

Influence of Bacterial Endotoxins on Bone Marrow and Blood Components in Rats

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Abstract

Endotoxins (Lipopolysaccharide, LPS) a component of the bacterial wall of gram-negative, 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 the production and release of several cytokines. In response to these cytokines, the different effects of endotoxins were seen. The effect of three types of endotoxins (*Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhimurium*) on bone marrow differential counts and peripheral blood parameters including erythrocytes, leukocytes (total and differential) and platelets count and hemoglobin content hematocrit were investigated in adult male rats. Male sprague Dawley albino rats weighting 220-250 gm were used in the study. Rats 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 hr of the injection. At 72 hr the bone marrow aspirations were harvested from 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 count, hemoglobin content and hematocrit percent. Data of the present study point out to important recommendation especially for the drugs. These drugs must have an amount of endotoxins not more than that allowed according to suitable pharmacopeia. Lemulus amebocyte lysate (LAL) test is the specific test used for determination of the endotoxins limit. This recommendation must be achieved to avoid the toxic effects of endotoxins.

Introduction

Bacteria express many different surface antigens and secrete a variety of virulence factors (e.g. toxins) that may trigger immune response. Bacterial exotoxins and endotoxins are important in the pathogenesis of specific diseases. Exotoxins are poisonous proteins that are secreted by many bacteria. Endotoxins are somatic lipopolysaccharide-protein complexes. These complex antigens are located in the outer membrane of all gram-negative bacteria (Jhon, 1997).

The presence or absence of hematologic changes in laboratory animals exposed to environmental chemicals or new pharmaceutical agents is an important element in the overall assessment of the

risks hazards of potential human or animal exposure. Blood and bone marrow are a complex mixture of cells that respond in different ways to various toxicologic insults (Bernard *et al.*, 2000 and John, 2000).

Bone marrow analysis can be a valuable aid diagnosis, prognosis and for monitoring of various conditions or diseases (Cowell and Tyler, 1992; Rusell *et al.*, 1994, Baskin, 1996 and Kathleen, 2000). Successful use depends on an adequate collection, smear preparation and staining (Morris *et al.*, 1993, Baskin, 1996 and Bernard *et al.*, 2000). Bone marrow aspiration is the most commonly used technique (Harvey, 1984, Grindem, 1989; Relford, 1991 and Weiss *et al.*, 1992).

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Thomas, *et al.*, (1989) 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 monokines induce daily neutrophilia and lymphopenia. T.N.F. also induce a slight decrease in early myeloid forms in the marrow.

Livingston *et al.*, (1990) reported that the proliferation of white blood cells is an important and necessary response to bacterial infection. The effect of hemorrhagic shock and Lipopolysaccharide (LPS) administration on myelopoiesis was investigated. They observed that the hemorrhagic shock had no effect on bone marrow. Also, Livingston *et al.*, (1992) described that the bone marrow white blood cell counts were unaffected by shock or LPS administration.

Dubois *et al.*, 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.* (1994) reported that intrathoracic injection of LPS (250 ng/cavity) induced a marker increase in the number of neutrophils at 1 hr, which reach to maximum within 6-12 hr and reduced after 24 hr. In parallel, an increase in blood neutrophils counts within 16- hr, was concomitant with a 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.*, (1996) observed that the megakaryocytes contribute to random selection of an excess of neutrophils in the bone marrow of rats treated with LPS.

William (1969) 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 (Erve *et al.*, 1978; Goodman *et al.*, 1979; Brigham and Meyrick, 1986 and Semedgard *et al.*, 1989).

Many studies have reported the existence of a several hematological changes in experimental endotoxemia. Lambalgen *et al.*, (1988); Egan *et al.* (1989); Hawes *et al.* (1983); Opdah *et al.* (1993); Kitajima *et al.* (1995); Shibayama *et al.* (1995); Pearson *et al.*, (1995); Kanayama *et al.*, (1996); Pham *et al.*, (2000) and Kosumi *et al.* (2001) showed that the administration of endotoxins from gram-negative bacteria to rats caused systemic hypotension, increased hematocrit and decreased numbers of circulating leukocytes, monocytes and platelets. Conversely, Metcalf *et al.* (2002) indicated that LPS caused a progressive decrease in hematocrit value of rats.

Magee and Beely (1991) reported that thrombocytopenia may be decreased production in bone marrow or increased destruction in the peripheral circulation. The mechanism of this drug-induced blood discrasia is either marrow depression or an immune reaction.

In contrast, Altenburg *et al.* (1997) indicated that the treatment of rats with single dose (250 µg/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.* (1998) 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 challenge (group B). Also, the latter 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 i.v infusion of LPS (Yun-Choi *et al.* 2002).

This study has been conducted to investigate the toxic effects of treatment by three types of bacterial endotoxins (*Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhimurium*) on bone marrow differential counts and peripheral blood

parameters including erythrocytes, leukocytes (total and differential) and platelets count and hemoglobin content and hematocrit percent.

Materials and Methods

1) Materials

1. Experimental Animals

For this study, forty healthy adult male Sprague Dawley albino rats were used. Animals were obtained from the animal house of National Organization For Drug Control and Research (Cairo-Egypt), between 220 and 250 g. They were fed with standard diet and preacclimated for one week prior to use.

2. Chemicals

The 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) were obtained from Sigma-Aldrich Chem, (Steinheim, Germany). All the previous endotoxins (LPS) were used as lyophilized 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.

3. Groups of Animals under investigation and plan of endotoxins injection:

Animals were divided into four main groups. Each group consists 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 *Escherichia 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 (1 mg/kg body weight).

4. Blood and tissue Sampling:

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

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 (William, 1969) and gently mixed.

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

II) Methods and Techniques

1-Examination of bone marrow smears:

Preparation of films of post-mortem bone marrow:

Films made of bone marrow post-mortem are seldom satisfactory. Berenbaum (1956) 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 12- 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 deposit. Films made of this suspension in the usual way (John and Lewis, 1991). Bone marrow smears were made and air dried, stained with Leischman's stain for 5 min, the diluted with distilled water for 10 min 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 sides.

2- 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 (1997). Hemoglobin (Hb) content was determined by using Randox reagent kits (Colorimetric method) according to the instruction manual (Kampen and Zijsta, 1961 and International Committee for standardization in hematology 1967).

Statistical Analysis:

The data obtained in the present work are represented in tables as mean \pm 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

I. Bone Marrow Cells: The data of bone marrow cells of control and endotoxins-treated rats are shown in Tables (1 and 2).

1. Normoblast: The results showed that following injection of LPS (1 mg/kg body weight), normoblast

cells significantly ($P < 0.001$) decreased at 72 hrs in the three groups of endotoxins. The percentage of control values recorded were 40.96%, 32.60% and 48.46% after 72 hr of *E. coli*, *K. pn.* and *S. ty.* post-treatment respectively. An ANOVA performed on the normoblast results from all groups revealed a significant treatment effect ($F=34.43$, $P < 0.001$) (Table 1).

2. 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 hr 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 hr from *S. ty.* injection

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 \pm S.E.M.	% of control	F-value	Significant difference between all groups
Normoblasts ^(S)	Control	22.70 \pm 1.54	-	34.43	0.001 #
	<i>E. coli</i>	9.30 \pm 0.76	40.96***		
	<i>K. pn.</i>	7.40 \pm 1.27	32.60***		
	<i>S. ty.</i>	11.00 \pm 0.98	48.46***		
Blasts ^(S)	Control	0.50 \pm 0.17	-	3.00	0.040 #
	<i>E. coli</i>	1.00 \pm 0.21	200.00*		
	<i>K. pn.</i>	1.30 \pm 0.2	260.00**		
	<i>S. ty.</i>	0.70 \pm 0.21	140.00		
Promyocytes ^(S)	Control	1.80 \pm 0.25	-	2.01	0.129 #
	<i>E. coli</i>	2.80 \pm 0.44	155.56*		
	<i>K. pn.</i>	2.20 \pm 0.20	122.22		
	<i>S. ty.</i>	2.20 \pm 0.20	122.22		
Myelocytes ^(S)	Control	13.50 \pm 1.05	-	10.95	0.0001 #
	<i>E. coli</i>	19.90 \pm 1.08	147.41***		
	<i>K. pn.</i>	16.50 \pm 0.69	122.22*		
	<i>S. ty.</i>	14.20 \pm 0.55	105.19		
Juveniles ^(S)	Control	5.30 \pm 0.34	-	23.92	0.0003 #
	<i>E. coli</i>	10.00 \pm 0.95	188.78***		
	<i>K. pn.</i>	12.50 \pm 0.95	235.85***		
	<i>S. ty.</i>	11.80 \pm 0.59	222.64***		
Staff ^(S)	Control	7.50 \pm 0.72	-	10.81	0.004 #
	<i>E. coli</i>	11.10 \pm 0.78	148.00**		
	<i>K. pn.</i>	12.50 \pm 0.43	166.67***		
	<i>S. ty.</i>	12.00 \pm 0.76	160.00***		

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

Values represent the mean number of cells \pm 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 $p > 0.05$.

(Table 1). ANOVA revealed a significant treatment effect ($F=3.00$, $P<0.04$) (Table 1).

3. 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 hr of *E. coli* post-treatment. However, non significant ($P>0.05$) increases of 22.22% and 22.22% was recorded in number of promyelocyte after 72 hr of *K. pn.* and *S. ty.* injection respectively. An overall ANOVA of the promyelocyte data revealed a non-significant effect ($F=2.01$, $P<0.129$) (Table 1).

4. Myelocytes: The data recorded in this work indicated highly significant ($P<0.001$) increase of 47.41% in myelocyte cells of bone marrow after 72 hr 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 of *S. ty.* post-treatment. The values (expressed as percentage of control) was 105.19%. An ANOVA performed on the myelocyte cells resulting from all groups revealed a significant effect ($F=10.95$, $P<0.0001$) (Table 1).

5. Juveniles: The present data of treated animals with endotoxins showed that juvenile cells significantly ($P<0.001$) increased in all treated groups after 72 hr of injection. The percentage 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).

6. 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 hr in rats treated with *E. coli.* also, significant ($P<0.001$) increase 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 72 hr 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).

7. Segmented: The effect of injection of 3 types of LPS on segmented cells of bone marrow in rats was

studied. The results exhibited significant ($P<0.001$) increase of 48.22%, 60.99% and 53.19% after 72 hr 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).

8. 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 values (expressed as a percentage of control) 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).

9. 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 hr 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 hr post-treatment respectively. Endotoxins treatment failed to modify the response of monocytes of bone marrow ($F=2.48$, $P<0.080$) (Table 2).

10. Eosinophils: The administration of bacterial endotoxins led to significant ($P<0.001$) decrease in eosinophil cells of bone marrow after 72 hr from injection of *E. coli* and *K. Pn.* Also, the number of this cells was significantly ($P<0.01$) decreased in *S. ty.* treated group. The values (expressed as a percentage of control) were 35.90%, 15.38% and 56.41% after 72 hr 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 hr of

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

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

Values represent the mean number of cells \pm 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.

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).

II. Blood Components: Results of the blood components of control and endotoxins-treated animals are shown in Tables (3 and 4).

(A) Blood Cells Count:

1. Red Blood Cells (RBC's): 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 hrs of injection. The values (expressed as percentage of control) were 85.75% (*E. coli*), 89.09% (*Kpn*) and 89.09% (*S. ty.*) at 24 hr of injection. Also, the red blood cells recorded values (expressed as percentage of control) were 64.81%, 66.37% and 68.82% at 72 hr in rats treated by *E. coli*, *K. pn.* and *S. ty.* respectively. ANOVA revealed significant differences between all groups

at 24 and 72 hr (F=24.30, P<0.001 and F=63.96, P<0.003 respectively) (Table 3).

2. White Blood Cells (WBC's): The data showed that following injection of LPS (1 mg/kg body weight, i.p.), white blood cells count was significantly (P<0.001) decreased. The percentage of control values recorded 35.71%, 30.86% and 29.21% after 24 and 72 hr 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 72 hr of *E. coli*, *K. pn.* and *S. ty.* administration respectively. An overall ANOVA of white blood cells data revealed a significant treatment effect at 24 and 72 hr (F=434.24, P<0.001 and F=356.45, P<0.001 respectively) (Table 3).

3. Differential White Blood Cells (WBC's):

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 cells number of WBC's as evidenced from their values (expressed as a percentage of control) 75.43%, 68.38% and 71.97% after 24 hrs of injection. Moreover, the decrease in the segmented cells was remanied 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 WBC's after 24 of endotoxins. 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 WBC's 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 WBC's revealed the occurrence of significant ($P<0.001$) decrease in its number after 24 and 72 hrs 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 afte 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 WBC's 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 was undertaken to examine the eosinophil cells number of WBC's in response to inection of endotoxins. he 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, number of eosinophil cells decreased non-significantly ($P>0.05$) of 6.36% in the group of animals treated with *E. coli*. Also, the value sof 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.* endotoxins. 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 hrs ($F=4.45$, $p<0.001$ and $F=19.21$, $P<0.003$) (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 nor after 72 hr of injection. The values of basophil cells in the control group was zero.

4. Platelets: The 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 hrs 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 performed on platelets count resulting from all groups revealed a significant treatment effects after 24 and 72 hrs ($F=109.35$, $P<0.001$ and $F=193.16$, $P<0.002$) (Table 3).

Table 3. Effect of an intraperitoneally single dose of 3 types of bacterial endotoxins (1 mg/kg body weight) after 24 and 72 hrs on red blood cells (RBCs)⁺, white blood cells (WBCs)⁺, and platelets⁺⁺⁺ count, hemoglobin⁵ content an dhemtocrit percent in adult male albino rats.

Blood	Time	Animal groups										F-value	Significant difference between all groups
		Control		<i>E. coli</i>		<i>K. pn.</i>		<i>S. ty.</i>		Treated			
		Mean ±S.E.M	Mean±S.E.M	% of control	Mean ±S.E.M	% of control	Mean ±S.E.M	% of control	Mean ±S.E.M	% of control			
Red blood cells	24 hr	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 hr	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 hr	9.69±0.21	3.46±0.1	35.71***	2.99±0.14	30.86***	2.830.17±	29.21***	434.24	0.001 #			
	72 hr	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 hr	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 hr	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 hr	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 hr	13.59±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 hr	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 hr	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 (RBC's) (x10⁹/mm³); ++; white blood cells (WBC's) (x10³/mm³); +++; platelets (x10⁹/mm³); S;hemoglobin content (g/dL) and %hematocrit percent (%). Values represent the mean number of RBC's (x 10⁹ / mm³) WBC's (x 10³ / mm³) and platelets (x 10⁹ / mm³); WBC's (x 10³ / mm³); 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-tests) at P<0.05.

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

Differential white Blood cells	Time	Animal groups										F-value	Significant difference between all groups
		Control		<i>E. coli</i>		<i>K. pn.</i>		<i>S. ty.</i>		Treated			
		Mean ±S.E.M	Mean±S.E.M	% of control	Mean ±S.E.M	% of control	Mean ±S.E.M	% of control	Mean ±S.E.M	% of control			
Segmented (S)	24 hr	2340.00±148.24	1765.00±74.15	75.43**	1600.00±104.82	68.38***	1684.00±117.12	71.97***	8.62	0.001 #			
	72 hr	2340.00±148.24	14797.00±22.89	63.97**	1040.00±109.23	44.44***	1048.00±116.62	44.79***	19.29	0.003 #			
Staff (S)	24 hr	255.00±131	342.00±27.56	134.12	270.00±19.49	105.88	306.00±29.63	120.00	0.70	0.557			
	72 hr	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 hr	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 hr	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 hr	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 hr	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 hr	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 hr	346.00±35.41	86.00±15.72	24.86***	259.00±22.52	4.86**	133.00±16.91	38.44***	19.21	0.003 #			

(S): Number of specific type of white blood cell. Values represent the mean number of specific type of white blood cells ± S.E.M. of 10 rats 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-tests) at P<0.05.

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 hrs of injection. The percentage of control values recorded 85.80%, 89.115 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 hr 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 hrs ($F=24.40$, $P<0.001$ 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 hr 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 31.455 after 72 hr of injection of *E. coli*, *K. pn.* and *S. ty.* respectively. ANOVA revealed significant difference between all groups at 24 and 72 hrs ($F=10.07$, $P,0.001$ 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 type except for eosinophils, normoblasts and lymphocytes. This effect may indicated 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 (Hryniewicz *et al.*, 1997).

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-CST) and a third cytokine called stem cell factor (SCF) (Lowell, 1997).

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 (Thomas *et al.*, 1989). Those authors indicates 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 1L-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 hematopoiesis and circulating number of leukocytes support the many lines of evidence that these monokines may contribute to the pathogenesis of acute and chronic inflammation (Thomas *et al.*, 1989).

Livingston *et al.* (1990) and Livingston *et al.* (1992) studied that the effect of bacterial infection on myelopoiesis 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 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 endotoxins-treated animals. These results are similar to those of Smedgard *et al.* (1989); Tvedten (1994) and Bernard *et al.* (2001), 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 arterial endotoxins were found induced an inhibitory effect on the hematopoiesis process. The impressive potency and diversity of their pharmacological, immunological, and toxic activities is 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 derived from a pool of pluripotent 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 does not 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 (Brigham & Meyrick, 1986 and Smedgard *et al.*, 1989).

Our findings are in accordance with other different studies indicating that LPS administration induce a decrease in circulating leukocytes and platelets. Matera *et al.*, (1988) 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.* (1989) 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.*, (1988) 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 minute.

Many studies have reported several, hemtological changes in experimental endotoxemia. Smedgard *et al.*, (1989) 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.*, (1993) showed that an early lymphopenia and monocytopenia was elicited by LPS or *E. coli* and and persisted throughout the experiment. Furthermore, Opdah *et al.*, (1993) reported that the infusion of 15- mg/kg LPS decreased the count of all types of leukocytes.

Shaibayama *et al.*, (1995) pointed to the existence of a relationship between the changes in neutrophils and platelets in the 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.*, (1995) showed that plasma fibrinogen concentration and number of platelets and leukocytes decrease after LPS injection. Also, Kanayama *et al.*, (1996) found a significant decrease in platelet count in endotoxin-treated pregnant rats compared with control rats. Furthermore, Altenburg *et al.*, (1997) indicated that treatment of rats with single dose (250 µg/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.*, (1998) showed that the experimental endotoxemia 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 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 µg/kg (infusion) caused proteinuria and thrombocytopenia in pregnant rats (Sakawi *et al.*, 2000).

Erve *et al.*, (1978) stated that, after three hours of endotoxemia in rats and monkey, there was a significant decrease in the number of circulating platelets. Moreover, Goodman *et al.*, (1979) showed that the leukocyte and platelet counts fell within 10 min of endotoxin treatment.

Pham *et al.*, (2000) showed that LPS induced a remarkable decrease in white blood cells and platelet counts, whereas lymphocytes increased. On the other hand, Kosumi *et al.*, (2001) found that the white blood cells count at 24 hrs and platelets count at 24, 48 and 72 hrs 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 long time (Yun-Choi *et al.*, 2002). 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 endotoxins, 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 (Moore *et al.*, 1987).

Endotoxins (LPS) and the trichothecenes are microbial 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 an important mediator of LPS plus DON-induced corticosterone and subsequent leukocyte apoptosis. Furthermore, IL-1 beta possibly acts through an ACTH-independent mechanism. (Islam and Pestka, 2003). Recently, LPS identified as a potent inducer of tumor necrosis factor, which play 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 (Cybulsky *et al.*, 1988).

In addition, the results of Kitajima *et al.*, (1995) 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), 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 has 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) (Prebeck *et al.*, 2003). Additionally, it has been reported that the rapid and selective accumulation of neutrophils into the lungs is thought to underline the pulmonary failure that leads to sepsis-related death. Moreover, pulmonary failure remains that most common cause of sepsis-related death. A key event 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 (Andonegui *et al.*, 2003).

However, stimulation of bone marrow-driven dendritic cells with LPS induced these cells to become mature dendritic cells with higher levels of surface major histocompatibility complex and costimulatory molecules and higher mRNA expression of Interleukin-1 alpha, Interleukin-1 beta, -6, and -12 (Jiang *et al.*, 2003). 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 (Tournier *et al.*, 2003).

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 endotoxin-poisoned rats. The impressive potency and diversity of their pharmacological, immunological and toxic activities is greatly related to active toxic moiety, lipid A, with variations on the characteristic backbone for different gram-negative species (Spemedgard *et al.*, 1989; Tvedten, 1994 and Bernard *et al.*, 2000).

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تأثير التوكسينات الداخلية للبكتيريا على النخاع العظمى و مكونات الدم في الجرذان

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ملخص

تعتبر التوكسينات الداخلية للبكتيريا واحدة من منتجات البكتيريا الفعالة التي تسبب التهابات حادة لأنسجة العائل. ويعتمد هذا البحث على دراسة تأثير ثلاثة أنواع مختلفة من التوكسينات الداخلية للبكتيريا وهى (اشيرشياكولى - سالمونيلا تيفيموريوم - كليسيلا نيمونيا) على خلايا النخاع العظمى وعلى مكونات الدم المختلفة التي تشمل خلايا الدم الحمراء، خلايا الدم البيضاء والصفائح الدموية ومحتوى الهيموجلوبين ونسبة الهيماتوكريت وذلك في ذكور الجرذان البيضاء. وقد تم اختيار الجرذان البيضاء من سلالة (سبراج - دولى) والتي تزن من ٢٥٠ - ٢٢٠ جرام كنموذج حيوانى للاختبارات التي أجريت في هذه الدراسة. لقد تم إعطاء ثلاثة أنواع مختلفة من التوكسينات الداخلية للبكتيريا عن طريق الحقن في الغشاء البريتونى (١ مجم / كجم من وزن الجرذ) جرعة واحدة لثلاثة مجموعات من الحيوانات بجانب المجموعة الضابطة والتي تم حقنها بريتونيا بنفس الجرعة (١ مجم / كجم) (بمحلول ملحي % ٩, ٠ . ولقد تم تجميع عينات الدم بعد ٢٤ ساعة وأيضاً بعد ٧٢ ساعة من بدء عملية الحقن . وبعد نهاية الـ ٧٢ ساعة تم قتل الجرذان ثم تم على الفور التشريح واستخراج عظمة الفخذ للحصول على النخاع العظمى منها. ولقد أظهرت النتائج أن التوكسينات الداخلية للبكتيريا سببت تغيرات مختلفة في خلايا النخاع العظمى وأيضاً سببت نقص ملحوظ في كلا من خلايا الدم الحمراء، خلايا الدم البيضاء والصفائح الدموية ومحتوى الهيموجلوبين ونسبة الهيماتوكريت. ونستخلص من نتائج هذه الدراسة الى التوصية بعدم استخدام عقاقير للحقن بها نسب من التوكسينات الداخلية للبكتيريا تتعدى النسب المسموح بها حتى نتجنب التأثيرات السامة لهذه التوكسينات.