

IMMUNE RESPONSE IN *Rattus rattus norvegicus* RATS AGAINST INFECTION WITH GIARDIASIS BY THE LIPOPOLYSACCHARIDE EXTRACTED FROM *Escherichia coli* II. BLOOD PICTURE.

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ABSTRACT

The study investigated, for the first time, the effect of the lipopolysaccharide (LPS) secreted by *Escherichia coli*, as an immunomodulator, against infection with giardiasis caused by *Giardia lamblia* in *Rattus rattus norvegicus*. The pathological changes occurred in the rats treated with LPS, were followed in comparison with the control groups, along the experiments period, depending on many criteria, included the changes in total and differentiated count of leukocytes. The results showed an elevation in the total count of leukocytes, expressed by elevation in neutrophils, variation in monocytes numbers accompanied with a decrease in lymphocytes numbers in treated rats in comparisons with the control groups. Results are provided that (LPS) modulates the immune defence of rats against infection with giardiasis.

الاستجابة المناعية في الجرذ النرويجي ضد الإصابة بداء الجيارديا باستخدام السكر المتعدد الدهني المستخلص من بكتريا *Escherichia coli* II. صورة الدم
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الخلاصة

تتاولت الدراسة الحالية، ولأول مرة، اختبار تأثير السكر المتعدد الدهني LPS المفرز من بكتريا *Escherichia coli*، بوصفه معدلاً مناعياً، في الجرذان النرويجية *Rattus rattus norvegicus* ضد الإصابة بداء الجيارديا لطفيل *Giardia lamblia*. تم متابعة التغيرات المرضية في الجرذان المعاملة بالسكر المتعدد الدهني مقارنة بجرذان السيطرة، طيلة فترة التجارب، وذلك بالاعتماد على عدة معايير متضمنة التغيرات الحاصلة في التعداد الكلي والتفاضلي لكريات الدم البيض. أظهرت النتائج ارتفاع معدل التعداد الكلي لكريات الدم البيض، من خلال ارتفاع أعداد الخلايا العدلة والتباين في أعداد الخلايا الوحيدة الذي صاحبه انخفاض في أعداد الخلايا اللمفاوية في الحيوانات المعاملة مقارنة بمجاميع السيطرة. وقد أثبتت النتائج أن السكر المتعدد الدهني LPS يعدل الدفاع المناعي للجرذان ضد الإصابة بداء الجيارديا.

INTRODUCTION

Giardia duodenalis (also referred to in the literature as *G. lamblia* or *G. intestinalis*) is an enteric protozoan parasite responsible for diarrhoea disease in a variety of host species, including humans. *G. duodenalis* is the most common intestinal parasite worldwide and is ranked in the top 10 parasites of men (1, 2). Giardiasis may cause acute or chronic diarrhoea, dehydration, abdominal discomfort, and weight loss (1-3). Despite the great prevalence of the infection, the patho-physiological processes responsible for giardiasis remain incompletely understood.

Many chemotherapeutic agents have been used, for fighting the disease such as Metronidazole, Albendazole, Nitazoxanide, Tizoxanide, Paromomycin, Tinidazole, Furazolidone, Quinacrine (4, 5). Side effects were noticed accompanied with use of these drugs e.g. dizziness, headache, nausea, abdominal pain and vomiting. Occasionally, treatment fails to eradicate *Giardia*. In such cases, the drug may be changed or a longer duration or higher doses may be used. Combination therapy also may be effective (e.g. quinacrine and metronidazole), (4, 5). Recently, and because of partial success of the mentioned drugs, another line of research was initiated, which is activation or stimulation of the immune system of the host. For this purpose, many substances obtained from different sources, such as fungi, plants and bacteria have been used (6-13). Among these substances are lipopolysaccharides which are derived from bacteria (14-20).

In the present study, immunomodulating activity of LPS, a lipopolysaccharide (endotoxin), secreted from bacteria *Escherichia coli*, is tested, for first time, against infection with giardiasis, the effect of treatment with this lipopolysaccharide, prior to infection, with giardiasis, on the total and differential count of leukocytes, is investigated.

MATERIALS AND METHODS

Bacterial sample: *Escherichia coli* strain was taken from Bacteriology lab/labs department/Al-Salam hospital in Mosul, and diagnosed by api20E technique.

Laboratory animals: 5-6 weeks old, laboratory bred, helminthes-free males of *Rattus rattus norvegicus* rats were used.

Preparation of the lipopolysaccharide (LPS): LPS was extracted from the walls of *Escherichia coli* according to (21), partially purified according to (22, 23). Sugar components of LPS was estimated according to [24], and lipids were estimated according to (25).

Cysts Isolation and Collection: Cysts of *Giardia lamblia* were obtained from infected stool according to (26-29). Cysts approximately 2×10^4 cysts/ml was inoculated orally according to (26).

Treatment Schedules: Six concentrations of LPS (50, 150, 250, 500, 1000, and 2000) μg were used. For each concentration, 30 rats, divided into 5 groups, each with 5 rats, were used, in addition to (-ve) control group (5 rats, for each experiment, neither infected nor treated with LPS) and (+ve) control group (5 rats, for each experiment, infected but not treated with LPS). Treatment protocols were carried out as follows:

Experiments (1-3): Rats were activated with all concentrations of LPS once only 72 hr (experiment 1) before infection, twice and four times at 72 hr intervals (experiments 2 and 3 respectively). The animals were anesthetized with diethyl ether and blood was obtained from the eye according to (30), four times for each experiment, for total and differential count of WBCs according to (31).

Statistical analysis: Duncan's multiple range test was applied to determine the significant differences between means.

RESULTS

Tables (1, 2, 3, 4, 5, 6, 7, 8, and 9) show the effect of LPS on total and differential count of WBCs in rats treated with different concentrations of this material, at different intervals, before infection.

Total WBCs count: In general, the results show a significant increase ($P<0.05$) in the total count of WBCs in animals treated with different concentrations of LPS (tables 1, 2, 3) in comparison with the control group. However, the highest significant increase appeared (27240), when rats treated with LPS twice at 72 hr interval, before infection, (experiment 2, the first week), at the concentration 2000 μg .

Differential WBCs count: A significant decrease in the lymphocytes was noticed, in most experiments, on treatment with the most concentrations of LPS (tables 5, 7, 9), with the maximum decrease at the concentration 50 μg when animals treated once 72 hr (experiment 1), before infection, in the first week of the experiment. This decrease was accompanied by a significant increase in neutrophils and monocytes (tables 4, 6, 8). No significant differences in eosinophiles and basophiles numbers, in treated animals, were noticed when compared with the control group.

Table (1): Effect of LPS on the total count of W.B.C. in rats treated once with LPS 72 hr before infection (experiment 1).

week	(-ve) Contr ol	(+ve) Contro l	Concentration (μg)					
			50	150	250	500	1000	2000
	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.
1	6860 \pm 296.7 k	9480 \pm 327.1i	8980 \pm 807.5j	10600 \pm 316.2h	12780 \pm 756.3fg	11760 \pm 1266.1g h	11380 \pm 511.8h	17930 \pm 319.3bc
2		9020 \pm 901.1j	11300 \pm 578h	17920 \pm 460.4b c	15180 \pm 558.6d	18010 \pm 2097.73 bc	16680 \pm 1961.4c	18990 \pm 665.6b
3		9808 \pm 864.9j	9000 \pm 158.1j	14080 \pm 719def	14080 \pm 804.4d ef	12920 \pm 2536.14 fg	18580 \pm 694.3b	20540 \pm 1295.4a
4		9480 \pm 327.1j	9140 \pm 397.5j	9420 \pm 286.35j	13240 \pm 554.97 ef	13480 \pm 1098.63 ef	15000 \pm 100.00d	14470 \pm 837.9cd

Similar letters indicate no significant differences between means. Different letters indicate significant differences between means.

Table (2): Effect of LPS on the total count of W.B.C. in rats treated twice at 72 hr interval before infection (experiment 2).

Week	(-ve) Control	(+ve) Control	Concentration (µg)					
			50	150	250	500	1000	2000
	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.
1	6860± 296.65e	9480± 327.11de	10140± 240.8de	12340± 740.2b- e	9900± 158.1de	11760± 230.2cde	11300± 339.1cd e	27240± 3723a
2		9020± 901.11de	15380± 443.8a- e	16800± 707.1a- e	26880± 676ab	18080± 258.8a-e	23080± 712.1a-d	13660± 421.9a-e
3		9808± 864.95de	14060± 753.6a- e	18440± 456.1a- e	19950± 254.9a-e	14920± 912.1a-e	20460± 1143.7a- e	11960± 594.1cde
4		9480± 327.1 de	9820± 192.3de	9840± 384.7de	11420± 895.5cde	7860± 606.6a-e	25840± 3594abc	8680± 1923de

Similar letters indicate no significant differences between means. Different letters indicate significant differences between means.

Table (3): Effect of LPS on the total count of W.B.C. in rats treated 4 times at 72 hrs intervals before infection (experiment 3).

Week	(-ve) Contr ol	(+ve) Contro l	Concentration (µg)					
			50	150	250	500	1000	2000
	Mean± SD.	Mean± SD.	Mean± SD.	Mean± SD.	Mean± SD.	Mean± SD.	Mean± SD.	Mean± SD.
1	6860± 297o	9480± 327.1n	9960± 573lmn	10720± 311klm	11740± 240.8j	9460± 343.5n	13320± 1458.4e fg	13780± 286.4de f
2		9020± 901.1n	12040± 1212h	2284± 426.6gh	13220± 286.4fg	11600± 316.2jk	14608± 751.6d	20080± 715.5a
3		9808± 847mn	13128± 1169fg	14340± 836.2de	13892± 349.5def	13220± 303.3fg	20160± 766.8a	20420± 1712.3a
4		9480± 327.1n	10880± 415jkl	13500± 663.3ef	12920± 798.1fgh	13040± 778fgh	16500± 689.2c	18562± 501.7b

Similar letters indicate no significant differences between means. Different letters indicate significant differences between means.

Table (4): Effect of LPS on differential count of granulocytes (neutrophils, eosinophiles, and basophiles) in rats treated once with LPS 72 hr before infection (experiment 1).

Type	(-ve)	(+ve)	Concentration (µg)					
	Control	Control	50	150	250	500	1000	2000
	Mean± SD.	Mean± SD.	Mean± SD.	Mean± SD.	Mean± SD.	Mean± SD.	Mean± SD.	Mean± SD.
neutrophils	17.60± 2.70cd	11.0± 2.35 j	23.0± 3.37a	23.0± 0.71a	10.6± 4.56j	13.8± 3.42d-j	4.0± 2.92abcd	2.8± 1.3f-j
		17.6± 3.36cd	22.6± 1.52a	23.2± 1.92a	18.8± 2.86bc	14.4± 0.55d-j	3.2± 5.2e-j	5.4± 2.61c-h
		12.6± 3.58g-j	21.4± 2.41ab	16.6± 1.34c-g	17.2 ± 48. cde	12.0± 3.46hj	16.8± 1.64c-f	5.0± 1.0c-h
		12.2± 2.05hj	23.6± 4.67a	15.4± 2.51c-h	18.6± 1.14bc	4.8± 1.1d1	6.4± 2.51c-g	17.2± 1.48cde
eosinophil	0.0± 0.0 b	0.2± 0.45ab	0.2± 0.45ab	0.4± 0.55ab	0.0 ± 0.0 b	0.4± 0.5477ab	0.4± 0.55ab	0.0± 0.0 b
		0.2± 0.45ab	0.2± 0.45ab	0.4± 0.45ab	0.6± 0.89ab	0.4± 0.55ab	0.4± 0.55ab	0.6± 0.55ab
		1.0± 1.0ab	0.6± 0.89ab	0.2± 0.55ab	0.4± 0.55ab	0.2± 0.55ab	0.2± 0.55ab	0.4± 0.55ab
		0.8± 0.45a	0.4± 0.55ab	0.2± 0.55ab	0.6± 0.55ab	0.6± 0.55ab	0.4± 0.55ab	0.2± 0.55ab
basophile	0.55± 0.2a	0.0± 0.0 a	0.0± 0.0 a	0.4± 0.55a	0.0± 0.0a	0.0± 0.0a	0.4± 0.55a	0.4± 0.55a
		0.2± 0.55a	0.2± 0.55a	0.2± 0.55a	0.2± 0.55a	0.4± 0.55a	0.4± 0.55a	0.4± 0.55a
		0.0± 0.0a	0.0± 0.0a	0.0± 0.0a	0.2± 0.55a	0.2± 0.55a	0.4± 0.55a	0.6± 0.55a
		0.4± 0.55a	0.0± 0.0a	0.2± 0.55a	0.2± 0.55a	0.6± 0.55a	0.4± 0.55a	0.4± 0.55a

Similar letters indicate no significant differences between means. Different letters indicate significant differences between means.

Table (5): Effect of LPS on differential count of non-granulocytes (lymphocytes, monocytes) in rats treated once with LPS 72 hr before infection (experiment 1).

Type	(-ve) Control	(+) Control	Concentration (μg)					
			50	150	250	500	1000	2000
	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.
lymphocyte	77.2 \pm 3.11a-d	84.2 \pm 4.0a	63.4 \pm 4.34def	66.8 \pm 7.5def	71.8 \pm 2.86ef	80.2 \pm 2.68abc c	79.6 \pm 6.27a-d	72.4 \pm 0.55abc
		72.4 \pm 8.14c-f	64.8 \pm 4.55ef	66.6 \pm 2.51ef	71.8 \pm 2.17a-f	77.2 \pm 1.79ab	80.4 \pm 2.7abc	73.4 \pm 2.88abc
		82.6 \pm 5.37ab	64.6 \pm 5.9def	70.8 \pm 2.59e-a	70.8 \pm 4.66a-f	79.8 \pm 4.0abc	75.6 \pm 3.21a-f	75.6 \pm 3.7abc
		82.6 \pm 1.82ab	65.4 \pm 5.73f	71.6 \pm 4.56ab	70.8 \pm 5.89b-f	78.2 \pm 5.67ab	75.2 \pm 2.49a-d	71.2 \pm 2.49a-d
monocytes	5.0 \pm 1.0cd	4.6 \pm 1.67j	11.6 \pm 1.67a	9.4 \pm 2.19a	8.2 \pm 2.49j	6.4 \pm 0.89d-j	6.0 \pm 3.24d-a	9.6 \pm 2.19f-j
		9.6 \pm 4.5056c	9.4 \pm 2.41a	9.2 \pm 0.45a	8.4 \pm 3.43bc	6.64 \pm 1.95d-j	8.0 \pm 5.05e-j	8.4 \pm 2.6c-h
		3.8 \pm 1.64g-j	9.8 \pm 2.95a	8.0 \pm 1.0c-g	9.0 \pm 4.24cde	7.2 \pm 2.95hj	5.4 \pm 1.95c-f	8.0 \pm 1.22c-i
		5.0 \pm 0.71hj	9.8 \pm 3.27a	7.2 \pm 4.1c-h	9.4 \pm 2.61bc	6.0 \pm 1.41d-i	6.4 \pm 1.34c-g	9.8 \pm 1.3cde

Similar letters indicate no significant differences between means. Different letters indicate significant differences between means.

Table (6): Effect of LPS on differential count of granulocytes (neutrophils, eosinophils, basophils) in rats treated twice at 72 hr interval (experiment 2) before infection.

Type	(-ve) Control	(+) Control	Concentration (μg)					
			50	150	250	500	1000	2000
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Neutrophils	17.6 \pm 2.7cd	11.0 \pm 2.35j	24.0 \pm 1.22a	22.8 \pm 2.59ab	20.2 \pm 3.83j	6.4 \pm 2.7bc	14.8 \pm 1.79d-a	13.6 \pm 3.13f-j
		17.6 \pm 3.36cd	23.6 \pm 3.36a	23.0 \pm 1.0	18.6 \pm 3.21bc	15.4 \pm 1.52d-j	14.8 \pm 1.79d-a	3.4 \pm 3.51c-h
		12.6 \pm 3.58g-j	21.6 \pm 4.39ab	19.2 \pm 2.59a	8.6 \pm 3.85bc	16.2 \pm 3.9hj	5.2 \pm 1.64e-j	14.0 \pm 1.22d-j
		12.2 \pm 2.05hj	22.8 \pm 2.77ab	18.4 \pm 2.7c-g	19.0 \pm 2.55cde	16.4 \pm 3.36d-i	12.8 \pm 1.79c-g	4.8 \pm 3.11cde1
Eosinophil	0.0 \pm 0.0c	0.2 \pm 0.45bc	0.0 \pm 0.0c	0.4 \pm 0.89bc	0.6 \pm 0.55bc	0.4 \pm 0.55bc	0.0 \pm 0.0 bc	0.4 \pm 0.55ab
		0.2 \pm 0.45bc	0.4 \pm 0.89a b	0.2 \pm 0.45ab	bc 0.0 \pm 0.0	0.4 \pm 0.89ab	0.4 \pm 0.89ab	0.6 \pm 0.55bc
		1.0 \pm 1.0a	0.4 \pm 0.55a b	0.8 \pm 0.45bc	0.4 \pm 0.89ab	0.2 \pm 0.45bc	0.2 \pm 0.45bc	0.6 \pm 0.55ab
		0.8 \pm 0.45ab	0.4 \pm 0.55a	0.2 \pm 0.45bc	0.4 \pm 0.55bc	0.2 \pm 0.45bc	0.4 \pm 0.89ab	0.2 \pm 0.45bc
basophil	0.2 \pm 0.45a	a 0.0 \pm 0.0	0.4 \pm 0.55a	0.2 \pm 0.45a	0.0 \pm 0.0a	0.4 \pm 0.55a	0.0 \pm 0.0 a	0.4 \pm 0.55a
		0.2 \pm 0.45a	0.2 \pm 0.45a	0.8 \pm 0.45a	0.2 \pm 0.45a	0.4 \pm 0.55a	0.4 \pm 0.55a	0.4 \pm 0.55a
		0.0 \pm 0.0 a	0.4 \pm 0.55a	0.0 \pm 0.0 a	0.2 \pm 0.45a	0.2 \pm 0.45a	0.0 \pm 0.0 a	0.4 \pm 0.55a
		0.4 \pm 0.55a	0.4 \pm 0.55a	0.2 \pm 0.45a	0.4 \pm 0.55a	0.2 \pm 0.45a	0.2 \pm 0.45a	0.6 \pm 0.55a

Similar letters indicate no significant differences between means. Different letters indicate significant differences between means.

Table (7): Effect of LPS on differential count of non-granulocytes (lymphocytes, monocytes) in rats treated twice at 72 hr interval (experiment 2) before infection.

Type	(-ve) Control	(+ve) Control	Concentration (μg)					
			50	150	250	500	1000	2000
	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.
lymphocyte	77.2 \pm 3.1a-d	84.2 \pm 4.02a	67.6 \pm 4.2def	67.6 \pm 2.5def	67.2 \pm 28.7ef	80.2 \pm 3.9abc	79.2 \pm 2.5abc	82.4 \pm 1.3abc
		72.4 \pm 8.14c-f	67.2 \pm 1.30ef	67.0 \pm 1.22ef	74.2 \pm 2.17a-f	79.0 \pm 3.94abc	79.2 \pm 2.5abc	78.8 \pm 3.6abc
		82.6 \pm 5.37ab	67.6 \pm 1.95def	76 \pm 2.45a-e	74.4 \pm 2.61a-f	81.4 \pm 4.98abc	75.2 \pm 2.77a-f	79 \pm 1.22abc
		81.6 \pm 1.82abc	65.4 \pm 8.35f	78.8 \pm 3.0abc	73.0 \pm 2.74b-f	78.2 \pm 1.8abc	77.4 \pm 4.9a-d	77.4 \pm 3.36a-d
monocytes	5.0 \pm 1.0cd	4.6 \pm 1.67j	9.2 \pm 1.3a	8.6 \pm 1.67a	2.2 \pm 2.59j	5.2 \pm 0.84d-j	6.0 \pm 3.0a-d	4.4 \pm 0.55f-j
		9.6 \pm 4.5cd	9.8 \pm 0.4472a	9.2 \pm 1.79a	6.2 \pm 1.64bc	7.0 \pm 0.71d-j	6.8 \pm 1.3e-j	4.6 \pm 1.52c-h
		3.8 \pm 1.64g-j	10.8 \pm 1.48ab	7.2 \pm 1.3c-g	7.8 \pm 1.64cde	6.2 \pm 1.79hj	7.2 \pm 3.19c-f	7.4 \pm 3.97c-i
		5.0 \pm 0.71hj	11.4 \pm 4.0a	5.4 \pm 0.55c-h	7.8 \pm 2.39bc	4.6 \pm 1.52d-i	5.4 \pm 2.51c-g	5.0 \pm 1.87cd e

*Similar letters indicate no significant differences between means.

**Different letters indicate significant differences between means.

Table (8): Effect of LPS on differential count of granulocytes (neutrophils, eosinophils, basophiles) in rats treated 4 times at 72 hr intervals (experiment 3) before infection.

Type	(-ve) Control	(+ve) Control	Concentration (μg)					
			50	150	250	500	1000	2000
	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.	Mean \pm SD.
Neutrophils	17.6 \pm 2.7cd	11.0 \pm 2.35j	22.6 \pm 3.84a	21.6 \pm 5.18a	17.4 \pm 2.510j	14.0 \pm 4.58d-j	15.0 \pm 4.58a-d	18.4 \pm 4.28f-j
		17.6 \pm 3.36cd	25.0 \pm 2.0a	22.6 \pm 2.51a	19.0 \pm 1.41bc	17.4 \pm 2.19d-j	14.0 \pm 3.32e-j	18.4 \pm 4.1c-h
		12.6 \pm 3.58g-j	23.4 \pm 3.58ab	19.8 \pm 2.35a	18.4 \pm 2.88cde	14.0 \pm 3.56hj	20.0 \pm 3.39c-f	17.0 \pm 5.61c-f
		12.2 \pm 2.05hj	23.2 \pm 2.77ab	18.4 \pm 1.34c-g	17.2 \pm 4.87bc	15.8 \pm 4.38d-i	19.0 \pm 3.39c-g	18.6 \pm 3.8cde
Eosinophil	0.0 \pm 0.0a	0.2 \pm 0.45a	0.4 \pm 0.55a	0.2 \pm 0.45a	0.2 \pm 0.45a	0.2 \pm 0.45a	0.2 \pm 0.45a	0.2 \pm 0.55a
		0.2 \pm 0.45a	0.2 \pm 0.45a	0.6 \pm 0.55a	0.2 \pm 0.45a	0.4 \pm 0.55a	1.4 \pm 2.1a	0.2 \pm 0.45a
		1.0 \pm 1.0ab	0.4 \pm 0.45a	0.2 \pm 0.45a	0.2 \pm 0.45a	0.2 \pm 0.45a	0.2 \pm 0.45a	0.8 \pm 0.45a
		0.2 \pm 0.45a	0.4 \pm 0.45a	0.2 \pm 0.45a	0.2 \pm 0.45a	0.2 \pm 0.45a	0.2 \pm 0.45a	0.4 \pm 0.89ab
Basophile	0.2 \pm 0.45b	0.0 \pm 0.0a	0.2 \pm 0.45ab	0.2 \pm 0.45ab	0.2 \pm 0.45a	0.0 \pm 0.0ab	0.0 \pm 0.0b	0.4 \pm 0.55ab
		0.2 \pm 0.45ab	0.2 \pm 0.45ab	0.4 \pm 0.45ab	0.2 \pm 0.45ab	0.2 \pm 0.45ab	0.2 \pm 0.45ab	0.2 \pm 0.45ab
		0.0 \pm 0.0b	0.2 \pm 0.45ab	0.2 \pm 0.45ab	40.0 \pm 0.55ab	0.2 \pm 0.45ab	0.0 \pm 0.0b	0.6 \pm 0.55ab
		0.4 \pm 0.55ab	0.2 \pm 0.45ab	0.2 \pm 0.45ab	0.0 \pm 0.0ab	0.0 \pm 0.0ab	0.0 \pm 0.0ab	0.2 \pm 0.45ab

Similar letters indicate no significant differences between means. Different letters indicate significant differences between means.

Table (9): Effect of LPS on differential count of non-granulocytes (lymphocytes, monocytes) in rats treated 4 times at 72 hr intervals (experiment 3) before infection

Type	(-ve) Control	(+ve) Control	Concentration (µg)					
			50	150	250	500	1000	2000
	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.
lymphocyte	77.2± 3.11b-c	84.2± 4.02a	68.6± 2.8g-h	69.2± 3.4fgh	71.8± 2.1e-h	76± 3.5b-e	79.8± 1.3abc	82± 3.74ab
		72.4± 8.14d-h	68.4± 2.0fgh	68.6± 2.0fgh	72± 4.4e- h	78.2±1.9 a-d	79± 1.8abc	82.4± 5.5abc
		82.6± 3.57ab	71.2± 5.9e-h	73.2± 1.2fgh	72.4± 4.5d-h	74.2±7.4 c-f	79.4± 1.6abc	79.0± 2.7abc
		81.6± 1.82ab	67.8± 5.6h	70.2± 2.86fgh	74± 2.3c- g	77.2±1.7 9b-c	814.2±3.7 ab	80.6± 4.61d-a
monocytes	5.0± 1.0cd	1.67± 4.6j	7.9± 1.8a	7.4± 1.52a	7.6± 1.82j	6.4± 2.7d-j	5.2± 0.84d=a	3.6± 1.34f-j
		9.6± 4.51cd	7.4± 1.82a	7.4± 2.07a	8.4± 2.88bc	5.8± 2.28d-j	5.8± 3.11e-j	3.8± 1.64c-h
		3.8± 1.64g-j	7.6± 1.67ab	7.0± 1.41c-g	6.36± 1.82cde	9.2± 4.15hj	5.0± 1.0c-f	5.2± 1.79c-d
		5.0± 0.71hj	8.8± 3.96a	10.0± 3.39c-h	6.8± 1.1bc	6.2± 4.44-d	3.4± 0.89c-g	4.0± 1.58cd

Similar letters indicate no significant differences between means. Different letters indicate significant differences between means.

DISCUSSION

In this study, we investigated the effect of lipopolysaccharide extracted from *Escherichia coli* on the total and differential count of leucocytes in *Rattus rattus norvegicus* rats treated with LPS prior to experimental infection with *Giardia* cysts, and to ensure that this lipopolysaccharide modulates the immune response of this host against infection with giardiasis. This lipopolysaccharide, found to be immunogenic and mitogenic for many immune cells such as B cells and T cells (14, 32, 16, 33, 19, and 20). Elevation in the total count of leucocytes observed in the present study, may be attributed to the specific mitogenic effect of LPS for the main immune cells (34, 16). Other studies found also that injection of LPS causes an increase in leucocytes of cerebrospinal fluid (35). Furthermore, LPS of *E. coli* stimulates accumulation of polymorphonuclear cells (PMNCs) in body fluids (36). This elevation is similar to that reported by (32, 9, 12) on the total leukocyte

count in rats treated with LPS extracted from 173a, and in mice treated with LPS extracted from *Pseudomonas aeruginosa*, respectively. On the other hand, a general reduction in the total count of leucocytes in the control group, observed in the present study, may be explained by increased infiltration of intraepithelial lymphocytes (IEL), which has been associated with giardiasis in a number of reports (37-39). Intriguingly, this increase was reported in immunocompetent as well as in athymic animals infected with *Giardia* (40), suggesting a role for extrathymic differentiation in this response. Recent report showed that the acute phase of *G. duodenalis* infection in mice is accompanied by an increase of intraepithelial and Lamina propria T-lymphocytes belonging to CD8+ subset (41). However, the significant increase in the number of neutrophils in rats treated with LPS, prior to infection, observed in the present study, in comparison with the control group, indicates the stimulation of production of these cells as phagocytic cells, have been enhanced by the lipopolysaccharide (42,14). These results are in agreement with [32] in rats treated with LPS extracted from *E. coli*173a, and with (19, 20). The variation of monocytes number in treated animals may be attributed to the diversity of LPS concentrations used, number of doses given and time of infection.

In control group, a decrease in neutrophils and monocytes number is attributed to the migration of these cells to the infection site. This result is in agreement with (43), in the mouse model of disease, who observed that invading *G. muris* trophozoites were found in the epithelium near dying or desquamating columnar cells, furthermore Macrophages beneath the basal lamina extended pseudopose into the epithelium, trapping invading *G. muris* trophozoites and enclosing them in phagolysosomes.

In general, it may well be concluded that LPS of *E. coli* can modulate immune response or defence of the host effectively against infection with giardiasis caused by *Giardia lamblia*.

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