EFFECTS OF BACTERIAL ENDOTOXIN ON SOME METABOLITES AND ENZYMES IN RAT SERUM

ABD EL RAHMAN A. EL GARAWANY*
OTHMAN A. AL SAGAIR**
EZZAT S. EL DALY***
KAMAL A. EL SHAIKH***
MAHMOUD H. EL GEBALY****
AHMED A. MOUSA*

SUMMARY : The effects of bacterial endotoxins (Escherichia coli, Klebsiella pneumoniae, and Salmonella typhimurium) on glucose and blood urea nitrogen (BUN) levels and aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and lactate dehydrogenase (LDH) activities were studied. Three groups of rats were injected (1 mg/kg body weight, i.p.) with three types of bacterial endotoxins (E. coli, K. pneumoniae and S. typhimurium) as a single dose. The control group was injected i.p. (1 mg/kg) in 0.9% normal saline. Blood sampling was performed from the orbital vein plexus after 24 and 72 hr of injection. Glucose level was increased significantly after 24 hr of after each 3 solutions of endotoxin. Its level showed non-significant decrease after 72 hr post-treatment. However, endotoxins caused significant increases in BUN, ASAT and LDH at 24 and 72 hr post-treatment. On the other hand, the ALAT activity was significantly decreased after the referred observation periods of endotoxins injection. The variation in serum glucose level after 24 and 72 hr post-treatment may be referred to different reasons. On the other hand, the increase of BUN concentration may be due to the toxic effect of bacterial endotoxins resembling to that occurring in renal damage and impairment of renal function. However, the changes in serum aminotransferases and LDH activities may be due to endotoxins induced hepatic microcirculatory disturbance and to the subsequent liver injury and tissue hypoxia.

Key Words: Endotoxin, enzyme activities.

INTRODUCTION

Endotoxins (lipopolysaccharides, LPS) are found only in the outer lipid bilayer that surrounds the cell walls of gram-negative bacteria, such as *Salmonella* and *Escherichia coli*. Each molecule of LPS consists of a core carbohydrate linked to a phospholipid (called lipid A) anchored in the bilayer, and to a long polysaccharide chain (called the O side chain) that extends outward from the bacterial surface (45). Endotoxin translocation might play an imporatant role in the development of shock, which may be mainly related to the accumulation of endotoxins in tissues. Endotoxin levels in hepatic homogenate were firstly increased 1.5 hours following shock, and were higher than those in pulmonary and renal homogenates. LPS levels in pulmonary homogenate were also higher than those in renal tissue following shock. The liver is the most important organ for endotoxin accumulation after hemorrhagic shock for; 1) the portal circulation is the prominent route for endo-

*Pyrogen Department, National Organization for Drug Control and Research (NODCAR), Giza, Egypt.
**Medical Microbiology Department, Faculty of Science, El Qassim University, EL Qassim, KSA. P.O. Box 237, Bureida 81999.
***Zoology Department, Faculty of Science, Helwan University, Cairo, Egypt.
****National Cancer Institute, Cairo University, Cairo, Egypt.

Table 1 : Effect of an intraperitoneally single dose of 3 types of bacterial endotoxins (1 mg/kg body weight) after 24 and 72 hr on blood glucose and blood urea nitrogen (BUN) in adult male albino rats.

Parameters	Time	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		
Blood Glucose (mg/dl)	24 hr	101.70±4.08	185.40±4.54	182.30***	243.90±12.178	239.82***	205.40±13.56	201.97***	39.09	0.001#
	72 hr	101.70±4.08	91.40±3.28	89.87	94.40±3.16	92.82	93.40±2.25	91.83	1.62	0.02
Blood Urea Nitrogen (BUN) (μg/dl)	24 hr	14.8±0.47	27.50±0.095	185.81***	31.90±1.75	215.54***	35.20±1.74	237.83***	44.51	0.003#
	72 hr	14.80±0.47	18.70±0.68	126.35**	23.70±0.99	160.14***	26.00±1.02	175.68***	37.33	0.003#

Values represent the mean of glucose (mg/dl) and BUN (μg/dl) levels ± S.E.M. of 10 rats per group. Statistically significant from normal control: ** $p < 0.01$; *** $p < 0.001$ 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 $p < 0.05$.

toxin in the intestine to enter the body after hemorrhagic shock; 2) the liver is the largest organ that have monocyte/macrophage system, from where endotoxin is mainly eliminated out of the body (19, 46).

Endotoxins translocated into the body after hemorrhagic shock was mainly distributed in the tissue organs and its bioactivities remained in tissues for a long time. To what extent, endotoxins in tissues are presently being discussed (19, 31).

Yelich and Janusek (51) found that the administration of Salmonella entritidis endotoxin to 10 day-old rats and 28 day-old rats at 2 and 30 mg/kg, respectively caused hyperglycemia as initial response to endotoxin followed by hypoglycemia. However, Morikawa *et al.* (29); Lomnitski *et al.* (27) and Kheir-Eldin *et al.* (23) mentioned that the endotoxin elevated the blood sugar level. In contrast, Horton *et al.* (16); Kaido *et al.* (20) and Dhuley and Naik (10) recorded that the LPS-induced decreases in blood sugar level in rats.

The administration of endotoxin to rats induces renal dysfunction characterized by increased blood urea nitrogen and plasma creatinine levels Endoh *et al.* (12); Haesgawa *et al.* (13); Yoshikawa *et al.* (52); Wellings *et al.* (47); She *et al.* (40) and Chen *et al.* (5).

Endotoxins caused rises in the serum levels of urea and creatinine (indicators of renal failure), aspartate and alanine aminotransferases (ASAT and ALAT); acid phosphatase and lactic dehydrogenase (LDH) (indicators of hepatocellular injury), bilirubin and gamma-glutamyl transferase (indicators of liver failure) and rise in serum levels of lipase (indicator of pancreas dysfunction) as well as nitrite (indicator of induction of nitric oxide synthase) (1, 3, 6, 9, 10, 12, 15,19, 21, 24-27, 30, 33, 36, 37, 41, 49, 53-55).

The treatment of rats with LPS resulted in increase in total protein content and LDH activity in bronchoalveolar lavage fluid associated with acute lung inflammation and injury (22). Also, Roelfsema *et al.* (35) and Kheir-Eldin *et al.* (23) reported that the LPS administration led to an increase in plasma concentrations of urea, cholesterol, triglyceride, corticosterone and insulin.

The objective of our investigation was to study the toxic effects of treatment by three types of endotoxins (*Escherichia coli*; *Salmonella typhimurium* and *Klebsiella pneumoniae*) on clinical chemistry values including blood glucose; blood urea nitrogen (BUN), aspartate aminotransferase (ASAT); alanine aminotransferase (ALAT) and Lactate dehydrogenase (LDH).

MATERIALS AND METHODS

Animals: Fourty adult male Spraguo Dawely albino rats weighing from 220- 250 g were used throughout the experiment. Animals were obtained from the animal house of National Organization For Drug Control and Research (Cairo - Egypt). They were

allowed free access to food and water and preacclimated for 1 week prior to use.

Endotoxins: Three types of endotoxins (lipopolysaccharide; LPS) were used during the experiments of this study. *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 (LPS) 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.

Injections: 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 (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).

Collection of Blood: Blood was collected from orbital venous plexus from each animal of the control group and treated groups after 24 and 72 hrs post-treatment in clean centrifuge tubes and centrifuged at 3000 r.p.m. for 15 min after complete clotting. Fresh serum samples were used for the determination of glucose level and blood urea nitrogen (BUN) levels and aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and lactate dehydrogenase (LDH) activities.

Methods: Determination of serum glucose concentration was carried out according to the method of Teuscher and Richterich (44) and Barham and Trinder (2) using RANDOX reagent kits. BUN was determined according to the method described by Windamn (48) and Hallet and Cook (14). The assay of BUN was performed using Scalvo diagnostics reagent kits and following their instructions. ASAT and ALAT activities were determined according to the method described by Pappas (32). The assay of transaminases was performed using reagent kits obtaind from BioMerieux Laboratory Reagents and Instruments, 69280, Marcy-I Etoile, France. LDH activity was estimated by UV kinetic method according to the method described by I.F.C.C. (17). The assay was performed using Sentil Ch reagent kits and following their instructions manual (Sample starter procedure).

Statistics: Results are expressed as the mean ± standard error (S.E.M.). Statistical analysis was done by using one-way analysis of variance (ANOVA) and F-test followed by Student's *t*-test and least significant difference (L.S.D.) at P<0.05. Differences with P<0.05 were considered significant.

RESULTS

Metabolites

Glucose: The glucose level in serum measured following endotoxins injection displayed significant ($P < 0.001$) increases after 24 hr of injection, where values of 82.30 %, 139.82 % and 101.97 % of control levels were observed in rats treated with *E. coli*, *K. pn.* and *S. ty.* respectively. Conversely, non-significant decreases of 10.13 %, 7.18 % and 8.17% of control were recorded after 72 hr in the glucose level of serum in animals treated by *E. coli, K. pn.* and *S. ty.* respectively. ANOVA revealed a significant treatment effect at 24 hr ($F= 39.09$, $P< 0.001$) whereas, endotoxins treatment failed to modify the response of the glucose level ($F=1.62$, $P< 0.02$) at 72 hr of injection (Table 1).

Blood urea nitrogen (BUN): Injection of bacterial endotoxins induced significant ($P<0.001$) increases in BUN concentration after 24 and 72 hrs. The concentrations measured as percentage of control were 185.81%, 215.54% and 237.83% after 24 hr in rats injected by *E. coli, K. pn.* and *S. ty.* respectively. Also, after 72 hr of *E. coli, K. pn.* and *S. ty.* injection, the BUN concentration showed values of 26.35% , 60.14% and 75.68% in relative to the control value respectively. ANOVA of BUN data revealed significant treatment effect after 24 and 72 hrs ($F= 44.51$, $P< 0.003$ and $F= 37.33$, $P < 0.003$ respectively) (Table 1).

Enzyme activities

Aspartate aminotransferase (ASAT): Considering the effect of bacterial endotoxins injection, it was found that the ASAT activity in serum exhibited significant increases after 24 and 72 hrs of injection. This is evident from the data expressed as percentage of control, which showed values of 118.07%, 129.16% and 128.67% after 24 hr from *E. coli, K. pn.* and *S. ty.* injections respectively. Also, the increases occurred in the ASAT activities after 72 hr measured as percentage of control were 192.04%, 204.34% and 205.54% after *E. coli, K. pn.* and *S. ty.* post-treatment respectively. ANOVA revealed significant treatment effect at 24 and 72 hrs ($F = 80.49$, $P < 0.003$ and $F = 83.78$, $P< 0.003$ respectively) (Table 2).

Alanine aminotransferase (ALAT): Injection of bacterial endotoxins induced significant ($P < 0.001$) decreases in ALAT activities after 24 and 72 hrs of injection. The

Table 2 : Effect of an intraperitoneally single dose of 3 types of bacterial endotoxins (1 mg / kg body weight) after 24 and 72 hr on aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and lactate dehydrogenase (LDH) activities in adult male albino rats.

Parameters	Time	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		
Aspartate amino-transferase (ASAT) (U/L)	24 hr	41.50±4.37	90.50±2.45	218.07***	95.10±2.49	229.1***	94.90±2.81	228.6***	80.49	0.003#
	72 hr	41.50±4.37	79.70±2.38	192.04***	84.80±2.25	204.34***	85.30±2.35	205.54***	83.78	0.003#
Alanine amino-transferase (ALAT) (U/L)	72 hr	29.60±1.63	20.50±0.76	69.26***	23.20±1.22	78.38***	23.30±1.17	78.72***	7.56	0.001#
	72 hr	29.60±1.63	16.60±0.88	56.08***	18.50±1.33	62.50***	20.30±1.75	68.58***	16.03	0.003#
Lactate dehydro-genase (LDH) (U/L)	24 hr	295.30±7.11	1370.80± 16.53	463.93***	858.90±11.05	290.86***	944.30±14.19	319.78***	1209.68	0.003#
	72 hr	295.30±7.11	951.30±13.65	322.15***	786.60±18.57	266.37***	861.0±13.46	291.73***	451.30	0.001#

Values represent the mean of ASAT, ALAT and LDH (U/L) activities ± S.E.M. of 10 rats per group. Statistically significant from normal control: ** $p < 0.01$; *** $p < 0.001$ 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 $p < 0.05$.

activities measured as percentage of control were 69.26%, 78.38 % and 78.72% after 24 hr of *E. coli, K. pn.* and *S. ty.* injections respectively. Furthermore, decreases after 72 hr of endotoxins *(E. coli, K. pn.* and *S. ty.*) indicated by the ALAT activities of 43.92%, 37.50% and 31.42% of control were recorded respectively. ANOVA showed significant treatment effect after 24 and 72 hrs (F=7.56, P < 0.001 and F=16.03, P < 0.003 respectively) (Table 2).

Lactate dehydrogenase (LDH): The study of the effect of bacterial endotoxins injection on the LDH activity in serum revealed the occurrence of a significant (P< 0.001) increase after 24 and 72 hrs. The activities showed values of 363.93%, 190.86% and 219.78 % when the activities were related to the controls, after 24 hr from *E. coli, K. pn.* and *S. ty.* injections respectively. With the continuous follow up of the effect of treatment the increase of LDH was maintained throughout till the 72 hr of injection of *E. coli, K. pn.* and *S. ty.*, where the LDH activities showed values of 222.15%, 166.37% and 191.73% in relative to the control value respectively. An ANOVA performed on the LDH resulting from all groups revealed a significant treatment effect after 24 and 72 hrs (F=1209.68, P< 0.003 and F= 451.30, P< 0.001 respectively) (Table 2).

DISCUSSION

The present data indicate that endotoxins administration of *E. coli, K. pn.* and *S. ty.* caused a highly significant increase in glucose level after 24 hr and then non-significantly decrease after 72 hr of endotoxins treatment. Our results are similar to those of Yelich and Janusek (51); Morikawa *et al.* (29); Lomnitski *et al.* (27) and Kheir-Eldin *et al.* (23). Yelich and Janusek (51) reported that *Salmonella entritidis* endotoxin caused hyperglycemia as initial response followed by hypoglycemia. In addition, bacterial endotoxins induced pancreatitis (49) which caused an increase in serum amylase associated with increase in glucose level. Moreover, it has been demonstrated that *in vivo* endotoxins treatment alter the ability of epinephrine to inhibit immunoreactive insulin secretion or to stimulate immunoreactive glucagon secretion (50). These findings

provide a partial mechanism to explain endotoxin-induced elevation in glucagon levels and hyperglycemia during endotoxicosis. Therefore, it is possible that the increase in serum glucose level in the present study after bacterial endotoxins treatment may be related to the elevation in glucagon levels during endotoxemia (50) and also may be due to pancreatitis, which resulted from LPS injection to rats (49).

However, the three types of endotoxins, used in the present study, induced a highly significant increases in BUN concentrations, ASAT and LDH enzyme activities after 24 hr of treatment and persisted for 72 hr. On the other hand, endotoxins treatment caused a noticeable inhibition in ALAT enzyme activity. Similar trends were previously reported by Endoh *et al.* (12); Haesgawa *et al.* (13); Yoshinori *et al.* (53); Jiang *et al.* (19); Yamano *et al.* (49); Liu *et al.* (25); Nakatani *et al.* (30); Sun *et al.* (41); Chiou *et al.* (6); Yun-Choi *et al.* (54) and Liu *et al.* (26) who demonstrated that endotoxins significantly increase serum ASAT, ALAT, LDH, BUN and creatinine levels.

The concomitant increase of BUN and glucose levels with the increased enzymatic activities of LDH and ASAT, in the present work, may give a qualitative indication to the enhanced cellular metabolic activity under endotoxemic stress. Alternatively, the increase in these parameters might provide an indication to the occurrence of hepatic, cardiac and renal dysfunction under endotoxicosis. This interpretation is in agreement with Chen *et al.* (5) and Pham *et al.* (33). Furthermore, increasing ASAT and LDH activities in bacterial endotoxins treated animals reported in the present work, may also suggest that endotoxins induce hepatic microcirculatory disturbances, which may cause liver injury (53).

Several studies indicate that endotoxemia increases the activities of the metabolic enzymes includes serum amylase and transaminase which are accompanied by rising metabolic products including BUN, and creatinine (51,52). In accordance, Wellings *et al.* (47) found that endotoxins administrations induce renal dysfunction characterized by increased BUN and plasma creatinine levels in treated rats. Additionally, endotoxin - treated pregnant rats exhibit increased blood pressure, norepinephrine, epinephrine, and ASAT, ALAT and LDH enzyme activities compared with control rats treated with saline (21). Interestingly, it was found that glycine totally prevented mortality and markedly minimized LPS- induced elevation of serum transaminase levels, hepatic necrosis, and lung injury (1,18).

Moreover, bacterial endotoxins treatment in rats was found to induce a significant activation of hepatic nitric oxide synthase correlated well with the increase in plasma nitrate plus nitrite concentration (16).

In agreement with our results, Ruetten and Thiemermann (36, 37) showed that endotoxemia for 6 hr caused a significant rise in the serum levels of ASAT, ALAT, gamma-glutamyl transferase and bilirubin reflecting the hepatic dysfunction. Furthermore, those authors reported that endotoxemia was found to induce a rapid increase in levels of urea and creatinine and lipase enzyme indicating the occurrence of renal and pancreatic dysfunction. Similarly, Jiang *et al.* (19) examined the effect of endotoxemia on ALAT and BUN levels in the plasma and the different tissues. They found that endotoxemia induced a significant elevation of ASAT and BUN. There was also a correlation between the aforementioned effect and the accumulation of endotoxins in the corresponding tissues, suggesting that there is a certain intrinsic relationship between accumulation of endotoxins in tissues and organ dysfunction following hemorrhagic shock.

In the present work, the data of the biochemical values were in agreement with all the previous studies except that of result of ALAT. This parameter was lower than those in the control group after 24 and 72 hrs of i.p. injection of bacterial endotoxins (1 mg / kg). The variation in these results may be due to (1) difference in bacterial endotoxins (LPS) doses; (2) difference in strain of bacterial endotoxins (LPS); (3) difference in strain of laboratory animal used and (4) difference in the biological response of rats.

Actually, acute inflammation constitutes the body's principal mode of defense against infection and other harmful agents, and neutrophils are the primary effector of the cells in this process. When inflammation occurs in response to infection with pathogenic microorganism, the damage that is often observed locally is a sacrifice aimed to prevent the spread of infectious agents throughout the body. Lipopolysaccharide, as an endotoxin immunomodulator, has been shown to stimulate many cellular substances, such as cytokines, vasoactive peptides, procoagulant factors and prostaglandins, *in vitro* and *in vivo* (4).

In addition, recent studies have demonstrated a crucial role for nitric oxide (NO) as a secondary mediator of endotoxemia. NO is synthesized from L-arginine by 2 types of NADPH-dependent enzyme systems. One involves a constitutive, Ca^{+2}/ calmodulin-dependent enzyme, which is associated with brain, endothelial cells and platelets. The other requires an inducible, Ca^{+2}-dependent enzyme and is found in many other cells, such as macrophages, polynuclear cells and smooth muscle cells (28). The effect of nitric oxide in the pathogenesis of endotoxin shock and multiple organ failure was examined by Takemura *et al.* (42). The last authors indicated that the LPS increased the activity of inducible nitric oxide synthase (NO synthase) in the liver, lung and spleen. These results suggested that suppression of endogenous nitric oxide might aggravate hepatic injury, partly caused by decrease in hepatic blood flow accompanied with oxidative stress in endotoxemia. Moreover, Horton *et al.* (1994) mentioned that the treatment of rats with bacterial endotoxin caused a significant induction of hepatic NO synthase correlated well with the increase in plasma nitrate plus nitrite concentration and also with inhibition of glucose synthesis. These results run in agreement with the data of glucose in the present work after 72 hr of endotoxins treatment.

In vitro, LPS stimulates production of NO in macrophages, glomerular mesangial cells and vascular smooth muscle cells via an inducible Ca^{+2} - independent NO synthase (7, 39). Moreover, there has been evidence supporting the relationship between nitric oxide increase and the hematological changes in immumnological function *in vivo*. Additionally, it has been suggested that LPS-induced increase in nitrate and nitrite levels and the hematological changes may compromise immune function (34,38,43). Consistently, antioxidants pretreatment were found to ameliorate the oxidative stress in LPS-treated rats by reducing levels of malondialdehyde, restoring glutathion content and normalizing the mitochondria / cytosolic hexokinase ratio, in addition to lowering levels of plasma corticosterone and glucose (23).

Our study indicated that endotoxins administration modulates the normal function in many organs including the heart, kidney, liver and immune system depending upon the alterations both in activities of the marker enzymes and the characteristic metabolites, and in bone marrow hemopoietic activity and blood components (11). In addition, these effects are thought to be mediated in part through the immuno-modulation and stimulation of many cellular substances, such as cytokines, vasoactive peptides, procoagulant factors and prostaglandins (4).

However, gram negative microorganisms elicit a brisk inflammatory reaction which is largely induced by one of their wall constituents, endotoxin. The infiltrating neutrophils phagocytose and kill the bacteria. The inflammatory reaction is often associated with severe local microvascular injury and abscess formation. Besides eliciting inflammation, endotoxin can predispose the local microvasculature to thrombosis upon subsequent systemic endotoxemia or complement activation. Both the inflammatory and thrombotic phenomena induced by endotoxins are mediated by the local generation of cytokines (8).

In conclusion, we may suggest that, the different changes in most of bone marrow cells, hematological parameters (11) and biochemical values in adult male albino rats which had been injected with bacterial endotoxins may be due to the disfunction of biological organs such as bone marrow, liver, kidney, heart and pancreas.

REFERENCES

1. Aoki Y, Ota M, Katsuura Y, Komoriya K, Nakagaki T : Effect of activated human protein C on disseminated intravascular coagulation induced by lipopolysaccharide in rats. Arzeneimittelforschung, 50: 809-815, 2000.

2. Barham D, Trinder P : Determination of serum glucose concentration. Analyst, 97:142, 1972.

3. Barton CC, Ganey PE, Roth RA : Lipopolysaccharide augments aflatoxin B (1)-induced liver injury through neutrophil-dependent and -independent mechanisms. Toxicol Sci, 58: 208-215, 2000.

4. Bone RC : The pathogenesis of sepsis. Ann Intern Med 115, 457-469,1991.

5. Chen CP, Yokozawa T, Kitani K : Beneficial effects of sanguisorbae radix in renal dysfunction caused by endotoxin in vivo. Biol Pharm Bull, 22:1327-1330, 1999.

6. Chiou WF, Ko HG, Chen CF, Chou CJ : Evodia rutaecapra protects against circulation failure and organ dysfunction in endotoxaemic rats through modulating nitric oxide release. J Pharm Pharmacol 54:1399-1405. Chride irritation of celiac ganglia in rats. Haemostasis, 27:149-156, 2002.

7. Cunha FQ, Assreuy J, Moncada S, Liew FY : Phagocytosis and induction of nitric oxide synthase in murine macrophages. Immunology 79, 408-411, 1993.

8. Cybulsky MI, William CMK, Hennery ZM : Acute inflammation and microthrombosis induced by endotoxin m interleukin-1, and tumor necrosis factor and their immlication in gram- negative infection. Laboratory investigation . 58:365, 1988.

9. Deaciuc IV, D'Souza NB, De Villiers WJ, Burikhanova R, Sarphie TG, Hill DB, Mcclain CJ : Inhibition of caspases in vivo protects the rat liver against alcohol-induced sensitization to bacterial lipopolysaccharide. Alcohol Clin Exp Res. 25:935-943, 2001.

10. Dhuley JN, Naik SR : Effect of rhinax on bacterial lipopolysaccharide induced endotoxemia in rats. Indian J Exp Biol, 36:315-317, 1998.

11. El Shaikh KA, Al Sagair OA, El Daly ES, El Garawany AA, El Gebaly MH, Mousa AA : Ifluence of bacterial endotoxin on bone marrow and blood components in Rat J Egypt Soc Toxicol (in press), 2005.

12. Endoh K, Ueno K, Miyashita A, Satoh T : An experimental model of acute liver injury using multicellular spheroids composed of rat parenchymal and non-parenchymal liver cells. Res Commun Chem Pathol Pharmacol, 82:317-329, 1993.

13. Haesgawa T, Nadia M, Wang L, Haghgoo S, Nabe ST, Kato N : Influence of endotoxin and lipid A on the renal handling and accumulation of gentamicin in rats. Biol Pharm Bull, 17:1651-1655, 1994.

14. Hallet CJ, Cook JGH : Enzymatic colorimetric determination of blood urea nitrogen. Clin Chem Acta, 35:33, 1971.

15. Hong KW, Kim KE, Rhim BY, Lee WS, Kim CD : Effect of rebamipide on liver damage and increased tumor necrosis factor in a rat model of endotoxin shock. Dig Dis Sci, 43:1545-1595, 1998.

16. Horton RA, Knowles RG, Titheradge MA : Endotoxin cause resprocal changes in hepatic nitric oxide synthsis, gluconeogensis, and flux through phosphoenolpyruvate carboxykinase. Biochem-Biophys-Res-Commun. 28 ; 204:659-665, 1994.

17. I.F.C.C. : Determination of lactate dehydrogenase. J Clin Chem Clin Biochem, 18, 521,1980.

18. Ikejima K, Iimuro Y, Forman DT, Thurman RG : A diet containing glycine improves survival in endotoxin shock in the rat. Am J Physiol, 271 (1 pt 1): G 97- 103, 1996.

19. Jiang J, Chen H, Diao Y, Tian K, Zhu P, Wang Z : Distribution of endotoxins in tissues and circulation and its effects following hemorrhagic shock. Chinese, Medical-Journal-English-Edition; 111(2) 118 - 122, 1998.

20. Kaido T, Yamaoka S, Seto S, Funaki N, Kasamatsu T, Tanaka J, Nakamura T, Imamura M : Continuous hepatocyte growth factor supply prevents lipopolysaccharide-induced liver injury in rats. FEBS Lett. 411(2-3):378-382,1997.

21. Kanayama N, She L, Maehara K, Kajiwara Y, Terao T : Induction of HELIP syndrome-like biochemical parameters by stimulation of the celiac ganglion in rats. J Hypertens, 14:453-459, 1996.

22. Kang JL, Lee HW, Lee HS, Pack IS, Chong Y, Castronova V, Koh Y : Genistein prevents nuclear factor-kappa B activation and acute lung injury induced by lipopolysaccharide. Am J Respir Crit Care Med 15; 164:2006-2012, 2001.

23. Kheir-Eldin AA, Motawi TK, Gad MZ, Abd-El Gawad HM : Protective effect of vitamin E, beta-carotene and N-acetylcysteine from the brain oxidative stress induced in rats by lipopolysaccharide. Int J Biochem Cell Biol, 33:475-482, 2001.

24. Kimura K, Moriyama M, Nishisako M, Kanna Y, Shiota M, Sakurada K, Musashi M, Sugano T : Modulation of platelet activating factor-induced glycogenolysis in the perfused rat liver after administration of endotoxin in vivo. J Biochem Tokyo, 123:142-149, 1998.

25. Liu B, Xiao N, Chen H, Zhou J : Effect of endothelin-1 on hepatic damage induced by endotoxin. Chin J Traumatol, 3:234-237, 2000.

26. Liu JJ, Wang JY, Zhang C, Nilsson A, Duan RD : Hepatic cirrhosis increases sensitivity of kidney to endotoxin in rats. Med Sci Monit, 8:BR 56-60, 2002.

27. Lomnitski L, Carbonatto M, Ben SV, Peano S, Conz A, Corradin L, Maronpot RR, Grossman S, Nyska A : The prophylactic effects of natural water soluble antioxidant from spinach and apocynin in a rabbit model of lipopolysaccharide-induced endotoxemia. Toxicol Pathol, 28:588-600, 2000.

28. Moncada S, Palmer RMJ, Higgs EA : Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev, 43, 109-142, 1991.

29. Morikawa S, Inubushi T, Takajhashi K, Ishii H, Ozawa K : Gluconeogenesis and phosphoenergetics in rat liver during endotoxemia. J Surg Res, 74:179-186, 1998.

30. Nakatani Y, Fukui H, Kitano H, Nagamato I, Tsujimoto T, Kuriyama S, Kikuchi E, Hoppou K, Tsujii T : Endotoxin clearance and its relation to hepatic and renal distribances in rats with liver cirrhosis. Liver, 21:64-70, 2001.

31. Olofsson P, Nylandr G, Olson P : Endotoxin-transport routes and Kinetics in intestinal ischemia. Acta Chir Scan, 151: 635, 1985.

32. Pappas NS : Enhanced cardiac enzyme-profile. Clin Lab Med, 9,4, 689-716, 1989.

33. Pham D, Jeng AY, Escher E, Sirois P, Battistini BX : Effects of a selective neutral endopeptidase and a nonselective neutral endopeptidase/endothelin-converting enzyme inhibitor on lipopolysaccharide-induced endotoxaemia in anaesthetized Sprague-Dawley rats. J Cardiovasc Pharmacol, 36:S362-366, 2000.

34. Quesenberry P, Halperin J, Ryan M, Stohlman F Jr. : Tolerence to the granulocyte-releasing and colony-stimulating factor elevating effects of endotoxin. Blood 45, 789 - 800, 1975.

35. Roelfsema V, Thomas GB, Lin H, Breier BH, Maxwell L, Oliver MH, Heineman E, Clark RC, Gluckman PD : The metabolic effects of endotoxin are differentially affected by the pattern of GH administration in the rat. J Endocrinol 17:173-181, 2001.

36. Ruetten H, Thiemermann C : Effect of selective blockade of endothelin ETB receptors on the liver dysfunction and injury caused by endotoxaemia in the rat. Br J Pharmacol, 119:479-486, 1996.

37. Ruetten H, Thiemermann C : Effect of tyrphostins and genistein on the circulatory failure and organ dysfunction caused by endotoxin in the rat: a possible role for protein tyrosine kinase. Br J Pharmacol, 122:59-70, 1997.

38. Satoshi K, Mitsuhiro T, Nozomi YM, Minoru S, Junko M, Yuji K : Lipopolysaccharide-assosiated elevation of serum and urinary nitrite / nitrate levels and hematological changes in rats. Toxicology Letters 78; 135 - 140, 1995.

39. Schultz PJ, Raiji L : Endogenously synthesized nitric oxide prevents endotoxin-induced glomerular thrombosis. J Clin Invest 90, 1718-1725, 1992.

40. She L, Kanayama N, El-Maradny E, Halim A, Maehara K, Terato T : Experimental hemolytic uremic syndrome induced by lipopolysaccharide irritation of celiac ganglia in rats. Haemostasis; 27:149-156, 1997.

41. Sun R, Zhang H, Si Q, Wang S : Protective effect of Ginko biloba extract on acute lung injury induced by lipopolysaccharide in D-galactose aging rats. Zhonghua. Jie He Hu Zi Zahi, 25:352-355, 2002.

42. Takemura S, Minamiyama Y, Inoue M, Kubo S, Hirohashi K, Kinoshita H : Nitric oxide synthase inhibitor increases hepatic injury with formation of oxidative DNA damage and microcirculatory disturbance in endotoxemia rat. Hepatogastroentrology, 47: 1364-1370, 2000.

43. Territo MC, Gold DW : Granulocyte function in experimental human endotoxemia. Blood 47, 549 - 544, 1976.

44. Teuscher A, Richterich P : Schweiz Med Wschr, 101: 345 and 390, 1971.

45. Tristran GP, Dorothy FB : In Medical Immunology Chapter 2 Page 26 - 27, 1997.

46. Vejchapipat P, Eaton S, Fukumoto K, Parkes HGK, Spitz L, Pierro A : Hepatic glutamine metabolism during endotoxemia in neonatal rats. Nutrition, 18:293-297, 2002.

47. Wellings RP, Corder R, Vane JR : Lack of effect of ET antibody or SB 209670 on endotoxin-induced renal failure. J Cardiovasc Pharmacol, 26 Suppl (3):S476-478, 1995.

48. Windamn FK : Enzymatic colorimeteric determination of blood urea nitrogen. Goodale's Clinical Interperetation of Laboratory Tests, FA Davis Co, Phildelphia, P 96, 1969.

49. Yamano M, Umeda M, Miyata K, Yamada T : Protective effect of the combined treatment of pancreatic and neutrophil elastase inhibitors on acute pancreatitis elicited by lipopolysaccharide in rats given intraductal injection of taurocholate plus trypsin. Naunyn Schmiedebergs Arch Pharmacol, 357:558-564, 1998.

50. Yelich MR : The effect of epinephrine on isuline and glugacon secretion from the endotoxic rat pancreas. Pancreas, 8:450- 458, 1993.

51.Yelich MR, Janusek WL : Glucose, lactate, insulin and somatostatin responses to endotoxin in developing rats. Shock, 2: 438-444, 1994.

52. Yoshikawa TTH, Takahashi S, Ichikawa H, Kondo M : Changes in tissue antioxidant enzyme activities and lipid peroxides in endotoxin-induced multiple organ failure. Circ Shock, 42: 53-58, 1994.

53. Yoshinori H, Kato S, Ohki E, Hamamatsu H, Fukumura D, Kurose I, Suzuki H, Suematsu M, Miura S, Ishii H : Effect of lipopolysaccharides on erythrocyte flow velocity in rat liver. J Gastroenterol, 32:783-790, 1997.

54. Yun-Choi HS, Pyo MK, Chang KC, Lee DH : The effects of higenamine on LPS-induced experimental disseminated intravascular coagulation (DIC) in rats. Planta Med, 68:326-329, 2002.

55. Zhang C, Walker LM, Mayeux PR : Role of nitric oxide in lipopolysaccharide-induced oxidant stress in the rat kidney. Biochem Pharmacol, 59:203-209, 2000.

Correspondence
Ezzat S. El Daly
Zoology Department,
Faculty of Science,
Helwan University,
Cairo, EGYPT.
e-mail: eeldaly@yahoo.com