INFLUENCE OF BACTERIAL ENDOTOXINS ON BONE MARROW AND BLOOD COMPONENTS

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SUMMARY: Endotoxin (Lipopolysaccharide, LPS) a component of the bacterial wall of gram-negative bacteria, has been recognized as one of the most potent bacterial products in the induction of host inflammatory responses and tissue injury and was used in this study to mimic infections. LPS induces production and release of several cytokines. In response to these cytokines, different effects of endotoxins are seen.

The effect of three types of endotoxins (Escherichia coli, Klebsiella pneumoriae and Salmonella typhimurium) on bone marrow, differential counts and peripherial blood parameters were investigated in adult rats.

Male spraguo Dawely albino rats weighing 220 - 250 g were used. They were injected i.p. (1 mg/kg body weight) with single dose of 3 types of endotoxins. Blood samples were collected from the experimental animals at 24 and 72 hours of the injection. At 72 hours the bone marrow aspirations were harvested from the femur of the rats for microscopic examination.

Endotoxins induced different changes in the cells of bone marrow. Also, lipopolysaccharide caused significant decreases in red blood cells, white blood cells and platelets counts, hemoglobin content and hematocrit percent.

Data of the present study point out to the dose of these toxins according to suitable pharmacopeia. Lemulus amebocyte lysate (LAL) test is specificly used for determination of the endotoxin limit. This recommendation should be observed to avoid the toxic effects of endotoxins.

Key Word: Endotoxins.

INTRODUCTION

Bacteria express many different surface antigens and secrete a variety of virulence factors (e.g. toxins) which may trigger immune responses. Bacterial exotoxins and endotoxins are important in the pathogenesis of specific diseases. Exotoxins are noxious proteins that are secreted by many bacteria. Endotoxins however are somatic lipopolysaccharide-protein complexes. These complex antigens are located in the outer membrane of all gram-negative bacteria (21).

The presence or absence of hematologic changes in laboratory animals exposed to environmental chemicals or new pharmaceutical agents are important elements in the overall assesment of the risks and hazards of potential human or animal exposure. As blood and bone marrow are complex mixtures of cells that respond in different ways to various toxicologic insults (6,24).

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Bone marrow analysis can be a valuable aid for diagnosis, prognosis and for monitoring of various conditions and diseases (4,9,27,44). Successful use depends on an adequate collection, smear preparation and staining (4,6,38).Thus bone marrow aspiration is the most commonly used technique (15,16,43,54).

Thomas *et al.* (50) indicated that the monokines may participate in the regulation of hematopoiesis and circulating numbers of leukocytes during chronic inflammation. It has been found that tumor necrosis factor (T.N.F.) as one of the monokines which induces daily neutrophilia and lymphopenia. T.N.F. also induces a slight decrease in early myeloid forms in the marrow.

Livingston *et al.* (31) reported that the proliferation of white blood cells is an important and necessary response to bacterial infection. The effects of hemorrhagic shock and Lipopolysaccharide (LPS) administration on myelopoisis were investigated. They observed that the hemorrhagic shock had no effect on bone marrow. Also, Livingston *et al.* (32) described that the bone marrow white blood cell counts were unaffected by shock or LPS administration.

Dubois *et al.* (11) noticed that the ability of interleukin-1 to enhance granulocyte differentiation *in vivo* is partly due to its ability to induce a cascade of cytokines and steroids which in turn regulate interleukin-1 receptors expression.

Bozza *et al.* (7) reported that intrathoracic injection of LPS (250 ng/cavity) induced a marked increase in the number of neutrophils at 1 hr, which reach to maximum within 6-12 hours and reduced after 24 hours. In parallel, an increase in blood neutrophil counts within 1-6 hr, revealed concomitant reduction in the number of these cells in the bone marrow. No change in blood or bone marrow eosinophil count was detected. However, the blood neutrophilia and the decrease in marrow neutrophil counts induced by intravenous injection of LPS (250 ng) were significantly lower than those observed after intrathoracic injection.

Tanaka *et al.* (49) observed that the megakaryocytes contribute to random selection of an excess of neutrophils in the bone marrow of rats treated with LPS.

William (53) noticed that the bone marrow pattern shows an increase [Myeloid: Erythroid (M:E) ratio] in chronic infections.

Administration of endotoxins to experimental animals results in a variety of pathophysiologic changes such as systemic hypotension and plumonary hypertension, as well as hematologic changes, manifested as a decrease in circulating leukocytes and platelets (8,13, 14,48).

Many studies have reported the existance of several hematological changes in experimental endotoxemia. Lambalgen *et al.* (30); Egan *et al.* (12); Hawes *et al.* (17); Opdah *et al.* (39); Kitajima *et al.* (28); Shibayama *et al.* (46); Pearson *et al.* (40); Kanayama *et al.* (26); Pham *et al.* (41) and Kosumi *et al.* (29) showed that the administration of endotoxin from gram-negative bacteria to rats caused systemic hypotension, an increased hematocrit and decreased numbers of circulating leukocytes, monocytes and platelets. Conversely, Metcalf *et al.* (36) indicated that LPS caused a progressive decrease in hematocrit value of rats.

Magee and Beely (34) reported that thrompocytopenia may be due to reduced production in bone marrow and increased destruction in the peripherial circulation. The mechanism of this drug-induced blood discrasia is either selective marrow depression and/or an immune reaction.

In contrast, Altenburg *et al.* (1) indicated that the treatment of rats with single dose (250 mg/kg) of LPS caused a dramatic increrase in number of circulating neutrophils concomitant with a decrease in the number of these cells in the bone marrow.

Andonova *et al.* (3) showed that the experimental endotoxemia was provoked via i.p. injection of 1 mg *E.coli* LPS/kg in rat (group A). Indomethacin was introduced (2.5 mg/kg) 30 min prior to LPS chalenge (group B). Also, the last authors reported that the dynamics of hematocrit and erythrocyte counts were similar in both groups with a decrease up to the second hr followed by an increase to maximum at post-treatment day 3.

The prolongation of prothrombin time was induced by the i.v. infusion of LPS. However, the prolongation of activated partial thrombin time and the decrease of platelet counts were suppressed after the i.v infusion of LPS (55).

This study has been conducted to investigate the toxic effects of treatment by three types of bacterial endotoxins (*Escherichia coli, Klebsiella pneumoriae* and

Salmonella typhimurium) on bone marrow differential counts and peripherial blood parameters including erythrocytes, leukocytes (total and differential) and platelets count and hemoglobin content and hematocrit percent.

MATERIALS AND METHODS

Experimental Animals

For this study, fourty healthy adult male Sraguo Dawely albino rats were used. Animals were obtained from the animal house of National Organization For Drug Control and Research Cairo - Egypt, weighing between 220 and 250 g. They were fed with standard diet and preacclimated for one week before using.

Chemicals

Three types of endotoxins (lipopolysaccharide; LPS) were used in this investigation. *Escherichia coli* endotoxin (LPS) serotype 055:B5, *Salmonella typhimurium* and *Klebsiella pneumoniae* were obtained from Sigma-Aldrich Chem. (Steinheim, Germany). All the previous endotoxins (LPS's) were used as lypholized powder prepared by phenol extraction. These types of endotoxins had to be dissolved in normal saline (sterile and pyrogen free 0.9 % NaCl) (El Nassr company) at pH 7.2 before they were injected.

Groups of animals under investigation and plan of endotoxins injection

Animals were divided into four main groups. Each group consisted of 10 rats.

Group 1: This group is the control animals. They were injected intraperitoneally (i.p.) with 0.9% normal saline (1 mg/kg body weight).

Group 2: The animals of this group received i.p. single dose of *Eshcerichia coli* endotoxin (Lipopolysaccharide, LPS) (1 mg/kg body weight).

Group 3: Rats of this group were injected i.p. with a single dose of *Klebsiella pneumoniae* endotoxin (1 mg/kg body weight).

Group 4: Animals of this group were injected i.p. with a single dose of *Salmonella typhimurium* endotoxin (1mg/kg body weight).

Blood and tissue sampling

Blood was collected from orbital venous plexus from each animal of the control and treated groups after 24 and 72 hours.

For determination of hematological parameters, such as red blood cells, white blood cells, platelets, hemoglobin, hematocrit and differential white blood cells count, a part of blood was collected into tubes containing dried 1 mg of EDTA/ml blood (53) and gently mixed.

After blood sampling (72 hours later), the animals were killed by using chloroform anaesthesia and dissected to obtain the femur for examination of the bone marrow.

Examination of bone marrow smears

Preparation of films of post-mortem bone marrow

Films made of bone marrow obtained post-mortem are seldom satisfactory. Berenbaum (5) described how the blood cells are much better preserved if the marrow is suspended in albumin before the films are made. He recommended that a small piece of marrow suspended in 1-2 ml of 5% bovine albumin (1 volume 30% albumin and 5 volume 0.9% NaCl). The suspension is then centrifuged and the deposited marrow cells are suspended in a volume of supernatant approximately equal to, or slightly less than that of the deposite. Films are made of this suspension in the usual way (23). Bone marrow smears were made and air dried, and stained with Leischman's stain for 5 minutes, then diluted with distilled water for 10 minutes and left to air dry. Examination of the bone marrow films were carried out using light microscope with an oil immersion lens. At least, 100 cells were counted in each slide.

Determination of hematological parameters

Hematological parameters including hematocrit and blood corpuscles and platelets count were carried out using the methods adapted by Simmons and Bernard (47). Hemoglobin (Hb) content was determined by using Randox reagent kits (Colorimetric method) according to the instruction manual (19,25).

Statistical analysis

The data obtained in the present work are represented in tables as mean ± standard error. The statistical analysis of experimental results was carried out by using one way analysis of variance (ANOVA) and F-test followed by Student's t-test. Thus the data presented, can be statistically evaluated. P value of less than 0.05 being considered to be statistically significant.

RESULTS

Bone marrow cells

The data of bone marrow cells of control and of endotoxins-treated rats are shown in Tables 1 and 2.

Normoblasts

The results showed that following injection of LPS (1 mg/kg body weight), normoblast cells significantly ($p<0.001$) decreased at 72 hours in the three groups of endotoxins. The percentages of control values recorded were 40.96%, 32.60% and 48.46% after 72 hours of *E. coli*, *K. pn.* and *S. ty.* post-treatment, respectively. An ANOVA performed on the normoblast results from all

Table 1: Effect of an intraperitoneally single dose of 3 types of bacterial endotoxins (1 mg/kg body weight) after 72 hr on 6 types of bone marrow cells in adult male albino rats.

Bone marrow cells	Groups	Mean ± S.E.M.	% of control	F-value	Significant difference between all groups
Normoblasts(S)	Control	22.70 ± 1.54	-	34.43	0.001#
	E. coli	9.30 ± 0.76	40.96***		
	K. pn.	7.40 + 1.27	32.60***		
	S. ty.	11.00 + 0.98	48.46***		
Blasts(S)	Control	0.50 ± 0.17	-	3.00	0.040#
	E. coli	1.00 ± 0.21	200.00*		
	K.pn.	1.30 ± 0.2	260.00**		
	S. ty.	0.70 + 0.21	140.00		
Promylocytes(S)	Control	1.80 ± 0.25	-	2.01	0.129 #
	E. coli	2.80 ± 0.44	155.56*		
	K. pn.	2.20 ± 0.20	122.22		
	S. ty.	2.20 ± 0.20	122.22		
Myelocytes(S)	Control	13.50 ±1.05	-	10.95	0.0001#
	E. coli	19.90 ± 1.08	147.41***		
	K. pn.	16.50 ± 0.69	122.22*		
	S. ty.	14.20 ± 0.55	105.19		
Juveniles(S)	Control	5.30 ± 0.34	188.78***	23.92	0.0003#
	E. coli	10.00 ± 0.63	235.85***		
	K. pn.	12.50 ± 0.95	222.64***		
	S. ty.	11.80 ± 0.59			
Staffs(S)	Control	7.50 ± 0.72	-	10.81	0.0004 #
	E. coli	11.10 ± 0.78	148.00**		
	K. pn.	12.50 ± 0.43	166.67***		
	S. ty.	12.00 ± 0.76	160.00***		

(s): Number of specific type of bone marrow cells per 100 cells bone marrow.
Values represent the mean number of cells ± S.E.M. of 10 rats per group.
Statistically significant from normal control: *p < 0.05; **p < 0.01 and ***p < 0.001 by using *t-test* followed by least significant difference (L.S.D.) at p < 0.05.
#: There is a significant difference between all groups by using one way ANOVA (*F-test*) at p < 0.05.

groups revealed a significant treatment effect (F=34.43, p<0.001) (Table 1).

Blasts

Considering the effect of the 3 types of endotoxins at single dose on blast cells of bone marrow, it was found a significant (p<0.05 and p<0.01, respectively) increase occurred after 72 hours in rats treated with *E. coli* and *K. pn.*; where an elevation of 100% and 160% was noted respectively. However, a non-significant (p>0.05) increase of 40% was recorded in blast cells after 72 hours from *S. ty.* injection (Table 1). ANOVA revealed a significant treatment effect (F= 3.00, p<0.04) (Table 1).

Promyelocytes

The effect of injection of the 3 types of bacterial endotoxins on promyelocyte of bone marrow in rats was studied. The data obtained showed a significant (p<0.05) increase of 55.56% after 72 hours of *E. coli* post-treatment. However, non significant (p>0.05) increases of 22.22% and 22.22% was recorded in number of promyelocytes after 72 hours of *K. pn.* and *S. ty.* injections respectively. An overall ANOVA of the

promyelocyte data revealed a non-significant treatment effect (F=2.01, p<0.129) (Table 1).

Myelocytes

The data recorded in this work indicating highly significant (p<0.001) increase of 47.41% in myelocyte cells of bone marrow after 72 hours of *E. coli* post- treatment. However, the number increased significantly (p<0.05) by 22.22% after *K. pn* post-injection. The myelocyte cells of bone marrow showed non-significant (p>0.05) increase after 72 hours of *S. ty.* post-treatment. The value of control was 105.19%. An ANOVA performed on the myelocyte cells resulting from all groups revealed a significant treatment effect (F=10.95, p<0.0001) (Table 1).

Juveniles

The present data of treated animals with endotoxins, showed that juvenile cells significantly (p<0.001) increased in all treated groups after 72 hours of injection. The percentages of control values recorded were 188.78% in *E. coli*, 235.85% in *K. pn.* and 222.64% in *S. ty.* treated rats (Table 1). ANOVA revealed significant treatment effect (F=23.92 , p<0.0003) (Table 1).

Staffs

Considering the effect of bacterial endotoxins single doses on staff cells of bone marrow, it was found significant (p<0.01) increase occurred after 72 hours in rats treated with *E.coli*. Also, significant (p<0.001) increases were recorded in the groups of animals injected with *K. pn.* and *S. ty.* The results obtained showed increases of 48.00% , 66.67% and 60.00% after 72 hours of *E.coli*, *K. pn.* and *S. ty.* injection respectively. An ANOVA performed on the staff cells resulting from all groups revealed a significant treatment effect (F=10.81, p<0.0004) (Table 1).

Segmented

The effect of injection of 3 types of LPS on segmented cells of bone marrow in rats was studied. The results exhibited significant (p<0.001) increases of 48.22%, 60.99% and 53.19% after 72 hours in the groups of animals injected with *E. coli*, *K. pn.* and *S. ty.* respectively. ANOVA revealed a significant effect of treatment (F=17.10, p<0.0001) (Table 2).

Lymphocytes

The results showed that following i.p. injection of endotoxins (1 mg/kg body weight), lymphocyte cells significantly (p<0.001) decreased in rats treated with *E. coli*. Also, the lymphocyte significantly (p<0.01) decreased in both groups of animals injected with *K. pn.* and *S. ty.* endotoxins. The control values were 73.41%, 79.36% and 76.19% after 72 hr of *E. coli*, *K. pn.* and *S. ty.* injection respectively. An overall ANOVA of the lymphocyte data revealed a significant treatment effect (F=5.30, p<0.004) (Table 2).

Monocytes

Considering the effect of bacterial endotoxins injection on monocyte cells of bone marrow, it was noticed that the number of cells exhibited non-significant (p>0.05) increases at 72 hours of the 3 types of endotoxins treatment. The percent of control values recorded were 130.07%, 134.62% and 103.85% in the groups of rats injected with *E. coli*, *K. pn.* and *S. ty.* after 72 hours post-treatment respectively. Endotoxins treatment failed to modify the response of monocytes of bone marrow (F=2.48, p<0.080) (Table 2).

Eosinophils

The administration of bacterial endotoxins led to significant (p<0.001) decrease in eosinophil cells of bone marrow after 72 hours from injection of *E. coli* and *K. pn.* Also, the number of these cells was significantly (p<0.01) decreased in *S. ty.* treated group. The control values were 35.90%, 15.38% and 56.41% after 72 hours of *E. coli*, *K. pn.* and *S. ty.* post-treatment respectively. An ANOVA performed on the eosinophils resulting from all groups revealed significant treatment effects (F=13.31, p<0.0003) (Table 2).

Myeloid: Erythroid (M:E) Ratio

The effect of bacterial endotoxins on the ratio of Myeloid: Erythroid (M:E) of bone marrow was examined in the present work. The results revealed the occurrence of highly significant (p<0.001) increases in the M:E ratio showing values of 233.33%, 438.09% and 190.48% in relative to control value after 72 hours of *E. coli*, *K. pn.* and *S. ty.* post-treatment respectively. ANOVA of M:E ratio revealed significant effect of treatment (F= 9.98, p<0.0001) (Table 2).

Table 2: Effect of an intraperitoneally single dose of 3 types of bacterial endotoxins (1 mg / kg body weight) after 72 hr on 4 types of bone marrow cells in adult male albino rats.

Bone marrow cells	Groups	Mean ± S.E.M.	% of control	F-value	Significant difference between all groups
Segmented(S)	Control	14.10 ± 1.13	-	17.10	0.0001#
	E. coli	20.90 ± 1.52	148.22***		
	K. pn.	22.70 ±1.10	160.99***		
	S. ty.	21.60 ± 0.91	153.19***		
Lymphocytes(S)	Control	25.20 ± 1.53	-	5.30	0.004 #
	E. coli	18.50 ± 1.26	73.41***		
	K. pn.	20.00 ± 1.43	79.36**		
	S. ty.	19.20 ± 1.02	76.19**		
Monocytes(S)	Control	2.60 ± 0.37	-	2.48	0.080
	E. coli	3.40 ± 0.22	130.07		
	K. pn.	3.50 ± 0.27	134.62		
	S. ty.	2.70 ± 0.30	103.85		
Eosinophils(S)	Control	7.80 ± 1.26	-	13.31	0.0003#
	E. coli	2.80 ± 0.36	35.90***		
	K. pn.	1.20 ± 0.47	15.38***		
	S. ty.	4.40 ± 0.67	56.41**		
M:E Ratio	Control	2.10 ± 0.28	-	9.98	0.0001#
	E. coli	7.00 ± 1.06	333.33***		
	K. pn.	11.30 ± 1.90	538.09***		
	S. ty.	6.10 ± 0.76	290.48***		

(S): Number of specific type of bone marrow cells per 100 cells bone marrow.

Values represent the mean number of cells ± S.E.M. of 10 rats per group.

Statistically significant from normal control **p<0.01 and ***p<0.001 by using *t-test* followed by least significant difference (L.S.D.) at p<0.05.

#: There is a significant difference between all groups by using one way ANOVA (*F-test*) at p<0.05.

Blood Components

Results of the blood components of control and endotoxins-treated animals are shown in Tables 3 and 4.

A. Blood Cells Count

Red Blood Cells (RBCs)

Considering the effects of 3 types of bacterial endotoxins on red blood cells, it was found that a significant (p<0.001) decrease occurred after 24 and 72 hours of injection. The control values were 85.75% (*E. coli*), 89.09% (*K. pn.*) and 89.09% (*S. ty.*) at 24 hours of injection. Also, the red blood cells recorded values (expressed as percentage of control) were 64.81%, 66.37% and 68.82% at 72 hours in rats treated by *E. coli*, *K. pn.* and *S. ty.* respectively. ANOVA revealed significant differences between all groups at 24 and 72 hours (F=24.30, p<0.001 and F=63.96, p<0.003 respectively) (Table 3).

White Blood Cells (WBCs)

The data showed that following injection of LPS (1mg/kg body weight, i.p.), white blood cells count was significantly (p<0.001) decreased. The percentages of control values recorded 35.71%, 30.86% and 29.21% after 24 and 72 hours of three types of endotoxins (*E. coli*, *K. pn.* and *S. ty.* respectively). Also, the white blood cells indicated significant (p<0.001) decreases of 72.45%, 76.68% and 79.46% in relative to control after

Table 3: Effect of an intraperitoneally single dose of 3 types of bacterial endotoxins (1 mg/kg body weight) after 24 and 72 hours on red blood cells (RBCs)+ white blood cells (WBCs)++ and platelets +++ count, hemoglobin$ content and hematocrit percent in adult male albino rats.

Blood	Time (hours)	Animal groups								
		Control	Treated							
			E. coli		*K. pn.*		*S. ty.*		F-value	Significant difference between all groups
		Mean ± S.E.M	Mean ± S.E.M	% of control	Mean ±S.E.M	% of control	Mean ± S.E.M	% of control		
Red blood cells	24	4.49±0.06	3.85±0.07	85.75***	4.00±0.03	89.09***	4.00±0.07	89.09***	24.30	0.001 #
	72	4.49±0.06	2.91±0.12	64.81***	2.98±0.07	66.37***	3.09±0.11	68.82***	63.96	0.003 #
White blood cells	24	9.69±0.21	3.46± 0.1	35.71***	2.99±0.14	30.86***	2.83±0.17	29..21***	434.24	0.001 #
	72	9.69±0.21	2.67± 0.12	27.55***	2.26±0.23	23.32***	1.99±0.20	20.54***	356.45	0.001 #
Blood platelet	24	452.70±10.09	97.70±5.96	65.76***	359.10±11.13	79.32***	224.60±8.95	49.61***	109.35	0.001 #
	72	452.70±10.09	40.80±6.60	53.19***	290.40±9.19	64.14***	191.20±6.04	42.23***	193.16	0.002 #
Hemoglobin	24	13.59±0.16	11.66±0.20	85.80 ***	12.11±0.09	89.11***	12.02±0.22	88.45 ***	24.40	0.001 #
	72	3.59±0.16	8.83±0.35	54.97 ***	8.82±0.24	64.90***	9.28±0.35	68.29 ***	65.06	0.003 #
Hematocrit percent	24	40.70±0.47	35.10±0.62	36.24 ***	35.30±1.29	86.73***	36.20±0.68	88.94 ***	10.07	0.001 #
	72	40.70±0.47	7.10±0.108	6.59 ***	26.70±0.72	65.60***	27.90±0.97	68.55 ***	64.11	0.004 #

+ : Red blood cells (RBCs) ($x10^6/mm^3$); ++ : white blood cells (WBCs) ($x10^3/mm^3$) and +++ : platelets ($x10^3/mm$); $:hemoglobin content (g/dL) and ¥:hematocrit percent (%).

Values represent the mean number of RBCs ($x10^6/mm^3$); WBCs ($x10^3/mm^3$) and platelets ($x10^3/mm^3$); hemoglobin content (g/dL) and hematocrit percent (%) ± S.E.M. of 10 rats per group. Statistically significant from normal control : ***p<0.001 by using *t-test* followed by least significant difference (L.S.D.) at p<0.05.

: There is a significant difference between all groups by using one way ANOVA (*F- test*) at p<0.05.

72 hours of *E.coli*, *K. pn.* and *S. ty.* administration, respectively. An overall ANOVA of white blood cells data revealed a significant treatment effects at 24 and 72 hours (F=434.24, p<0.001 and F=356.45, p<0.001, respectively) (Table 3).

Differential White Blood Cells (WBCs)

a) Absolute Segmented Cells: Injection of three types of endotoxins showed a significant (p<0.01) decrease (*E. coli*) and significant (p<0.001) decreases (*K. pn.* and *S. ty.*) in the segmented cell numbers of WBCs as evidenced from their values (expressed as a percentage of control) 75.43%, 68.38% and 71.97% after 24 hours of injection. Moreover, the decrease in the segmented cells was remained after 72 hr of injection. The values (expressed as percentage of control) were 63.97%, 44.44% and 44.79% in rats treated with *E. coli*, *K. pn.* and *S. ty.* respectively. ANOVA revealed a significant treatment effect after 24 and 72 hr (F=8.62, p<0.001 and F=19.29, p<0.003, respectively) (Table 4).

b) Absolute Staff Cells: The response of blood cells for the action of bacterial endotoxins showed non-significant (p>0.05) increase in the number of staff of WBCs after 24 of endotoxins injection. Conversely, non-significant (p>0.05) decrease in the number of staff cells were seen after 72 hr of *E. coli*, *K. pn.* and *S. ty*. injection. The values expressed as percentage of control were 134.12%, 105.88% and 120.00% after 24 hr of *E. coli*, *K. pn.* and *S. ty.* post-treatment. Also, after 72 hr, the staff number of WBCs showed 91.37%, 85.0% and 81.19% in rats treated with *E. coli*, *Kpn* and *Kpn* respectively. ANOVA of the staff data revealed non-significant treatment effect after 24 and 72 hr (F= 0.70, p<0.557 and F= 0.19, p<0.898) (Table 4).

c) Absolute Lymphocyte Cells: The study of injection effect of endotoxins on lymphocyte number of WBCs

Table 4: Effect of an intraperitoneally single dose of 3 types of bacterial endotoxins (1 mg/kg body weight) after 24 and 72 hours on differential white blood cells (segmented; staff; lymphocyte; monocyte and eosinophil) in adult male albino rats.

Differential white blood cells	Time (hours)	Animal groups								
		Control	Treated							
			E. coli		*K. pn.*		*S. ty.*		F-value	Significant difference between all groups
		Mean ± S.E.M	Mean ± S.E.M	% of control	Mean ± S.E.M	% of control	Mean ± S.E.M	% of control		
Segmentel(S)	24	2340.00±148.24	1765.00±74.15	75.43**	1600.00±04.82	68.38***	1684.00±117.12	71.97***	8.62	0.001 #
	72	2340.00±148.24	14797.00±122.89	63.97***	1040.00±109.23	44.44***	1048.00±116.62	44.79***	19.29	0.003 #
Staff(S)	24	255.00±21.31	342.00±27.56	134.12	270.00±l9.49	105.88	306.00±29.63	120.00	0.70	0.557
	72	255.00±21.31	233.00±18.38	91.37	217.00±29.74	85.09	209.00±22.63	81.19	0.19	0.898
Lymphocyte(S)	24	6078.00±310.72	852.00±40.49	14.02***	799.00±52.79	13.15***	568.00±53.54	9.35***	275.02	0.003 #
	72	6078.00±310.72	559.00±48.45	9.86***	564.00±63.79	9.28***	443.00±72.86	7.29***	285.22	0.003 #
Monocyte(S)	24	644.00±40.74	144.00±19.41	22.36***	132.00±20.97	20.49***	87.00±30.29	13.51***	31.38	0.001 #
	72	644.00±40.74	116.70±18.97	18.12***	153.00±25.21	23.76***	139.00±22.28	21.58***	32.29	0.001 #
Eosinophil(S)	24	346.00±35.41	324.00±29.59	93.64	183.00±24.86	52.89***	178.00±25.21	51.45***	4.45	0.001 #
	72	346.00±35.41	86.00±l5.72	24.86***	259.00±22.52	4.86 **	133.00±l6.9	38.44***	19.21	0.003 #

(S): Number of specific type of white blood cells

Values represent the mean number of specific type of white blood cells ± S.E.M. of 10 rats per group.

Statistically significant from normal control: ***p<0.001 by using *t-test* followed by least significant difference (LS.D.) at p<0.05.

: There is a significant difference between all groups by using one way ANOVA *(F-test)* at p<0.05.

revealed the occurrence of significant (p< 0.001) decrease in its number after 24 and 72 hours of administration. The lymphocyte number (expressed as percentage of control) were 14.02%, 13.15% and 9.35% after 24 hr of *E. coli*, *K. pn.* and *S. ty.* post- treatment respectively. Moreover, the values were 9.86%, 9.28% and 7.29% of controls after 72 hr of injection of *E. coli*, *K. pn.* and *S. ty.* respectively. ANOVA performed on the absolute lymphocyte cells revealed a significant treatment effect after 24 and 72 hr (F= 275.02, p<0.003 and F= 285.22, p<0.003) (Table 4).

d) Absolute Monocyte Cells: The monocyte cells number in WBCs counted following endotoxins injection displayed significant (p<0.001) decrease of 77.64% (*E. coli*), 79.51% (*K. pn.*) and 86.49% (*S. ty.*) in relative to the control value after 24 hr of endotoxins injection. Where, a significant (p<0.001) decrease of 81.88% 76:24% and 78.42% of control was observed in the monocyte number after 72 hr of *E. coli*, *K. pn.* and *S. ty.* post-treatment respectively. ANOVA revealed a significant treatment effect after 24 and 72 hr (F= 31.38, p<0.001 and F= 32.29, p<0.001) (Table 4).

e) Absolute Eosinophil Cells: This study was undertaken to examine the eosinophil cells number of WBCs in response to injection of endotoxins. The results revealed the occurrence of significant (p<0.001) decrease after 24 hr of injection of 47.11% (*K. pn.*) and 48.55% (*S. ty.*) in relative to the control value. Conversely, the number of eosinophil cells decreased nonsignifcantly (p>0.05) of 6.36% in the group of animals treated with *E. coli.* Also, the values of eosinophil cells decreased significantly (p<0.001) after 72 hr in *E. coli* and *S. ty.* post-treatment and significantly (p<0.01) decreased in the group of rats which injected with *K. pn.* endotoxin. The values expressed as percentage of control were 24.86%, 74.86 and 38.44% of *E. coli*, *S. ty.* and *K. pn.* post-treatment respectively. ANOVA of the eosinophil data revealed a significant treatment effect after 24 and 72 hours (F= 4.45, p<0.001 and F= 19.2, p<0.003) (Table 4).

f) Absolute Basophil Cells: Basophil count did not disclose any variation due to the treatment with endotoxins (*E. coli, K. pn.* and *S. ty*) neither after 24 hr nor after 72 hr of injection. The values of basophil cells in the control group was zero.

Platelets

Changes occurred in the platelets count in response to bacterial endotoxins administration showed that rats exhibited significant ($p<0.001$) decreases after 24 and 72 hours post-treatment. The values (expressed as percentage of control) were 65.76%, 79.32% and 49.61% after 24 hr of injection of *E. coli*, *K. pn.* and *S. ty.* respectively. However, after 72 hr the platelets count recorded 53.19%, 64.14% and 42.23% in relative to the control in *E. coli*, *K. pn.* and *S. ty.* treated rats respectively. An ANOVA test performed on platelets count resulting from all groups revealed a significant treatment effects after 24 and 72 hr ($F=109.35$, $p<0.001$ and $F=193.16$, $p<0.002$) (Table 3).

B. Hemoglobin Content

Analysis of hemoglobin content in blood under the effect of injection of bacterial endotoxins revealed the occurrence of significant ($p<0.001$) decrease after 24 and 72 hours of injection. The percentage of control values recorded 85.80%, 89.11% and 88.45% at 24 hr after *E. coli*, *K. pn.* and *S. ty*. administration respectively, whereas, 64.97%, 64.90% and 68.29% of control were recorded at 72 hours in rats injected with *E. coli*, *K. pn.* and *S. ty.*, respectively. ANOVA performed on the hemoglobin content resulting from all groups revealed significant treatment effect at 24 and 72 hours ($F=24.40$, $p<0.001$ and $F=65.06$, $p<0.003$, respectively) (Table 3).

C. Hematocrit Percent (PCV)

The effect of bacterial endotoxins injection on hematocrit percent showed that the hematocrit would exhibit significant ($p<0.001$) decreases of 13.76%, 13.27% and 11.06% after 24 hours of *E. coli, K. pn.* and *S. ty.* post-treatment respectively. Also, the hematocrit showed significant ($p<0.001$) decrease of 33.41%, 34.40% and 31.45% after 72 hours of injection of *E. coli, K. pn.* and *S. ty.* respectively. ANOVA revealed significant difference between all groups at 24 and 72 hours ($F=10.07$, $p<0.001$ and $F=64.11$, $p<0.004$, respectively) (Table 3).

DISCUSSION

Blood and bone marrow comprise a complex mixture of cells that respond in different ways to various toxicologic insults. In addition, the presence or absence of hematologic changes in laboratory animals exposed to environmental chemicals or new pharmaceutical agents may provide valuable tools to evaluate the toxicity of different agents.

The present results concerned with the effect of bacterial endotoxins (*E. coli, K. pn.* and *S. ty*.) administration on bone marrow activity, reflected in the differential investigation of various cell types, showed that, these bacterial endotoxins induce a significant increase in most bone marrow cell types except for eosinophils, normoblasts and lymphocytes. This effect may indicate that a stimulatory action of the bacterial endotoxins upon bone marrow activity occurred. Because of their nature, endotoxins interact with cell membranes and have major effects on cell growth and functions. These effects can be caused by the insertion of lipopolysaccharide (LPS) into the cell membrane and its binding to cellular receptors or to soluble proteins. Similar stimulatory effects of endotoxins on bone marrow activity has been observed in rats (18).

Notably, hematopoiesis stem cells (HSCs) could be induced to proliferate by treating them with large doses of certain hormonal factors, called cytokines, which are present in the marrow environment. Cytokines are a diverse family of polypeptide hormones that can be secreted individually by cells of one or more types and each have specific effects on the growth, differentiation, or functions of other cells. There are several different classes of cytokines; most of those that are known to regulate hematopoiesis belong to subgroups called the colony stimulating factors (CSFs) or the interleukins. The cytokines that can promote HSC growth in vitro include interleukin 3 (IL-3), granulocyte - monocyte colony - stimulating factor (GM-CSF) and a third cytokine called stem cell factor (SCF) (33).

In contrast, endotoxemia was reported to have no effect or decrease bone marrow activity. Monokines

(cytokines produced by monocytes) may contribute to the regulation of hematopoiesis and circulating numbers of leukocytes during inflammation. The hematological effects of daily intravenous injection of recombinant monokines tumor necrosis factor (TNF), interleukin-1 (IL-1) and granulocyte-colony stimulating factor (G-CSF) were therefore studied in the bone marrow and circulation of rats over the course of a week (50). Those authors indicate that the bone marrow on day 8 at 24 hours after the last injection of TNF demonstrated a slight decrease ($p<0.05$) in the average number of immature myeloid forms (myeloblast and myelocyte), but a slight statistically not significant average increase in number of mature neutrophils. Most strikingly, the marrow exhibited an increase in the late normoblasts of erythroid series. No significant changes in the number of pronormoblast was occurred. The bone marrow of IL-1-treated rats demonstrated an increase in the average number of all myeloid forms, a statistically significant increase in the number of myeloblasts and promyelocytes, and specially mature segmented neutrophils. G-CSF administration, intravenously induced neutrophilia in bone marrow. In conclusion, the effects of the chronic administration of exogenous TNF, IL-1 and G-CSF on hematopoieses and circulating number of leukocytes support many lines of evidence that these monokines may contribute to the pathogenesis of acute and chronic inflammation (50).

Livingston *et al.* (31,32) studied that the effect of bacterial infection on myelopoiosies after hemorrhagic shock. The proliferation of white blood cells is an important and necessary response to bacterial leukocytosis although bone marrow and spleen cellularity was unaffected by either shock or LPS.

The results obtained in the present work exhibited dramatic decrements in white blood cells (Leucopenia), red blood cells, hemoglobin content, and platelets count (Thrombocytopenia) of the endotoxin-treated animals by a dose of 1 mg/kg. The changes in the hematological parameters seemed to be dose-dependant, as the anemic status was severe and persistent in the endotoxin-treated animals. These results are similar to those of Semedgard *et al.* (48); Tvedten (52) and Bernard *et al.* (6), who regarded the effect of bacterial endotoxins of *Escherichia coli, Klebsiella pneumoniae* and *Salmonella typhimurium* (*E. coli, K. pn.* and *S. ty.*) administration on the blood components. Where bacterial endotoxins were found induced an inhibitory effect on the hematopoiesis process. The impressive potency and diversity of their pharmacological, immunological, and toxic activities are greatly related to active toxic moiety, lipid A, with variations on the characteristic backbone for different gram-negative species.

The process by which blood cells grow, divide, and differentiate is called hematopoiesis. Three cell lines are produced: (1) red blood cells (erythrocytes), responsible for oxygen transport; (2) platelets, responsible for the control of bleeding; and (3) white blood cells (leukocytes), the vast majority of which are involved in host defense. All of the three classes are ultimately drived from a pool of pleuripotent hematopoietic stem cells (HSCs), which reside in the marrow and have the unique ability to give rise to all of the different mature blood cell types, under the appropriate conditions. The HSCs are self-renewing cells, when they proliferate at least some of their daughter cells remain as HSCs, so that the pool of stem cells do not become depleted. Administration of endotoxins (LPS) from gram-negative bacteria to experimental animals results in variety of pathophysiologic findings. These impacts include hemodynamic changes such as systemic hypotension and pulmonary hypertension, as well as hematologic changes, manifested as a decrease in circulating leukocytes and platelets (8,48).

Our findings are in accordance with other different studies indicating that LPS administration induce a decrease in circulating leukocytes and platelets. Matera *et al.* (35) noticed that the intravenous injection of Salmonella enteritidis endotoxin (25 mg/kg i.v.) produced significant decreases at 30 min in white blood cell count ($p>0.001$); in platelets count ($p>0.01$); and increase of hematocrit ($p>0.03$). Moreover, Egan *et al.* (12) found that the injection of LPS was accompanied by the increased of heart rate and a reduced circulating platelets count (23% of initial).

Also, Lambaigen *et al.* (30) found that rats showed characteristic changes in hematocrit during endotoxemia; an increase from 20 to 45 minutes followed by a decrease to pre-shock values or less at time of 120 minutes.

Many studies have reported several, hematological changes in experimental endotoxemia. Semedgard et al. (48) indicated that the administration of endotoxin from

gram-negative bacteria to rats results in systemic hypotension, an increased hematocrit and decreased numbers of circulating leukocytes, monocyte and platelets. Also, Hawes *et al.* (17) showed that an early lymphopenia and monocytopenia was elicited by LPS or E.coli and persisted throughout the experiment. Furthermore, Opdah *et al.* (39) reported that the infusion of 1-5 mg/kg LPS decreased the count of all types of leukocytes.

Shaibayama *et al.* (46) pointed to the existence of a relationship between the changes in neutrophils and platelets in peripheral blood and the degree of focal hepatocellular necrosis and serum transaminase in rats after endotoxin injection. In addition, the number of platelets in the peripheral blood decreased rapidly after endotoxin injection. In rats, Pearson *et al.* (40) showed that plasma fibrinogen concentration and number of platelets and leukocytes decrease after LPS injection. Also, Kanayama *et al.* (26) found a significant decrease in platelet count in endotoxin-treated pregnant rats compared with control rats. Furthermore, Altenburg *et al.* (1) indicated that the treatment of rats with single dose (250 mg/kg) of LPS caused a dramatic increase in number of circulating neutrophils concomitant with a decrease in the number of these cells in the bone marrow .

Andonova *et al.* (3) showed that the experimental endotoximea was provoked via i.p. injection of 1 mg *E.coli* LPS/kg in rats. In addition, it is reported that the dynamics of hematocrit and erythrocyte counts, greatly decrease up to the 2nd hr followed by an increase to maximum post-treatment at day 3. Furthermore, administration of endotoxin at doses of 2 and 10 mg/kg (infusion) caused proteinuria and thrombocytopenia in pregnant rats (45).

Erve *et al.* (13) stated that, after three hours of endotoxemia in rat and monkey, there was a significant decrease in the number of circulating platelets. Moreover, Goodman *et al.* (14) showed that the leukocyte and platelet counts fell within 10 min of endotoxin treatment.

Pham *et al.* (41) showed that LPS induced a remarkable decrease in white blood cells and platelet counts, whereas lymphocytes increased. On the other hand, Kosumi *et al.* (29) found that the white blood cells count at 24 hours and platelets count at 24, 48 and 72 hours in the LPS group were lower than those in the control animals.

It is well known that vast numbers of mature blood cells are produced daily in the marrow, but the rate of production of each type is precisely controlled and responsive to physiologic demands. For example, production of leukocytes often increases markedly during systemic infections.

It has been recently found that the majority of LPS could rapidly enter the tissues from the circulation within several minutes after intravenous injection of LPS and keep its toxicity for along time (55). Those authors found that i.v. infusion of LPS induced a prolongation of prothrombin time (PT), prolongation of activated partial thrombin time and suppression of platelets count.

Much clinical and experimental data suggest that infection and graft-versus-host disease (GVHD) are intimately associated and bacterial endotoxin, a potent immunostimulant, influence the severity of GVHD. These observations support the hypothesis that endotoxin influence the pathogenesis of GVHD, and provide a useful model for studying the effects of endotoxins in a well-defined immunological system (37).

Endotoxins (LPS) and the trichothecenes are microbial toxins that are frequently encountered in food and environment. Coexposure to LPS (0.1 mg/kg, i.p) and the trichothecene deoxynivalenol (DON, vomitoxin) (12.5 mg/kg, p.o induces corticosterone-dependent apoptosis in thymus, Peyer's patches, and bone marrow in mice. In addition, interleukin-1 beta has been suggested to be an important mediator of LPS plus DON-induced corticosterone and subsequent leukocyte apoptosis. Furthermore, IL-1 beta possibly acts through an ACTH-independent mechanism (20). Recently, LPS was identified as a potent inducer of tumor necrosis factor, which plays a major role in the pathogenesis of endotoxin-induced shock in patients severely infected by gram-negative bacteria. Notably, the development of fever is a specific physiological response to endotoxins. Another biological property of endotoxins and lipopolysaccharides is the ability to produce tolerant states when administered in repeated sublethal doses (10).

In addition, the results of Kitajima *et al.* (28) mentioned that lipopolysaccharide decrease white blood cells (80% of control), lymphocytes (40% of control) and platelets (35% of control), while, a significant increase of neutrophils (330% of control) and monocytes (650% of control) occurred during the 24 hr post-treatment.

Bacterial endotoxins, LPS-protein complexes released from gram-negative bacteria, are potent agents in the in vivo induction of endogenous cytokines, such as Interleukin-1, Tumor Necrosis Factor, Interleukin-6 and colony stimulating factor. It has become apparent that many of the LPS-related physiologic responses are mediated by these endogenous cytokines. In addition, chlamydial endotoxin and *Escherichia coli* 055:B5 endotoxins have been found to depend on Toll-like receptor 4 without depending on Toll-like receptor 2 to stimulate bone marrow-derived dendritic cells to secrete tumor necrosis factor (TNF) (42). Additionally, it has been reported that the rapid and selective accumulation of neutrophils into the lungs is thought to underlie the pulmonary failure that leads to sepsis-related death. Moreover, pulmonary failure remains the most common cause of sepsis-related death. A key event that, is thought to explain this pathology is the rapid accumulation of neutrophils in the narrow lumen of lung capillaries. Indeed, depletion of neutrophils in animal models preserves the lung during endotoxemia (2).

However, stimulation of bone marrow-derived dendritic cells with LPS induced these cells to become mature dendritic cells with higher levels of surface major histocompatibility complex and co-stimulatory molecules and higher mRNA expression of Interleukin-1 alpha, Interleukin-1 beta, -6, and -12 (22). Additionally, fever is one of the most frequent clinical signs encountered in pathology, especially with respect to infectious diseases. It is currently thought that the role of fever on immunity is limited to activation of innate immunity; however, its relevance to activation of adaptive immunity remains unclear. Interestingly a recent study found that fever-like thermal conditions regulate the activation of maturing dendritic cells (51).

In view of the findings reported in this work and in other relevant studies, we may suggest that decrements in hematological parameters after administration of *E. coli, K. pn.* and *S. ty.* may be due to the inhibitory effect of these bacterial endotoxins on the hematopoiesis process. Our data also reflect serious injury of bone marrow in bacterial endotoxins-poisoned rats. The impressive potency and diversity of their pharmacological, immunological and toxic activities are greatly related to active toxic moity, lipid A, with variations on the characteristic backbone for different gram-negative species (6,48,52).

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