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EFFECTS OF DIABETES ON CYTOKINES AND OXIDATIVE ORGAN INJURY IN A RAT MODEL OF SEPSIS

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Abstract

We aimed to investigate how Diabetes Mellitus (DM) affects myeloperoxidase activity, antioxidant status, and lipid peroxidation using biochemical approaches in heart, liver, and lung and serum cytokine analyses, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in rat with sepsis induced by a cecal ligation and puncture-induced (CLP) sepsis. The rats were divided into four groups: control group, diabetic group, sepsis group, and diabetic+sepsis group. DM was induced in the male Wistar albino rats by administration of alloxan. Polymicrobial sepsis was induced by cecal ligation and two-hole puncture. After alloxan administration, all groups of rats were allowed to recover for 1 month. CLP model was applied after 1 month recovery to group 3 and 4. IL-6 and TNF-α, were measured. Effects of antioxidant defenses on the DM and/or sepsis process, the antioxidant levels superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) were evaluated in heart, lung and liver tissues. The oxidant levels, such as lipid peroxidation (LPO) and myeloperoxidase (MPO) levels were also evaluated in tissues. We demonstrated DM to augment the level of oxidant and proinflammatory cytokines in lung, liver, and heart and also to exacerbate oxidative injury as assessed by increased LPO and MPO, and decreased GSH and SOD levels in a sepsis model. DM increased levels of proinflammatory cytokines while DM also resulted in significantly increased levels of proinflammatory cytokines following CLP. DM-increased plasma proinflammatory cytokines levels correlated positively with tissue oxidant levels, such as MPO and LPO levels in a rat abdominal sepsis model, based on CLP, which resulted in the exacerbation of oxidative organs injury.

Key words: Diabetes Mellitus, Sepsis, Oxidant stress, Rat, Cytokine.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder, increasing a patient's risk for a variety of bacterial infections, such as postoperative wound infections (32), coronary heart disease, and peripheral vascular diseases (53). DM is caused by an absolute or relative loss of insulin secretion, and is characterized by hyperglycemia, which may lead to complications including severe infections, multiple organ failure, and death (12). Under hyperglycemic conditions, many recent studies have shown that production of cytokines, such as tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), and interleukin 10 (IL-10) is increased. Alloxan is an antibiotic and a chemotherapeutic agent that kills β cells in the pancreas via reactive oxygen intermediates (47); in other words, a kind of diabetogenic. It is widely used to create animal models of insulin-dependent DM. DM, by itself, increases the production of tissue damaging reactive oxygen species (ROS) which also have been suggested as a contributing factor in the pathogenesis of DM (2-4, 31, 40, 57). Our interest was in determining whether under the diabetogenic conditions as a second oxidative stress would alter the antioxidant defense system under sepsis conditions. Leonidou et al. demonstrated that, under stress conditions, hyperglycemia correlates with increased cytokine production following sepsis (36).

CLP (cecal ligation and puncture) is an animal model that closely mirrors the clinical course of human abdominal sepsis, in which an endogenous septic focus results in a polymicrobial infection with systemic inflammatory response syndrome (SIRS) (61). Bacterial invasion of the peritoneal cavity due to intestinal leakage leads to organ failure, septic shock, and death (6). Sepsis is a complex inflammatory response of the host to bacterial infection and remains the leading cause of death, despite appropriate antimicrobial therapy. It frequently occurs after hemorrhage, trauma, or abdominal surgery, causing varying degrees of infection, ranging from SIRS to septic shock (16). Mortality in SIRS, which continues to be the most common cause of morbidity and mortality in infectious diseases, is about 26% (49). In the United States, sepsis results in 200,000 deaths per year (5). Sepsis is frequently associated with acute multiple tissue injury (14, 28, 58). Increased vascular permeability of the endothelium occurs in multiple or-

gans during sepsis, leading to plasma extravasations, and subsequent bacterial translocation may play an important role in the development of multiple tissue injury (55). Also large numbers of neutrophils, which accumulate in the injured tissues, can trigger the inflammatory process (37). This septic host response results in the over-expression of inflammatory cytokines (23), such as TNF-α and IL-1 which lead to SIRS, multiple organ failure, and death. It has been shown that one of the important mechanisms that is associated with increase in tissue and plasma oxidative stress, due to the generation of free radicals, correlates in intra-abdominal sepsis (18, 35). It has been known for over 30 years that oxidants play a major role in inflammation and multiple tissue injury (51). Under normal physiological conditions, a homeostatic balance exists between the formation of oxygen free radicals and their removal by endogenous scavenging antioxidants (30). During sepsis, there is an overproduction of oxygen free radicals, the natural scavenging mechanisms are unbalanced, and the processes which are implicated in microvascular dysfunction are followed by organ dysfunction (46). In a clinical study, Starkopf et al. demonstrated a sepsis-induced increase in lipid peroxidation levels and decrease in serum antioxidant capacity (54). Uyanik et al. also showed that DM increased sepsis induced kidney injury in ovariectomized rats (58). Although oxidative stress and DM have been studied in different sepsis models, to our knowledge there has not been a comparative study of the combined relationship of tissue oxidative stress, antioxidative response, and plasma cytokines on DM and sepsis.

In the light of the established findings, we aimed to investigate how DM affects myeloperoxidase (MPO) activity, antioxidant status, and lipid peroxidation using biochemical approaches in heart, liver, and lung and serum cytokine analyses, such as IL-6 and TNF-α in rats with sepsis induced by CLP.

MATERIALS and METHODS

Animals

In the present study, sixty, 12-week-old, male Wistar rats were used for all experiments, each weighing 150–170 g, obtained from Ataturk University's Experimental Animal Laboratory of Medicinal and Experimental Application and Research Center (ATADEM). Animal experiments and procedures were performed in accordance with the national guidelines for the use and care of laboratory animals, and were approved by Ataturk University's local animal care committee. Rats were housed in standard plastic cages on sawdust bedding in an air-conditioned room at 22±1°C under lighting controls (14 h light/10 h dark cycle). Standard rat chow and tap water were given ad libitum.

Chemicals

All chemicals for laboratory experimentation were purchased from Sigma Chemical Co. (Germany).

Experimental Design

The rats were divided into four groups, each containing fifteen individuals: a control group (intact), a diabetic control group, a CLP-induced sepsis group, and a diabetic + CLP-induced sepsis group. The groups were kept separated in different cages. All groups of rats weighed and summarized in Table 5 as presence initial weight. After alloxan administration, all groups of rats were allowed to recover for 1 month (groups 1, 2, 3, 4). After 1 month of groups of rats weighed again and summarized in Table 5 as presence final weight. A CLP model of polymicrobial sepsis was applied after 1 month recovery to group 3 and 4.

Alloxan-induced DM

DM was induced in the male Wistar albino rats by intraperitoneal administration of aqueous alloxan monohydrate (Sigma-Aldrich Co.) at a single dose of 150 mg/kg body weight as previously described (29). The alloxan was dissolved in 0.9% NaCl solution, freshly prepared, and injected intraperitoneally to rats that had fasted for one night. In the non-diabetic group, 0.9% NaCl solution was injected intraperitoneally at the same volume. After alloxan administration, the pancreas secretes insulin at high levels; fatal hypoglycemia can occur. To prevent this adverse effect, 4–6 h after the alloxan treatment, 5 ml of 20% glucose solution were injected intraperitoneally. Then 5% glucose solution was added to the drinking water of the rats for 24 h to prevent possible hypoglycemia. Fasting blood samples were collected 72 h later, via the tail vein, and blood glucose levels were measured with an Accu-Check Active blood glucose monitor (21). At the end of third day, animals that had serum glucose levels higher than 200 mg/dl were considered diabetic and were included in the study. Body weight, blood glucose and appetite were monitarized 30 days after alloxan injection. Daily food intake of the rats was decreased in diabetic rats groups and related weight loss was observed. However, blood glucose levels were always higher that 200 mg/dl during the experiment.

Sepsis Model

A CLP model of polymicrobial sepsis was applied in the rats. Polymicrobial sepsis was induced by cecal ligation and two-hole puncture, as described by Chaudry, with minor modification (61). Anesthesia was induced by intraperitoneal administration of tiopenthal 25 mg kg^{-1}. After shaving the abdomen, the peritoneum was opened. Once the diaphragm exposed the abdominal organs, the cecum was isolated and ligated with a 3/0 silk ligature just distal to the ileocecal valve. Two punctures were made with a 12-gauge needle through the cecum distal to the point of ligation and the cecum was returned to the peritoneal cavity. The abdominal incision was then closed with a 4/0 sterile synthetic absorbable suture. The wound was bathed in 1% lidocaine solution to provide analgesia. All of the animals were given 2 ml/100 g body weight of normal saline subcutaneously at the time of surgery, and also 6 h after their operations, for fluid resuscitation. The rats were deprived of food postoperatively, but had free access to water for the next 24 h until they were killed. All 4 groups of rats were killed 24 h later by an overdose of a general anaesthetic (thiopental sodium, 50 mg/kg). Cardiac blood samples were collected immediately and transferred to the laboratory for estimation of TNF-α and IL-6 levels in serum. The lungs, livers, and hearts were rapidly removed from all rats and washed in ice-cold saline. They were la-

beled and stored at –80 ^{0}C until time of analysis.

TNF-α and IL-6 Cytokine Measurements in Serum

Sera from the 4 rat groups were separated and stored at −80 ^{0}C until thawing at time of assay. IL-6 and TNF-α were measured from one sample with highly-sensitive ELISA kits (Biosource, USA), specific for rat cytokines, according to the manufacturer's instruction. Cytokine assays for each animal and matched control were run in the same lot.

Biochemical Investigation of Liver, Lung, and Heart Tissues

After macroscopic analyses, superoxide dismutase (SOD), catalase (CAT), myeloperoxidase (MPO), lipid peroxidation (LPO) activity, and glutathione (GSH) enzyme levels in rat liver, lung, and heart tissues were determined. To prepare the tissue homogenates, tissues were ground with liquid nitrogen in a mortar. The ground tissues (0.5 g each) were then treated with 4.5 mL of the appropriate buffer. The mixtures were homogenized on ice using an Ultra-Turrax Homogenizer for 15 min. The homogenates were filtered and centrifuged using a refrigerated centrifuge at 4 ^{0}C. These supernatants were then used for the determination of enzymatic activities. All assays were performed at room temperature in triplicate.

SOD Activity

Measurements were made according to Sun et al. (56). SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitro blue tetrazolium (NTB) to form formazan dye. SOD activity was then measured at 560 nm by the degree of inhibition of this reaction, and was expressed as millimole per minute per milligram of tissue (mmol. min-1. mg tissue-1).

CAT Activity

Decomposition of H_2O_2 in the presence of catalase was performed at 240 nm (1). CAT activity was defined as the amount of enzyme required to decompose one nanomole of H_2O_2 per minute at 25 ^{0}C and pH 7.8. Results are expressed as millimole per minute per milligram of tissue (mmol. min-1. mg tissue-1).

MPO Activity

MPO activity was measured according to the modified method of Bradley et al. (11). The homogenized samples were frozen and thawed three times, and then centrifuged at 1500 g for 10 min at 4 ^{0}C. MPO activity was determined by adding 100 µl of the supernatant to 1.9 ml of 10 mmol/L phosphate buffer (pH 6.0) and 1 ml of 1.5 mmol/L o-dianisidine hydrochloride containing 0.0005% (wt/vol) hydrogen peroxide. The changes in each sample's absorbance at 450 nm were recorded on a UV–vis spectrophotometer. MPO activity in all tissues was expressed as micromole per minute per milligram of tissue (µmol min^{-1} mg tissue^{-1}).

Determination of LPO

LPO in tissue was determined by estimating the level of malondialdehyde (MDA) using the thiobarbituric acid test (42, 44). The rats' tissues were promptly excised and rinsed with cold saline. To minimize the possibility of interference of haemoglobin with free radicals, any blood adhering to the mucosa was carefully removed. The corpus mucosa was scraped, weighed, and homogenized in 10 mL of 100 g/L KCl. The homogenate (0.5 mL) was added to a solution containing 0.2 mL of 80 g/L sodium laurylsulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate, and 0.3 mL distilled water. The mixture was incubated at 98 ^{0}C for 1 h. Upon cooling, 5 mL of n-butanol:pyridine (15: l) was added. The mixture was centrifuged for 30 min at 4000 rpm. The supernatant was measured at 532 nm and a standard curve was obtained using 1,1,3,3-tetramethoxypropane. The recovery was over 90%. The results were expressed as nanomole of MDA per gram of tissue (nmol MDA/g).

Total GSH Determination

The amounts of GSH in the tissues were measured according to the method of Sedlak and Lindsay (50). The mucosal surface of the tissue was collected by scraping, and was then weighed and homogenized in 2 mL of 50 mM Tris–HCl buffer containing 20 mM EDTA and 0.2 M sucrose, pH 7.5. The homogenate was immediately precipitated with 0.1 ml of 25% trichloroacetic acid and the precipitate was removed after centrifugation at 4200 rpm for 40 min at 4 ^{0}C. The supernatant was used to determine GSH using 5,5'-dithiobis (2-nitrobenzoic acid). Absorbance was measured at 412 nm using a spectrophotometer. The results of the GSH levels in the tissues were expressed as nanomoles per milligram of tissue (nmol mg tissue^{-1}).

Determination of serum enzymes like aspartataminotransferase (AST), alanine aminotransferase (ALT)

The separated serums were used for the determination of ALT (24) and AST (25) activities with an autoanalyzer (Comas C-501).

Statistical Analysis

Data for serum cytokine levels measured by ELISA were subjected to one-way analysis of variance (ANOVA) using SPSS 13.0 software. Differences among the groups were obtained using the LSD option and were considered significant at $p<0.05$. A statistical analysis of oxidant and antioxidant enzymes was carried out using one-way ANOVA followed by Duncan's multiple range test (DMRT) using the SPSS software package, version 12.00 and were considered significant at $p<0.05$. All the results were expressed as mean ± SE for the 6 rats in each group. Significance between mortality rates was determined by the aid of chi-square test and Fisher exact test.

RESULTS

Mortality

In the sepsis group, 6 (6/15 – 40%) rats died between

12 and 24 h after CLP-induced sepsis ($p<0.02$), while no mortality was observed in the intact control group or the DM control group. In the DM with sepsis group, 9 (9/15 – 60%) of the rats were died between 6 and 24 h (Figure 1). When we compared the mortality rates in the DM+sepsis group with DM group, we could see that the survival rate in the DM+sepsis group was significantly decreased ($p<0.007$). At the end of the experiment there were 6 rats in the CLP+DM group, 9 rats in the sepsis group, 15 rats in the intact group and 15 rats in the DM group. We did not handle samples from dead animals for histopathology, serum examination and biochemistry. Thus, we selected 6 randomized animals from each groups for further experiments in histopathology, serum examination and biochemistry (6 rats from CLP group, 6 rats from DM+CLP group, 6 rats from intact group and 6 rats from DM group).

Biochemical Results for Oxidant and Antioxidant Levels of Lung Tissue in Rats

In order to explore the effects of antioxidant defenses on the DM and/or sepsis process, the antioxidant levels (SOD, CAT, GSH) were evaluated in all lung tissues. The oxidant levels, such as lipid peroxidation levels and the enzymatic activities of MPO were also evaluated in all lung tissues. The results that are presented in Table 1 show that SOD activities and GSH levels for the DM group, sepsis group, and DM + sepsis groups were lower and that the CAT, MPO, and LPO levels were higher than those of the healthy intact rat group. DM had a similar effect on the SOD and CAT activities, but not much more than in the sepsis and DM + sepsis groups. The results showed that there was no significant alteration in the SOD activities of the sepsis and sepsis+DM groups when compared with each other. There was no significant alteration of the LPO activities of the DM and sepsis groups when compared with each other. On the other hand LPO activities of the sepsis + DM group were higher when compared with both DM and sepsis groups in the lung tissue.

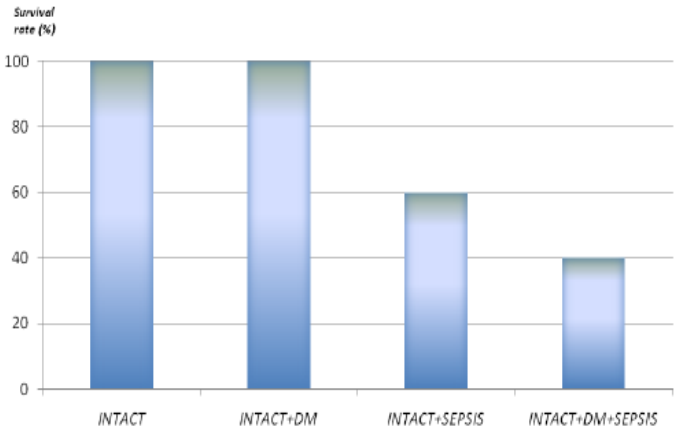

Figure 1 Survival rates (%) of the experimental groups.

Biochemical Results for Oxidant and Antioxidant Levels of Liver Tissue in Rats

In order to explore the effects of antioxidant defenses on the DM and/or sepsis process, antioxidant levels (SOD, CAT, GSH) were evaluated in all liver tissues. The oxidant levels, such as lipid peroxidation levels and the enzymatic activities of MPO were also evaluated in all liver tissues

The results that are presented in Table 2 show that GSH levels for the sepsis group and the DM + sepsis groups were lower and the CAT, MPO, and LPO levels were higher than those of the healthy intact rat group. SOD activities of the DM group were lower when compared with the DM + sepsis and sepsis groups. SOD level for sepsis and DM+sepsis were both higher than intact.

Biochemical Results for Oxidant and Antioxidant Levels of Heart Tissue in Rats

In order to explore the effects of antioxidant defenses on the DM and/or sepsis process, antioxidant levels (SOD, CAT, and GSH) were evaluated in all heart tissues. The oxidant levels, such as lipid peroxidation and enzymatic activities of MPO were also evaluated in all heart tissues.

The results that are presented in Table 3 show that the GSH levels for the DM group, the sepsis group, and the

Table 1. Effects of diabetes and/or sepsis on changes in total glutathione (GSH), lipid peroxidation levels, enzymatic activities of myeloperoxidase (MPO), catalase (CAT) and superoxide dismutase (SOD) of lung tissues.

	Number of Animal	CAT activity (mmol/min/mg tissue)	SOD activity (mmol/min/ mg tissue)	MPO activity (µmol/min/ mg tissue)	Amount of LPO (nmol/g tissue)	Amount of GSH (nmol/mg tissue)
(LUNG)						
Intact	6	166.1±2.8a	165.2±0.3c	14.49±0.23a	14.59±0.08a	3.22±0.04c
Intact+Diabetes	6	176.3±1.2b	154.2±1.5b	19.45±0.35c	15.61±0.26b	3.05±0.03c
Intact+Sepsis	6	182.1±1.2c	148.8±0.9a	17.23±0.23b	15.71±0.18b	2.76±0.05b
Intact+Diabetes+Sepsis	6	197.1±1.1d	148.2±1.2a	25.98±0.47d	16.79±0.08c	2.58±0.08a

Means in the same column by the same letter are not significantly different to the One Way ANOVA-Duncan test ($\alpha=0.05$). Results are means ± SE of three measurements.

Table 2. Effects of Diabetes and/or sepsis on changes in total glutathione (GSH) ve lipid peroxidation levels, enzymatic activities of myeloperoxidase (MPO), catalase (CAT) and superoxide dismutase (SOD) of liver tissues.

	Number of Animal	CAT activity (mmol/min/mg tissue)	SOD activity (mmol/min/ mg tissue)	MPO activity (µmol/min/ mg tissue)	Amount of LPO (nmol/g tissue)	Amount of GSH (nmol/mg tissue)
(LIVER)						
Intact	6	90.2±0.4a	115.9±1.6b	14.4±0.7a	12.36±0.71a	3.48±0.01d
Intact+Diabetes	6	98.9±0.7b	106.3±0.7a	20.1±0.9b	31.82±0.24b	3.26±0.01c
Intact+Sepsis	6	108.6±0.3c	136.1±1.4d	38.4±1.4c	59.93±1.48c	3.03±0.02b
Intact+Diabetes+Sepsis	6	123.3±1.8d	131.4±0.5c	43.8±0.7d	74.09±0.87d	2.83±0.02a

Means in the same column by the same letter are not significantly different to the One Way ANOVA-Duncan test (α=0.05). Results are means ± SE of three measurements.

Table 3. Effects of Diabetes and/or sepsis on changes in total glutathione (GSH) ve lipid peroxidation levels, enzymatic activities of myeloperoxidase (MPO), catalase (CAT) and superoxide dismutase (SOD) of heart tissues.

	Number of Animal	CAT activity (mmol/min/mg tissue)	SOD activity (mmol/min/ mg tissue)	MPO activity (µmol/min/ mg tissue)	Amount of LPO (nmol/g tissue)	Amount of GSH (nmol/mg tissue)
(HEART)						
Intact	6	122.3±2.6a	142.0±0.8b	7.99±0.28b	15.60±0.05a	3.83±0.01d
Intact+Diabetes	6	154.5±1.0b	132.8±0.1a	6.57±0.28a	30.09±1.75b	3.56±0.02b
Intact+Sepsis	6	1173.1±1.3c	157.9±0.8d	17.61±0.67c	42.42±1.18c	3.62±0.02c
Intact+Diabetes+Sepsis	6	202.1±0.4d	153.0±1.3c	16.41±0.30c	55.36±0.18d	3.20±0.02a

Means in the same column by the same letter are not significantly different to the One Way ANOVA-Duncan test (α=0.05). Results are means ± SE of three measurements.

DM + sepsis group were lower from healthy intact rat group. SOD, CAT, MPO, and LPO levels for the sepsis group, and the DM + sepsis group were higher than those of the healthy intact rat group. For the DM group CAT, and LPO levels were higher; SOD and MPO levels are lower than intact group.

SOD activities of the DM group were lower when compared to the DM + sepsis and sepsis groups. The results showed that there was no significant alteration in the MPO activities of the sepsis and sepsis + DM groups when compared with each other.

Effects of DM and/or Sepsis in CLP-induced Sepsis of Rats on Serum IL-6 and TNF- α Levels

The data in Table 4 show that CLP-induced sepsis significantly increased the serum TNF-α and IL-6 levels, when compared to the intact. DM caused a significant increase in serum levels of TNF-α and IL-6 in comparison to the intact group. The strongest production of TNF-α and IL-6 in serum was observed in the group that had CLP-induced sepsis + DM.

The data in Table 4 show that the DM group had significantly increased serum levels of IL-6 and TNF-α in comparison to the intact group. CLP-induced sepsis increased the serum levels of IL-6 and TNF-α to 43.60±2.0 pg/mL and 230.57±14.5 pg/mL, respectively; compared to 13.25±0.8 pg/mL and 39.37±2.6 pg/mL in the intact control group. The serum levels of IL-6 and TNF-α in the DM + CLP-induced sepsis groups increased to 220.24±21.4 pg/mL and 374.20±18.6 pg/mL, respectively; compared to values of 33.25±2.1 pg/mL and 77.56±12.6 pg/mL in the DM control group. Table 4 also shows that the CLP-induced sepsis groups had significantly increased serum IL-6 and TNF-α level compared to the DM control groups.

Table 4. Effects of Diabetes and/or sepsis on serum IL-6 and TNF- α levels (pg/mL) were compared. *Significant at $p<0.05$; **Significant at $p<0.001$ in LSD test. (N:Number of Animals)

Groups	**N**	**IL-6**	**TNF-α**
Intact	6	13,25±0,8	39,37±2,6
Intact + Sepsis	6	43,60±2,0*	230,57±14,5**
DM	6	33,25±2,1*	77,56±12,6*
DM + Sepsis	6	220,24±21,4**	374,20±18,6**

Effects of DM and/or Sepsis in CLP-induced Sepsis of Rats on Serum Glucose, ALT, AST and Weights of the Rats

As shown in Table 5 and 6 alloxan-treated animals pre-

Table 5. Differences between weights of the rats before (initial weight) and after waiting period one month later (final weight).

Groups	Number of Animals	Initial weight	Final weight	Difference (%)
Intact	15	155±5	176±11	+13.5
Intact+Diabetes	15	161±8	140±6	-13.0*
Sepsis	15	159±5	183±9	+15.1
Sepsis+diabetes	15	163±7	139±7	-14.7*

* means p<0.001 when compared to intact group.

Table 6. Blood glucose levels of the rats after waiting period

Groups	Number of Animals	Before Alloxan application	After Alloxan application
Intact	15	103±7.1	99.8±4.4
Intact+Diabetes	15	98.2±4.7	398.7±22.1*
Sepsis	15	106.4±5.5	101.2±3.9
Sepsis+diabetes	15	102.3±6.1	415±31.3*

* means p<0.001 when compared to intact group.

sented increased blood glucose levels and reduced weight significantly when compared the intact group. The effects of sepsis and DM on the serum enzymes ALT and AST are shown in Figure 2. Alloxan administration significantly increased the activities of the serum enzymes when compared with the control (P<0.05). The strongest production of ALT and AST serum levels was observed in the group that had both DM + CLP-induced sepsis (P<0.05). Also sepsis significantly increased the activities of the serum enzymes when compared with the control (P<0.05).

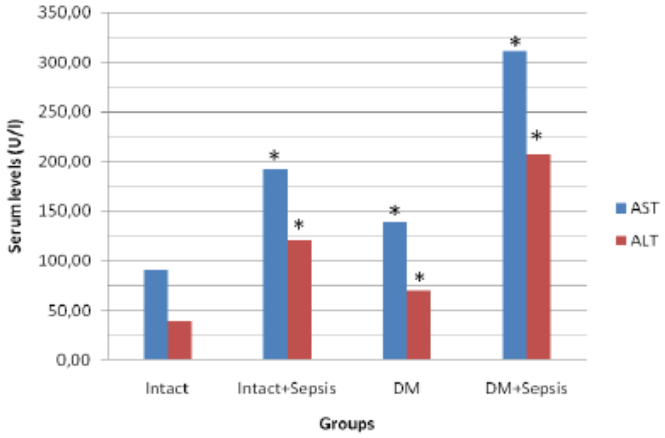

Figure 2. Serum levels of AST and ALT in experimental groups. N=6 for each group, * means p<0.01 when compared to intact group according to LSD option of one way ANOVA.

DISCUSSION

Many of the problems in DM involve inflammation, sepsis, tissue damage, and oxidative stress. This study investigated the relationship between tissue levels of LPO, GSH, CAT, SOD and plasma proinflammatory cytokines, such as IL-1, and TNF-α in a rat intra-abdominal sepsis model. An accepted animal model was chosen, cecal ligation and perforation, which mimics the clinical situation of bowel perforation and bacterial infection in humans (9). In the present study, the late phase of sepsis was accepted to be equal to the experimental model at the 24th hour. In our study, the sepsis group of animals suffered a mortality rate of 40% (6/15) and the DM + sepsis group of animals suffered a mortality rate of 60 % (9/15).

In the present study, we demonstrated DM to augment the level of oxidant and proinflammatory cytokines in lung, liver, and heart and also to exacerbate oxidative injury as assessed by increased LPO and MPO, and decreased GSH and SOD levels in a CLP-induced sepsis model. Exposure of rats to CLP increased MPO activity and LPO levels in lung, liver, and heart tissue, indicating the infiltration of polymorphonuclear neutrophils and the development of oxidative tissue injury. One of the key features of sepsis is inflammation, which is characterized by tissue infiltration and activation by phagocytic cells, a process involving the secretion of an array of inflammatory cytokines (37, 60). Following tissue infiltration and activation by phagocytic cells, the release of a cascade of pathophysiologically uncontrolled proinflammatory mediators occurs, such as TNF-α (10), which can be responsible for the ongoing interactions of different cell types and can aggravate the inflammation and cause multisystem organ failure in experimental models of sepsis and in clinical settings (17). Increased serum early release of proinflammatory cytokines is important in the pathogenesis of septic shock (8, 18) . TNF-α, which plays a pivotal role in the inflammatory process, was also augmented by DM. Similarly, in our study, DM induced by alloxan for 1 month resulted in increased levels of proinflammatory cytokines (TNF-α, IL-6), while DM also resulted in significantly increased levels of proinflammatory cytokines (TNF-α, IL-6) following CLP. These data suggest that the ability of these rats (DM+sepsis) to produce more inflammatory cytokines in response to CLP-induced sepsis may, in part, account for a significant increase in the survival of

"septic-only" rats. The mechanisms by which DM exerts a stimulator effect on proinflammatory cytokine levels may involve the activation of this proinflammatory expression. Many studies have shown that infection is perceived by activated immunologic cells, which in turn secrete cytokines, such as TNF-α, and activate inflammatory cells that release large amounts of reactive oxygen species (ROS), which cause cellular injury via peroxidation of membrane lipids (20). One of the important mechanisms associated with the increase in tissue and plasma oxidative stress due to the generation of free radicals correlates in intra-abdominal sepsis (35). It has been known for over 30 years that oxidants play a major role in inflammation and multiple tissue injury (51). Polymorphonuclear leukocytes (PMN) and monocytes/macrophages respond to septic stimulation by producing ROS (e.g., superoxide, hydrogen peroxide) (26). Additionally, endothelial cells are influenced by ROS that are produced during sepsis (39). Under normal physiological conditions, a homeostatic balance exists between the formation of oxygen free radicals and their removal by endogenous scavenging antioxidants (30). During sepsis, there is an overproduction of oxygen free radicals, while the natural scavenging mechanisms are unbalanced; processes which are implicated in microvascular dysfunction followed by organ dysfunction (46). In a clinical study, Starkopf et al. demonstrated an increase in the lipid peroxidation levels and a decrease in serum antioxidant capacity induced by sepsis (54). In septic shock, the levels and activities of SOD and GSH are due to the oppressive production of free radicals (13, 38). Atahan et al. suggested that overproduction of reactive oxygen species during tissue injury may cause a consumption and depletion of the endogenous antioxidant enzymes (7). Therefore, taking these established results into account, we decided to offer insight into the possible mechanism that explains the role of oxidative stress in DM in sepsis. The results are shown in our data, and they are in accordance with our hypothesis that DM exerts aggravating effects, by the increase of LPO and MPO activities as markers of lipid peroxidation. Furthermore, MPO activity in lung, liver, and heart tissue were also regressed by the effects of DM. Increased concentrations of LPO and MPO are found in rats with sepsis (27), and tissue MPO is a marker of lipid peroxidation levels and is increased in septic shock induced by CLP in rats (43). MPO and LPO activities, markers of neutrophil accumulation in tissue, were found to be elevated in the CLP group, while in the DM + sepsis groups, MPO and LPO activities were elevated much more than in the sepsis groups. GSH is an important constituent of intracellular protective mechanisms against oxidative stress (19). Ortoloni et al. showed that plasma GSH was decreased in septic shock patients (45). Another study has shown that plasma GSH levels are decreased in children with sepsis (52). Carbonell et al. showed that depletion of liver GSH potentiated the oxidative stress induced by endotoxins in rats, in which plasma lipid peroxide levels were raised (15). Ritter et al. showed that MDA and plasma SOD levels are markers of early mortality in septic rats (48). Our study showed increased tissue LPO and MPO levels and decreased GSH and SOD, 24 h after CLP, consistent with the literature (27). Our results are in concordance with those of Jacobs et al., where it was demonstrated that a decrease of SOD and GSH activities in the experimental model of septic shock induced by fecal peritonitis in rats, which provides further evidence of the important role of these antioxidant enzymes in the control of oxidative stress in diabetic sepsis patients (33). Liver, lung, and heart LPO, MPO, and GSH concentrations correlated with plasma proinflammatory cytokines concentrations. The main enzymes involved in protection against the damage caused by oxidative stress are CAT, SOD, and GSH. All of these antioxidants restrict the cytotoxic effects of toxic free radicals, which are expressed at low levels during normal conditions (34). Sauza et al. showed an increase of macrophage CAT activities, probably to prevent oxidative damage secondary to CLP in rats (22). Victor and de la Fuente demonstrated that, several times after lipopolysaccharide (LPS) was given to animals, SOD and CAT activities decreased from 4 to 24 h in peritoneal macrophages (59). We found that CAT activity, one of the protector oxidants in tissue, elevated in the CLP group, while in the DM + sepsis groups CAT activity elevated much more than in the sepsis groups in all tissues. The differences monitored from our results might be secondary to the differences between the LPS and CLP models.

Our data show that treatment with alloxan for induction of DM led to the development of hepatic injury in rats. Serum enzymes such as ALT and AST were significantly increased in the DM+sepsis group. These serum enzymes according to Zimmerman have been identified to toxic and cholestatic hepatic injuries (62). Elevation of AST has been reported to be an index of hepatocellular injury in rats, while ALT elevation is more associated with necrotic state (41). The increment in the activities of AST and ALT in the plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, giving an indication of the hepatotoxic effect of alloxan. But according to our data sepsis with DM are the most toxic effect on the liver tissue. Also our antioxidant and oxidant parameters have been supported these findings.

We conclude that DM-increased plasma proinflammatory cytokines levels correlated positively with tissue oxidant levels, such as MPO and LPO levels in a rat abdominal sepsis model, based on CLP, which resulted in the exacerbation of oxidative lung, heart, and liver injury. Hyperglycemia severely increased plasma cytokines and oxidant levels with the stages of our sepsis model. Further prospective and randomized clinical controlled trials are required to investigate oxidative stress and the antioxidative response in intra-abdominal sepsis with DM.

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