours. Overall, a comparison between SLPS and ELPS showed that there was no significant variation on circulating midocytes, and the intraperitoneal route of injection has a meaningful alteration in midocytes count rather than the intravenous route. Moreover, the dose-effect showed no serious effect circulating midocytes of all studied group animals. table

Table (5): Midocyte count in response to ELPS or SLPS in different times and routes of injection

Dose	Route of injection	Experimental groups	Time			Average of dose, route of injection, LPS type	Average LPS type Standard vs. Extract	Average of Route of injection effect Ip vs. IV	Average Dose effect
			6 h	12 h	24 h				
High	IP	G5	0.94 ± 0.79 ^{cdef}	1.15 ± 0.63 ^{cdef}	2.20 ± 1.93 ^{abcd}	1.43 ±1.22 ^{ab}	1.76 ±1.18	1.88 ± 1.21*	1.44 ± 1.23
		G1	1.23 ± 0.55 ^{cdef}	3.26 ± 2.12 ^{a}	3.05 ±1.06 ^{a}	2.51 ±1.59 ^{a}			
	IV	G7	0.73 ± 0.45 ^{def}	1.00 ± 0.78 ^{cdef}	1.23 ± 0.11 ^{cdef}	0.98 ± 0.50^{b}			
		G3	0.20 ± 0.17 ^{f}	0.40 ± 0.69 ^{ef}	1.90 ± 0.36 ^{abcdef}	0.82 ± 0.89 ^{b}			
Low	IP	G6	1.30 ± 0.79 ^{bcdef}	1.13 ± 1.02 ^{cdef}	2.56 ±0.66 ^{abc}	1.66 ± 0.99 ^{ab}	1.38 ±0.98	1.25 ± 0.86	1.70 ± 0.91
		G2	0.96 ± 0.25 ^{cdef}	1.83 ± 0.35 ^{abcdef}	3.00 ± 0.5 ^{ab}	1.93 ± 0.94 ^{ab}			
	IV	G8	1.13 ±1.23 ^{cdef}	1.03 ± 0.46 ^{cdef}	2.06 ± 1.25 ^{abcde}	1.41 ± 0.98 ^{ab}			
		G4	1.10 ± 0.36 ^{cdef}	1.86 ± 0.35 ^{abcdef}	2.43 ±0.66^{abcd}	1.80 ±0.71 ^{ab}			
	Time average		0.95 ±0.65 C	1.47 ± 1.17 B	2.30 ± 0.98 A				

-The (G1 and G5) groups received high LPS dose using IP, (G2 and G6) received low LPS dose using IP method; (G3 and G7) groups have received high LPS dose using IV, (G4 and G8) received low LPS dose using IV route.

- The average of LPS type (Standard Vs. Extract), an average of the route of injection effect IP vs. IV, average dose effect, and time-average indicates the mean of all groups that represented Standard LPS, IP, dose, times vs. the mean of all groups that represented Extracted LPS, IP, dose time.
- All data represented as mean × 10^9/L ± standard deviation of midocyte cells count.
- (*) means there was a significant difference when $P \leq 0.05$.
- the different letter indicates a significant difference when $P \leq 0.05$.

Polymorphonuclear leukocytes PMNLs (neutrophils) also affected by LPS injection by showing a significant increase in circulating G6 blood samples after 12 hours of injection compared to G3 and G5 at the same time as well as after 6 hours of injection in blood samples of G3, and this effect was linked to average dose, route of injection and type of LPS. Despite the highest elevation in the count of PMNLs was noticed in G6 animals, it exhibited no variations within the groups and the fluctuations in PMNLs count was noticed throughout the study time with no significant variations except after 6 hours, and 12 hours of injection of the same group which showed decline and elevation in number respectively. Meanwhile the intraperitoneal route of injection showed a rise in PMNLs count compared to the intravenous route regardless of the type of LPS whether standard or extracted. Furthermore, low LPS dose gave increases in leukocyte number in comparison with high LPS dose at $P \leq 0.05$. table

Table (6): Neutrophils count in response to ELPS and SLPS at different times and routes

Dose	Route of injection	Experimental groups	Time			Average of dose, route of injection, LPS type	average LPS type Standard vs. Extract	average of Route of injection effect Ip vs. IV	Average Dose effect
			6 h	12 h	24 h				
High	IP	G5	3.30 ± 2.13 cde	6.85 ± 4.73 abcde	7.83 ± 5.46 abcde	5.99 ±4.00 abc	5.58 ±2.76	6.85 ±3.04 *	5.08 ±3.09
		G1	6.13 ± 2.07 abcd	8.83 ± 1.26 abc	4.50 ±3.11 abcde	6.48 ± 2.55 ab			
	IV	G7	3.76 ± 1.32 bcd	5.16 ± 2.89 abcde	4.90 ± 0.20 abcde	4.61 ± 1.71 bc			
		G3	2.23 ±1.95 d	2.70 ± 4.67 de	5.36 ± 1.41 abcde	3.43 ± 3.01 b			
Low	IP	G6	5.36 ± 0.97 bcde	9.76 ± 3.20 a	8.96 ±0.89 ab	8.03 ± 2.66 a	6.00 ±3.27	4.73 ±2.67	6.50 ± 2.79*
		G2	7.90 ± 1.99 abcd	7.46 ± 3.00 abcde	5.93 ± 2.13 abcde	7.10 ± 2.28 ab			
	IV	G8	5.13 ±4.77 abcde	7.23 ± 4.63 abcde	4.83 ± 1.41 abcde	5.73 ± 3.58 abc			
		G4	4.50 ± 1.30 abcde	6.46 ± 2.35 abcde	4.50 ± 1.34 abcde	5.15 ± 1.79abc			
	Time average		4.79 ± 2.57 B	6.82 ±3.55 A	5.85 ±2.59 AB				

-The (G1 and G5) groups received high LPS dose using IP, (G2 and G6) received low LPS dose using IP method; (G3 and G7) groups have received high LPS dose using IV, (G4 and G8) received low LPS dose using IV route.

-The average of LPS type (Standard Vs. Extract), an average of the route of injection effect Ip vs. IV, average dose effect, and time-average indicates the mean of all groups that represented Standard LPS, IP, dose, times vs. the mean of all groups that represented Extracted LPS, IP, dose time.

- All data represented as mean × 10^9/L ± standard deviation of granulocyte cells count.

- (*) means there was a significant difference when $P \leq 0.05$.
- The different letter indicates a significant difference when $P \leq 0.05$.

Discussion:

Innate immunity is preserved phylogenetically as biological defense mechanisms directed toward any foreign material that inter to the body; it plays an important role in activation other specific immunity of the immune system. White Blood cells are an important part of the blood system and consider as the evolutionary innate immune system responsible for response and protect the body from pathogen infection by moving some of its cells (neutrophils) to the infection site for fighting the invading pathogen (20). So, the study of WBCs changes during the LPS challenge gives good information about how innate immunity works.

Our results showed diverse levels of significance between different groups injected with a different type of LPS and with different times and routes, but as a general, there was an initial transient decrease in total WBCs count, Lymphopenia, granulopenia, monocytopenia (midocyte) at 6 hours after LPS injection (time × different dose) which raised after 24 hours. This result was similar to (21) in rats and with that recorded by (22-25). Results of this study differed from that recorded by (26) how noted an increase in neutrophils, lymphocytes, and monocytes after 8 hours in mice injected LPS intraperitoneally, also (19) indicated an increase in WBCs, lymphocytes, and decrease in neutrophils, while the number of basophils and eosinophils changed according to dose concentration during intraperitoneal injection of LPS in mice. Animals in the above study received only a single dose and this explains the most non-significant differences between total and differential WBCs as reported (19). The result of our study is in agreement with (20), who found that the repeated rat treatment with LPS caused a high number of WBCs and lymphocytes which affect immune response either directly or indirectly.

The early transient decrease in total WBCs, lymphocytes, Midocytes, and neutrophils resulted from the administration of LPS which affects the leukocyte adhesion and inflammation process (22). Many studies in animals concluded that LPS rapidly removed from circulation and enter the tissues within minutes which resulted in producing its adviser effect for a long period (27). The intravenous injection of LPS causes increases in adhesion of WBCs to vascular endothelial cells in mesentery microvesicles and an increase in ICAM-1 in plasma (22). The endothelial cells derived TLR4, ICAM-1, ELAM-1 which are associated with L-selectin to mediate leukocyte vascular endothelial interaction and binding of neutrophils to endothelial cells and finally sequestration to endothelium and migration to the inflamed area; in case of LPS injection, the neutrophils response to rechange cytoskeleton and formation of microfilaments and increased the density of neutrophils in pulmonary capillaries. Finally, leukocytes became trapped in pneumonic microcirculation (22, 28, 29), this result augmented the idea that during LPS administration rapid sequestration of WBCs especially neutrophils trapped in narrow lung capillary in animals to protect lung during endotoxemia (27). The further decrease may occur due to apoptosis of WBCs as the effect of LPS mediated by suicide bags in neutrophils that contain internalized LPS which poured its content into neutrophils cytoplasm and induced its death.

The early transient decrease in lymphocytes and midocytes maybe increase the demand of these cells to counter-attack the LPS bad effect which is mediated by LPS /CD14 - TLR4 pathways that cause an increase in endothelial production of pro-inflammatory cytokines as TNFα which chemotaxis the lymphocyte to the LPS site (30). The increase of WBCs, Lymphocytes, midocytes, and granulocytes after 24 hours of LPS injection resulted from LPS ability to produce an inflammatory response (24), and initiation of the immune response as a reply to its immunogen properties (31). After the LPS enters the circulation the pro-inflammatory cytokines increase including IL1-B, IL6, and TNFα (20), and increase of granulocyte / macrophage-

colony stimulating factor which increases differentiation and releases the PMNC from bone marrow. These monokinis can affect the bone marrow and induced more neutrophils, this was mediated by attachment of LPS with cellular receptors and stimulation of bone marrow (27) causing rapid releases of neutrophils to circulation which manifested by increasing band cells and shift to the left of WBCs (28). Also, LPS is a potent mitogen causing lymphocytes to transform into a different group as B lymphocyte or T- helper cell (32). Furthermore, LPS have a positive effect on the life span of lymphocytes and LPS stimulate lymphocytes and monocytes to be further secretion of inflammatory cytokines, so LPS can increase the lymphocytes in peripheral circulation after 24 hours of injection (19), and collectively the LPS injection produces an elevation in WBCs in mice (16).

Results of the current study agree with (31) who recorded a similar effect of LPS extracted from *E. coli* O157:H7 isolated from different animal's sources that affect differential WBCs, the present study indicated that extracted LPS increased lymphocytes, that appear more susceptible to the effect of LPS as well as they are more predominant in circulating in rats (33) and/ or LPS has different mitogenesis activity to lymphocytes (32).

The responses of WBCs, lymphocytes, midocytes in G1 and granulocytes at G6 animals' group was higher than other animal groups and this may be due to the differences between the ELPS and the SLPS structure which affect its attachment with cell surface receptors especially CD14, TLR4 found in neutrophils and lymphocytes, midocytes (34), and without LPS attachment to these receptors, the consequent event is absent or very little (30).

This study showed that a high dose of LPS produces less effect in total WBCs, neutrophils due to its strong toxic effect (31). Also, a similar conclusion was reported by (29) who revealed that a low dose of *E. coli* LPS has a milder toxic effect and lower intensity in the rabbit.

The present study showed that the intraperitoneal route of administration gave more elevation than intravenous in all WBCs, lymphocytes, midocytes, and granulocytes, this result agreed with (33) who studied the effects of intravenous and intraperitoneal routes of administration of LPS on lymphocytes and recorded that both of them produce lymphopenia, but the intravenous route augmented its persistence for more time. One of the principal results in this study was an increase in the phagocytic index (after 6, 12, 24 hours), this agrees with (25) who showed that there was an initial decrease followed by an increase in the phagocytic index after 4 hours when treated piglet with 10 µg/kg of LPS**,** also, a similar increase showed by (19) after 48 hours when using different concentrations of LPS from *Pseudomonas aeruginosa* and reached a high level in a dose of 15mg/kg in mice, while (35) noted an increase in the phagocytic index in alveolar macrophages after different doses used in the intravenous injection. On another hand (36) concluded that a low dose of Leptospira LPS caused increases of the phagocytic index, also (37) showed that a low dose of *E. coli* LPS was able to prime phagocyte at 45 min. A similar result was noted in the current study when a low dose gave an increased phagocytic activity rather than a high dose. All these studied the effect of LPS in different routes and all then induced increase in the phagocytic index, and this led to the conclusion that the route of injection did not affect the phagocytic activity of phagocytes that stimulated with *E. coli* LPS as appeared in the current study.

An increase in the phagocytic index in rats after LPS injection may come from the LPS effect on activation of phagocytes by more than one surface receptor that affects phagocytoses such as CD18 or scavenger receptors or most important LPB protein – CD14 receptors (37). The LPS can activation of complement factors C5a and C3b that are responsible for opsonization and attracting of neutrophils to the site of infection and finally increase of phagocytic index in LPS treated mice (8,19). The significant difference observed between extracted and standard LPS in the current study may be related to the difference in the chemical structure of both types of LPS which is reflected by the different

bands in SDS PAGE occur by the difference between O-polysaccharides, oligosaccharides, and Lipid A of both LPS type (14). A similar observation was reported by (36) who recorded that the chemical structure of LPS may influence the level of macrophage activation.

The bactericidal activity of phagocytes is the most important mechanism for removing the invaders. The current study recorded the increased activity of respiratory bursts of peripheral leukocytes of rats in both experiments (after 6, 12, 24 hours after injection of both types of LPS), this is similar to that recorded by (37). This indicates the direct effect of LPS on stimulation ROSs in white blood cells. The *E. coli* LPS produce ROSs by multi mechanisms, TLR4 which was ligand for LPS mediated activation of downregulation signaling of mitogen-activated protein kinase 3 MKK3 which produce its effect within 20 minutes after LPS exposure, leading to phosphorylation of p38 mitogen-activated protein kinase (MAPK), also the CD14 associated with nucleotide regulatory Giα2 subunit of G proteins which when activated produce activation of p38 MAPK and ERK1/2; these double activation (MKK3, G proteins) resulted in partial phosphorylation of P47phox and p67phox cytoplasmic component of NADPH oxidase and production assembly of NADPH oxidase and finally production of respiratory burst (34,39,40). Another method for generation ROSs under cytokine response such as TNFα which can increase the Ca^{+2} influx and plasma membrane depended on the mobilization of p38 MAPK and lead finally to a conformational change in P47phox and p67phox and increased of NADPH oxidase (40) and TNFα mediated the most systemic effect of LPS including ROSs (41). Bacterial LPS causes inducing TNFα and also nitric oxide (NO) (42). The nitric oxide synthase also requires any exogenous stimulation as LPS even in low dose (43) nitric oxide exaggerates the effect of ROSs by combining with superoxide anion leading to the formation of peroxynitrite ($ONOO^-$) (44). The SLPS give priming action in respiratory burst activation and this may relate to differences in some chemical composition in LPS used. In the study of (45), they noted that Brucella may require 100 more times of LPS to produce similar ROSs that occur by Salmonella LPS, while (46) recorded that rough Brucella cause more oxidative stress than smooth Brucella.

Conclusion

The existing study concluded that the extracted *E. coli* LPS (local strain) was able to elevation the phagocytic and respiratory burst of the blood leukocytes with different effects in low and high doses and routes of injection, also, the total and differential white blood cells. So, the extracted *E. coli* LPS (local strain) can be used to produce innate immunomodulation in rats.

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