A study of the use of celecoxib in a rat model of septic shock

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Background
Septic shock is a leading cause of mortality in critically ill patients. Despite recent advances in the understanding of septic shock pathophysiology, its management remains a therapeutic challenge.
Aim
We aimed to investigate the effects of partial and selective cyclooxygenase (COX)-2 inhibition without affecting constitutive COX-1 and basal COX-2 activities in septic rats induced by single cecal ligation and puncture (SCLP).
Materials and methods
A total of 24 Sprague Dawley rats were allocated into four groups: sham, SCLP, sham+celecoxib, and SCLP+celecoxib. At 2 h after sham and SCLP operations, celecoxib (0.5 mg/kg) or vehicle (saline; 1 ml/kg) was administered orally to rats. At 18 h after drug administrations, mesenteric artery blood flow and aortic activity were measured. Blood and tissue samples were obtained for biochemical and histopathological examinations. Furthermore, survival rate was monitored throughout 96 h.
Results
Celecoxib ameliorated mesenteric perfusion and aortic activity. Survival rate was 0% at 49th hour in the SCLP group, but in the SCLP+celecoxib group, it was 42.8% at the end of 96 h. Celecoxib prevents the increase of serum aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, blood urea nitrogen, creatinine, and inflammatory cytokine (tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 levels). The decreases in tissues glutathione levels and the increases in liver, lung, spleen, and kidney malondialdehyde levels in the SCLP group were prevented by celecoxib. The histopathological protective effects of celecoxib on organ injury owing to SCLP were also observed.
Conclusion
Celecoxib showed improvement of clinical outcomes of sepsis through effects on mesenteric perfusion, aortic function, and its anti-inflammatory and antioxidative effects.

Keywords:
celecoxib, mesenteric arterial blood flow, multiple organ damage, sepsis, survival, vascular hyporeactivity

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Introduction

Septic shock is a leading cause of mortality in ICUs [1]. Its management remains a therapeutic challenge despite recent advances in understanding its pathogenesis. Cardiovascular dysfunction plays a key role in the development of multiple organ failure, the ultimate stage of septic shock, and is associated with poor outcomes. This includes marked hypotension with cardiac dysfunction (and involve many cellular and molecular pathogenesis) [2–4]. The time window for intervention is short, and treatment must promptly control the source of infection and restore hemodynamics [5]. But the point of the problem during septic shock is that vasodilation is different in the diverse vascular beds with a significant difference in vasomotor states depending on the size of the vessels. Facts state that hemodynamics does not reflect the state of microcirculation [6]. Even in the same organ, blood flow distribution can vary regionally: for example, in the kidney, endotoxemia causes significant reduction in renal cortical but not in medullary perfusion [7]. So, there is the possibility of different mechanisms involved in the regulation of microvascular and macrovascular tone.

β receptors are present in both smooth muscles and endothelial cells of vessels and are responsible for regulation of the vascular tone. A decrease of β-adrenergic functions and density in arteries was shown invarious cardiovascular pathologies such as

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hypertension, myocardial infarction, and heart failure [7–11].

Patients with septic shock are liable to adrenergic storm owing to excessive sympathetic nervous system activation [12] and the infusion of exogenous catecholamines for resuscitation. Classically, septic shock is defined as a vasodilatory shock characterized by hypotension owing to peripheral reduction of vascular resistance but also by poor response to vasoconstrictor [13]. Vascular alterations result from an impairment of vascular smooth muscle cell contraction capacity [14] and/or an endothelium [15]. Septic shock results in systemic vasodilation, vasoplegia, hyperresponsiveness to vasoconstrictors [3], and hypoperfusion of vital organs that lead to multiple organ dysfunction [4]. Correspondingly, diminished blood flow to the mesenteric vascular bed, increased vascular resistance, and impaired responsiveness to exogenously applied vasoconstrictor agents are related to hypoperfusion and multiple organ failure. The translocation of intestinal bacteria from the intestinal lumen to the systemic circulation following mesenteric ischemia was shown to contribute to high mortality rates.

Prostanoids (prostaglandins and thromboxane A2) play an important role in septic shock. Two isoforms of cyclooxygenase (COX), COX-1 and COX-2, catalyze the rate-limiting step of prostanoid biosynthesis [13,14]. Under physiological conditions, COX-1 is constitutively expressed in most tissues, whereas COX-2 is expressed at low levels in only a few tissues such as brain and kidney. The prostanoids are normally produced at low levels that regulate the homeostasis, gastrointestinal epithelial cytoprotection, intestinal barrier maintenance, mucosal secretion, smooth muscle function, and vascular tone [15]. The expression of COX-2, an inducible enzyme, is increased in inflammation, organ damage, and malignancy [6,16]. The COX-2 overexpression is associated with increased vascular and intestinal permeability, systemic dissemination of the microorganisms, massive and uncontrolled secretion of inflammatory cytokines and free radicals, development of multisystem hypoperfusion, multiple organ failure, and eventual death in sepsis [8]. Partial and selective COX-2 inhibition without affecting the constitutive COX-1 and basal COX-2 expressions is a good therapeutic target in sepsis.

Celecoxib, a selective COX-2 inhibitor, provides partial inhibition of COX-2 when it is administered to rodents by oral gavage [16]. Owing to these findings, we aimed to investigate the possible beneficial effects of celecoxib in sepsis induced by single cecal ligation and puncture (SCLP) with particular attention to the decreases in survival, mesenteric blood flow and vasopressor response of the aorta, and histopathological and biochemical injury in target organs such as liver, lung, kidney, and spleen [5].

Materials and methods

Drugs

Celecoxib in powder form was obtained from Pfizer, New York, USA.

Xylazine in solution was obtained from Adwia, Cairo, Egypt.

ELISA kits were used for biochemical measurements.

Animals

A total of 48 male albino rats (weighing 200–250 g) were purchased from the animal house of the ophthalmology institute, Giza, Egypt. The animals were housed in a temperature-controlled and humidity-controlled room (21±2°C and 30–70%, respectively) under a 12-h light/12-h dark illumination sequence with ad libitum access to tap water (drinking bottle) and standard pellet dairy chow. All experimental procedures were performed according to the recommendations from the Institutional Animal Care and Use Committee of Cairo University. Before the commencement of any intervention, this study was approved by the Institutional Animal Care and Use Committee. Animals were allocated into four groups.

Animal grouping and design of the work

Group I: sham operated and saline administrated (sham group).
Group II: SCLP operated and saline administrated (SCLP group).
Group III: sham operated and celecoxib treated [0.5 mg/kg, oral gavage (o.g.)] (sham+celecoxib group).
Group IV: SCLP operated and celecoxib treated (0.5 mg/kg, o.g.) (SCLP+celecoxib group).

Methods

Rats were administered with celecoxib (0.5 mg/kg/day, o.g.) 2 h after sham and SCLP operations. The dose of celecoxib was chosen on the basis of previous studies. Low-dose celecoxib (0.5 mg/kg) administration inhibits the overexpression of COX-2 selectively and partially. All rats were fasted 2 h before operations but

were allowed ad libitum access to drinking water [16,17].

For survival assessment, 24 rats were observed at 6-h intervals until 96 h after the sham and SCLP operations and divided into the same four groups. Another 12 rats were used for mesenteric artery blood flow (MABF) and isometric measurements, as well as histopathological and biochemical analyses. In these 24 rats, MABF was measured with a Doppler ultrasound flowmeter 20 h after the sham and SCLP operations, where 20 h after the SCLP operation represents the late phase of polymicrobial sepsis [18]. At the end of MABF measurement, blood samples were collected from retroorbital venous plexuses and were stored at −80°C until performance of the assays. After collection of the blood samples, aorta was removed for isometric measurements and the samples of liver, spleen, kidney, and lung tissues were removed for biochemical and histopathological analyses. The tissue pieces of liver, spleen, kidney, and lung were transferred to Eppendorf tubes and were stored at −80°C until the time of malondialdehyde (MDA) and total glutathione (GSH) analyses. The other tissue pieces were fixed in 10% neutral buffered formaldehyde solution for histopathologic examination.

Model of polymicrobial sepsis: interventions

Polymicrobial sepsis was induced in rats using the SCLP method as described previously.

Single cecal ligation perforation model

In brief, the rats were intraperitoneally anesthetized with xylazine (20 mg/kg) and ketamine (100 mg/kg). A 2-cm ventral midline incision was then performed, and the cecum was carefully exposed. The free distal cecum was marked using methylene, and then the cecum was punctured after the ligation. However, in the SCLP-SCLP model, the puncture holes were kept opened with a drainage bar. The cecum was tightly ligated with a 3.0-silk suture just below the ileocecal valve and punctured twice with an 18-G needle. A small amount of feces was extruded from the perforation sites via gentle squeeze of the cecum (Linlin *et al.*, 2018) [33].

The cecum was returned to its normal intra-abdominal position, and the laparotomy site was then closed in two layers using traumatic 3-0 silk sutures. After the operations, normal saline (3 ml/100 g, s.c.) was injected immediately to the nape of the neck of rats for fluid resuscitation. For the sham procedure, all of the same steps were applied, without ligation and puncture of the cecum.

Confirmation of the septic model

SCLP-operated rats began to show signs of sepsis 8–10 h after surgical manipulation, which were diarrhea, secretion from the nose and eyes, and decreased spontaneous movement. Compared with SCLP animals, sham-operated rats were active and appeared normal. After autopsy, we found in the peritoneal cavity of septic rats that there were large amounts of foul-smelling, purulent peritoneal fluid and the ligated portion of the cecum was dilated and gray-black. In the abdominal cavity of sham rats, the intestine was pink and healthy.

Measurements

Mesenteric artery blood flow measurement

The animals were anesthetized with xylazine, and a ventral midline incision was then performed again 20 h after sham and CLP operations. The mesenteric artery was carefully exposed, and blood flow was measured by a Doppler ultrasound flowmeter, which provides absolute blood flow readings (ml/min) and is located around the common mesenteric artery trunk. The animals were stabilized for 15 min before recording MABF values [19].

Isometric measurements

The changes in alpha-receptor-mediated vasoconstriction during sepsis were evaluated on isolated rat aortic rings via cumulative administration of phenylephrine, an a_1-receptor agonist, into the organ bath. After thoracotomy, the thoracic part of aorta was removed and put into a cold Krebs-Henseleit solution [as mM; sodium chloride 118, sodium bicarbonate 25, potassium chloride 4.7, calcium chloride 2, monopotassium dihydrogen phosphate 1.2 magnesium sulfate 1.2, and glucose that was gassed with carbogen (95% O_2/5% CO_2)] and kept at 37°C. The aorta preparations were allowed to stabilize in the Krebs-Henseleit solution for about 45 min with washing out of the muscles in successive 15-min intervals [20]. After this period, cumulative concentrations of phenylephrine were added into the organ baths [21,22].

Biochemical measurements

Serum activities of aspartate aminotransferase (AST, a nonspecific marker for hepatic injury), alanine aminotransferase (ALT, a specific marker for hepatic parenchymal injury), and lactate dehydrogenase (LDH, a nonspecific marker for tissue damage) and serum levels of blood urea nitrogen (BUN, a marker for the glomerular renal function) and creatinine (Cr, a marker for the glomerular filtration rate and hence

renal failure) were measured using ELISA kits (Sigma-Aldrich, USA).

Serum tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) concentrations were measured with ELISA kits that were specifically designed for rat cytokines, and all measurements were performed in accordance with the manufacturer's instructions.

As a result of oxidative damage, lipids are degraded by free radicals and end products of lipid peroxidation chain such as MDA occur. To evaluate the oxidative organ damage, MDA levels in the liver, lung, spleen, and renal tissues were assayed with a lipid peroxidation assay kit (Sigma-Aldrich) according to the manufacturer's protocol.

For antioxidant status, total GSH levels in the liver, lung, spleen, and renal tissues were measured with a NADPH linked enzymatic colorimetric GSH assay kit (Sigma-Aldrich) and analyzed in accordance with the manufacturer's protocol. The total GSH levels of samples were expressed as nmol/mg tissue protein concentration.

Histopathological examination

Microscopy

Presentative sections were sampled from dissected organs in cassettes and were fixed in formalin 10% for 24 h and then processed in a tissue processor overnight. Paraffin blocks were prepared. One section was cut from each paraffin block by microtome at 5-μm thickness and then stained with hematoxylin and eosin for routine histopathological examination.

Hematoxylin and eosin

Hematoxylin and eosin staining included the following steps: (a) deparaffinize in xylene – two changes; (b) absolute alcohol; (c) 95% alcohol; (d) rinse in tap water; (e) Harris hematoxylin – 6 min; (f) wash in tap water; (g) decolorize with 1% acid–alcohol – quick dips; (h) wash in tap water; (i) blue in 1% lithium carbonate – two to three dips; (j) if lithium carbonate is not available, use ammonia water; (k) wash with tap water (check under the microscope – nuclei should be distinct blue and the background light or colorless;) l) if it is not well stained, stain again in Harris hematoxylin for 2–3 min; (m) if overstained, decolorize again with 1% acid–alcohol – quick dips; (n) wash with tap water; (o) counterstain in 1% eosin – two to three quick dips; (p) wash quickly with tap water; (q) 95% alcohol; (r) absolute alcohol – two changes; (s) xylene – two changes; and (t) mount.

Results

Nuclei represent blue and cytoplasm represents pale pink.

Photography

The digital images of the selected tissue preparations were photographed using an Olympus DP26 digital net camera attached to a Olympus CX31 microscope.

Statistical analysis

Data were transferred to the Statistical Package of the Social Sciences Software program, version 24 (SPSS Inc., NewYork, USA), to be statistically analyzed.

Data were summarized using mean and SD for quantitative variables and frequency and percentage for qualitative ones.

Comparison between groups was performed using one-way analysis of variance with Tukey's post-hoc test for quantitative variables and χ^2 or Fisher exact test for qualitative ones.

P values less than 0.05 were considered statistically significant and less than 0.01 were considered highly significant.

Results

Group I: sham operated and saline administrated (sham group)

The 96-h survival rate was 100% (Fig. 1).

MABF obtained from anesthetized rats was 17.33 ±0.56 (Fig. 2).

The contractile response of aortic smooth muscles to phenylephrine was normal.

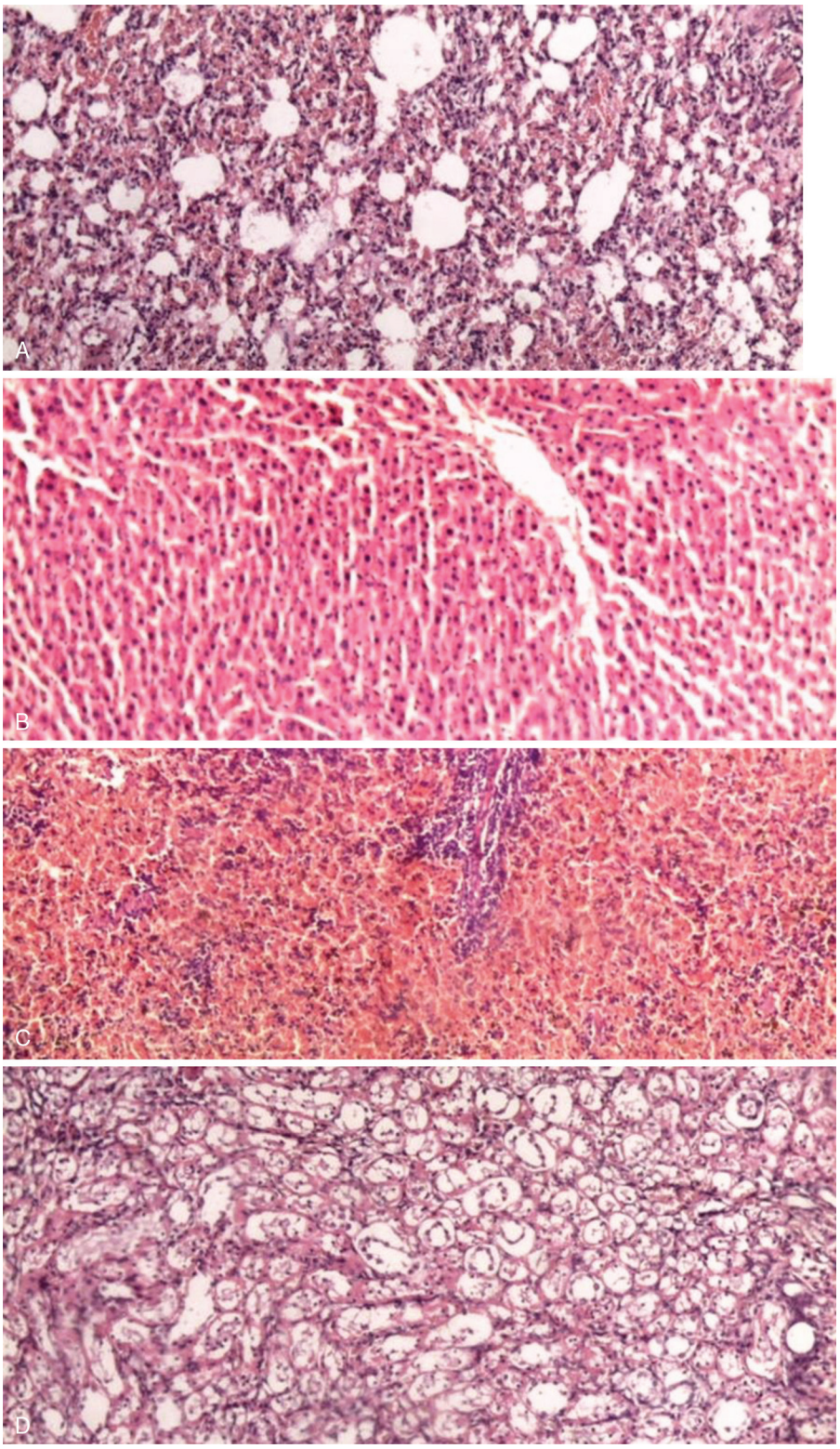
Serum TNF-α, IL-1β, and IL-6 as well as AST, ALT, LDH, BUN, and Cr were found at normal levels in the blood of rats after 20 h of start (Tables 1 and 2) . The MDA and GSH levels in the liver, lung, kidney, and spleen tissues were in the normal range (Table 3).

Histopathologically, the structures of lung, liver, spleen, and kidney tissues were normal in the sham group (Fig. 3a–d).

Group II: single cecal ligation and puncture operated and saline administrated (single cecal ligation and puncture group)

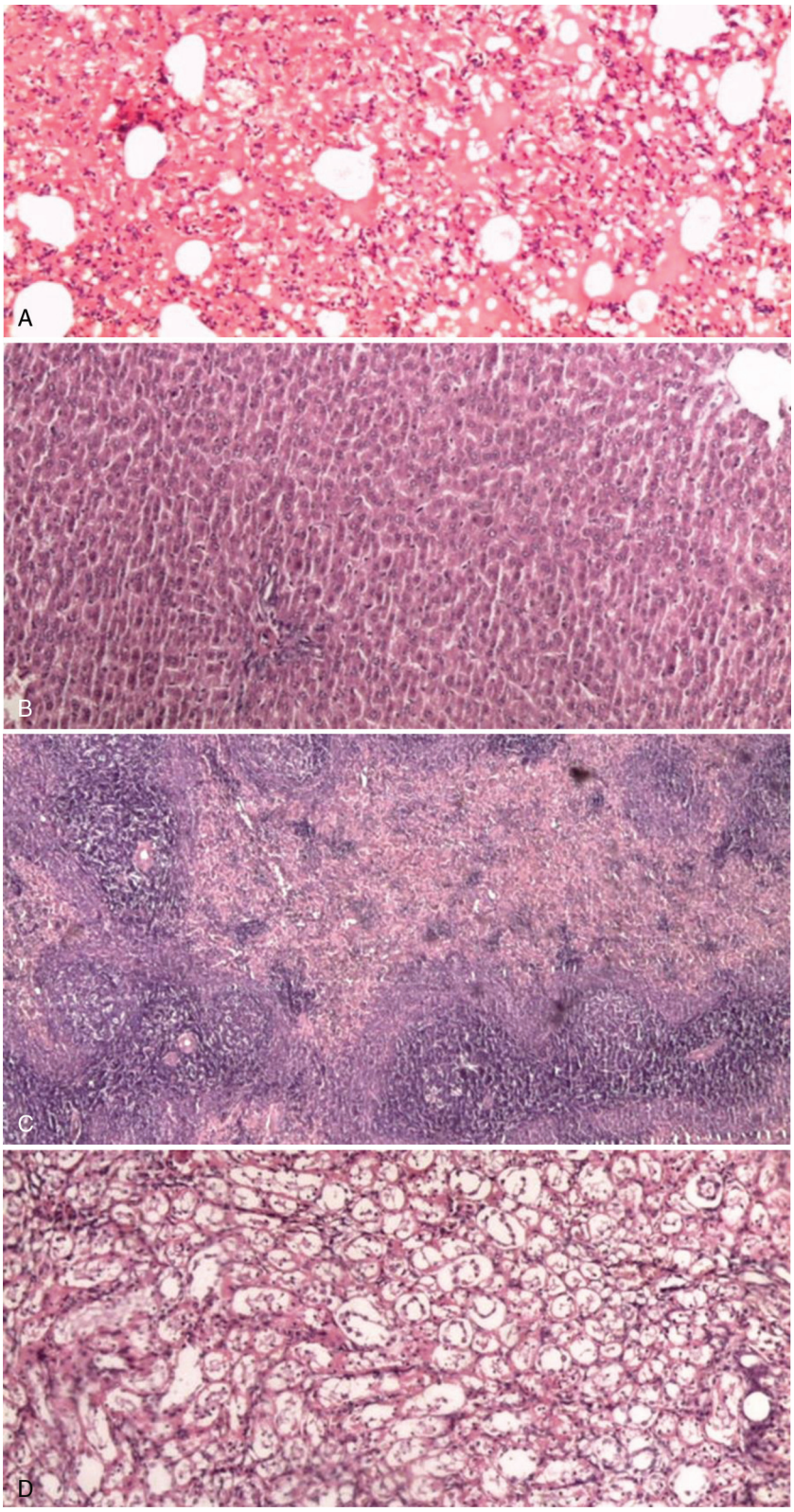
All rats died within 51 h after the SCLP operation (Fig. 1).

Figure 1



(a) Section in lung tissue. The alveoli are patent, with normal interstitium (H&E, ×200). (b) Sections revealed normal liver tissue. The portal tracts are within normal (H&E, ×200). (c) Sections in spleen showing normal tissues (H&E, ×200). (d) Section of kidney showing tubules. The interstitium showed no inflammation. The vessels are within normal (H&E, ×100). (a–d) The sham group (group I) showed normal histopathological structures of liver lung, kidney, and spleen tissues. H&E, hematoxylin and eosin.

Figure 2



(a) Section in lung tissue. The alveoli showed intra-alveolar fluid exudation and moderately congested edematous interstitium (H&E, ×200). (b) Sections revealed liver tissue. The portal tracts are expanded by mixed inflammatory cellular infiltrate with focal interface hepatitis. The hepatocytes showed focus of spotty necrosis (H&E, ×100). (c) Sections in spleen showing congested red pulp (H&E, ×100). (d) Section of kidney showing tubules with frequent sloughing. The interstitium showed no inflammation. The vessels are normal (H&E, ×100). (a–d): group II (SCLP group). H&E, hematoxylin and eosin; SCLP, single cecal ligation and puncture.

MABF obtained from anesthetized rats was 4.35±0.26 ($P<0.05$ vs. sham) (Fig. 2).

The contractile response of aortic smooth muscles to phenylephrine showed hyporeactivity.

Serum TNF-α, IL-1β, and IL-6 as well as AST, ALT, LDH, BUN, and Cr showed increased levels in the blood of rats after 20 h after SCLP (vs. sham group, $P<0.05$) (Tables 1 and 2).

MDA levels in the liver, lung, kidney, and spleen tissues showed increased levels (Table 3).

GSH levels in the liver, lung, kidney, and spleen tissues showed decreased levels (Table 3) ($P<0.05$ vs. sham group).

Histopathologically, the septic lung tissues showed moderate intra-alveolar hemorrhage, interstitial and alveolar edema, as well as mild peribronchial and interstitial inflammation, which was concentrated near the vessel wall (Fig. 4a). In the septic liver tissues, there were moderate venous hemocongestion and mild inflammatory mononuclear cell infiltration in the parenchyma and periportal area hepatocytes showed focus of spotty necrosis (Fig. 4b). In the septic spleen tissues, there were congested red pulp (Fig. 4c). Septic kidney showed frequent sloughing parenchyma (Fig. 4d).

Group III: sham operated and celecoxib treated (0.5 mg/kg, o.g.) (sham+celecoxib group)

The 96-h survival rate was 100% (Fig. 1).

MABF obtained from anesthetized rats showed significant higher MABF (22.41±0.64, $P<0.05$ compared with sham) (Fig. 2).

The contractile response of aortic smooth muscles to phenylephrine was normal.

Serum TNF-α, IL-1β, and IL-6 as well as AST, ALT, LDH, BUN, and Cr were found at normal levels in the blood of rats after 20 h of start (Tables 1 and 2). The MDA and GSH levels in the liver, lung, kidney, and spleen tissues were in the normal range (Table 3).

Histopathologically, the structures of lung, liver, spleen, and kidney tissues were normal in the sham +celecoxib group (Fig. 5a–d).

Group IV: single cecal ligation and puncture operated and celecoxib treated (0.5 mg/kg, o.g.) (single cecal ligation and puncture+celecoxib group)

Celecoxib increased the survival rate in the SCLP +celecoxib group to 46% ($P<0.01$, vs. SCLP group) (Fig. 1).

Table 1 Biochemical blood parameters

	Group I	Group II	Group III	Group IV
AST	140±18	272±31*	153±20	170±26#
ALT	60±10*	120±20**	70±14	60±10##
LDH	560±70	1120±110**	575±70	480±130#
BUN	40±10	110±7**	45±10	42±12##
Cr	0.5±0.03	0.7±0.2*	0.5±0.07	0.52±0.05#

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; LDH, lactate dehydrogenase. *Significant difference between sham versus other groups. #Significant difference between cecal ligation and puncture versus cecal ligation and puncture+celecoxib group (*,#, $P<0.05$; **,##, $P<0.01$).

Table 2 The serum interleukin-1 beta, interleukin-6, and tumor necrosis factor-alpha levels

	Group I	Group II	Group III	Group IV
IL-1β	29±4	300±98**	40±10	81±23##
IL-6	25±8	435±97***	20±6	40±9###
TNF-α	70±14	390±113***	65±14	104±14##

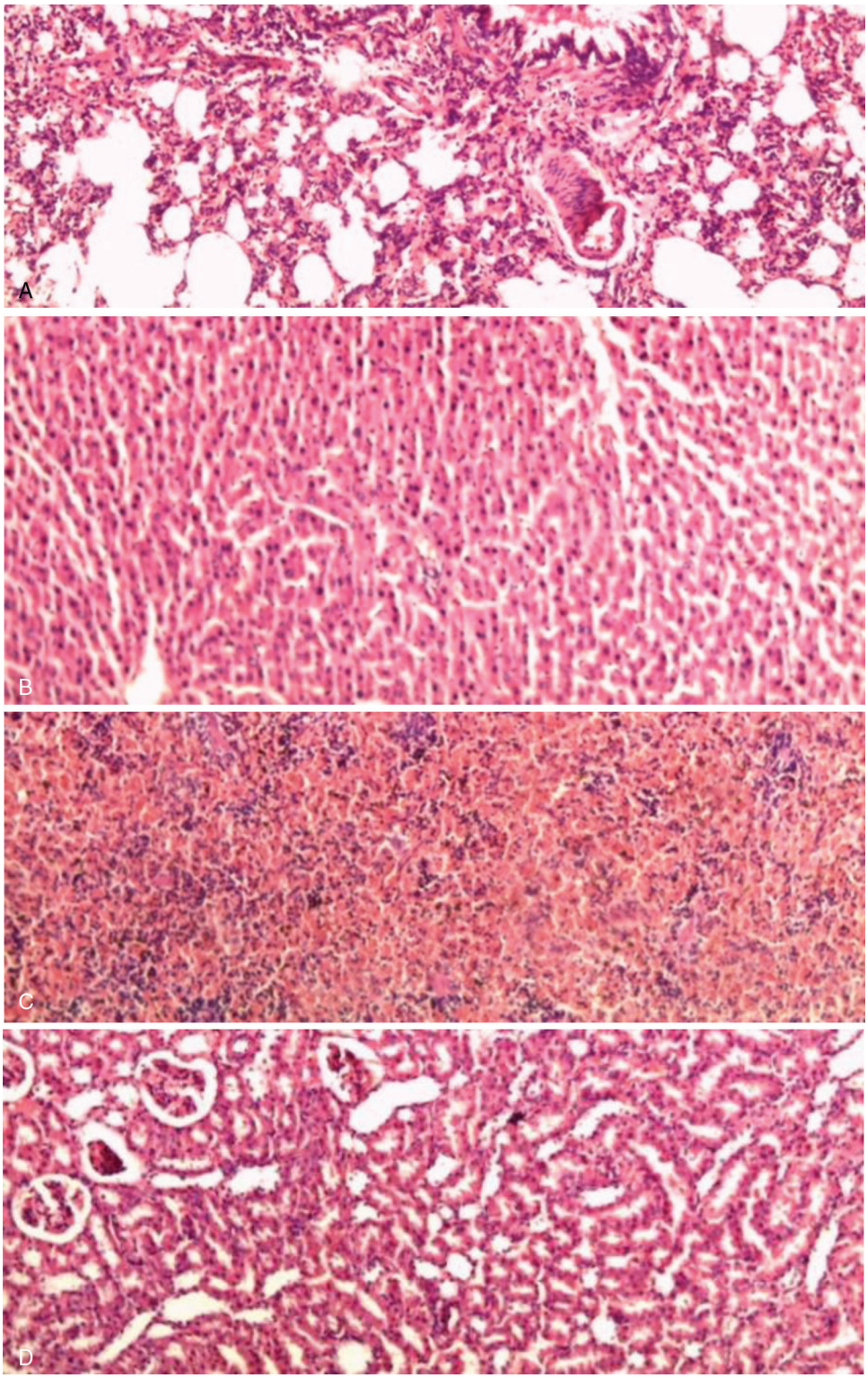
Data are shown as mean±SD. IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor-alpha. Cecal ligation and puncture versus cecal ligation and puncture+celecoxib group. *Significant difference between sham versus other groups. #Significant difference between cecal ligation and puncture versus cecal ligation and puncture+celecoxib group (**, ##, $P<0.01$; ***, ###, $P<0.001$).

Table 3 Tissue malondialdehyde and total glutathione levels

	Liver MDA	Lung MDA	Spleen MDA	Kidney MDA	Liver GSH	Lung GSH	Spleen GSH	Kidney GSH
Group I	5±0.4	4.4±0.3	3±0.27	4.7±0.7	120±21	14±2	7.4±1.2	11.65±1.3
Group II	8±1**	7.6±0.7**	5.6±0.8**	8±1.2**	33±8**	7.7±1.3**	3±0.61**	6.28±0.68**
Group III	4.8±0.6	5±0.4	3.3±0.4	5±0.79	163±16	12.7±2.83	6.6±0.87	12.66±1.4
Group IV	5.2±0.6	4.9±0.46##	3.8±0.43##	6.1±0.46##	119±18##	11±0.9	5.23±1.1#	9.5±1.4#

Data are shown as mean±SD. GSH, glutathione; MDA, malondialdehyde. Sham versus other groups. A significant difference between single cecal ligation and puncture versus single cecal ligation and puncture+celecoxib group. *Significant difference between sham versus other groups. #Significant difference between cecal ligation and puncture versus cecal ligation and puncture+celecoxib group (*,# $P<0.05$; **, ##, $P<0.01$; ***,###, $P<0.001$).

Figure 3

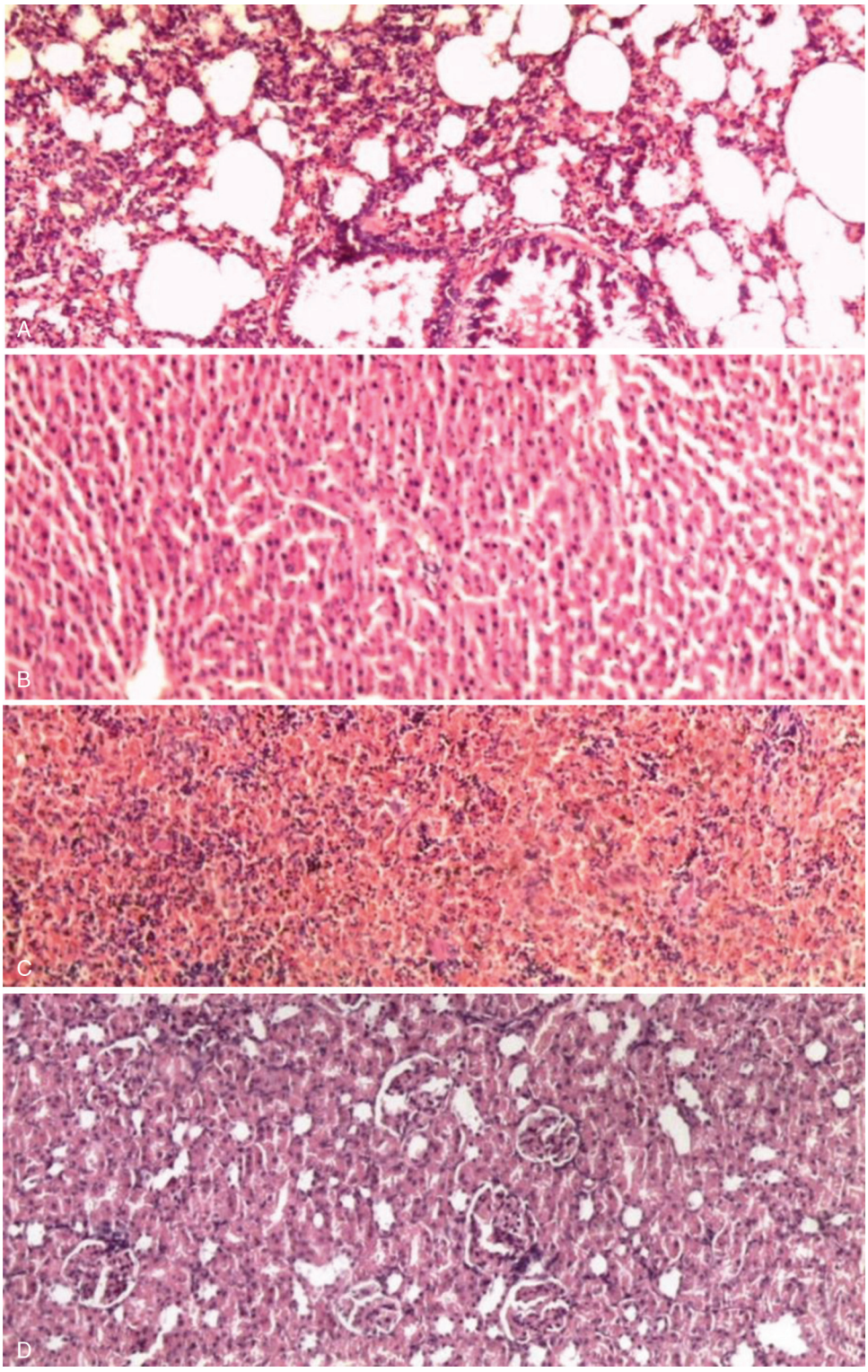


(a) Section in lung tissue. The alveoli are patent, with mildly congested edematous interstitium (H&E, ×200). (b) Sections revealed liver tissue. The portal tracts are within normal (H&E, ×200). (c) Sections in spleen showing normal structure (H&E, ×200). (d) Section of kidney showing within normal glomeruli. The tubules were within normal apart from mild epithelial sloughing. The interstitium showed no inflammation. The vessels are within normal (H&E, ×100). (a–d): the sham+celecoxib group (group III) showed normal histopathological structure of liver lung, kidney, and spleen tissues. H&E, hematoxylin and eosin.

MABF obtained from anesthetized rats was increased (19±0.53) ($P<0.01$ compared with the SCLP group) (Fig. 2).

The contractile response of aortic smooth muscles to phenylephrine showed increased activity compared with the SCLP group.

Figure 4

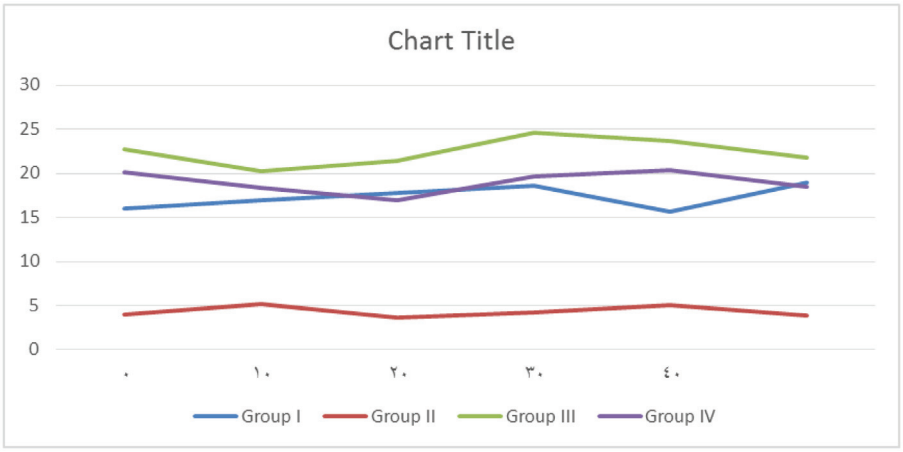


(a) Section in lung tissue. The alveoli are patent, with mildly congested edematous interstitium (H&E, ×200). (b) Sections revealed liver tissue. The portal tracts are within normal (H&E, ×200). (c) Section in spleen showing normal structure (H&E, ×200). (d) Section of kidney showing within normal two glomeruli. The tubules were within normal apart from mild epithelial sloughing. The interstitium showed no inflammation. The vessels are within normal (H&E, ×200). (a–d): group IV. H&E, hematoxylin and eosin.

Celecoxib prevents the increase of serum levels of TNF-α, IL-1β, and IL-6 as well as AST, ALT, LDH, BUN, and Cr in the blood of rats after 20 h after SCLP (Tables 1 and 2) ($P<0.01$ vs. SCLP group).

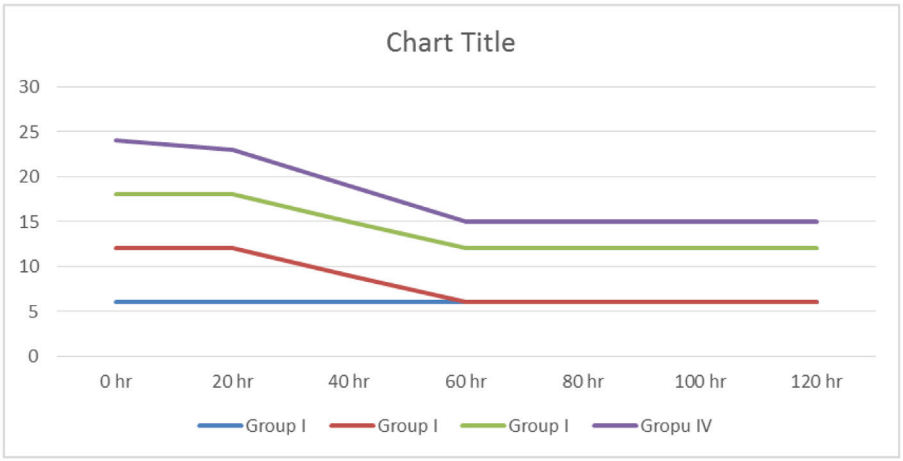
Treatment with celecoxib prevented SCLP-induced elevations of MDA levels in all of the tissues. The celecoxib treatment prevented the SCLP-induced reductions of GSH levels in lung and kidney, liver, and spleen tissues (Table 3).

Figure 5



Mesenteric arterial blood flow. A significant difference was found between sham versus other groups ($P<0.05$). A significant difference was found between SCLP versus SCLP+celecoxib group ($P<0.01$). SCLP, single cecal ligation and puncture.

Figure 6



Survival rate. The survival rate was assessedfor 96 hforthe groups.A significant differencewasfound between sham versus other groups.A significant difference was found between SCLP versus SCLP+celecoxib group ($P<0.05$; $P<0.01$; $P<0.001$). SCLP, single cecal ligation and puncture.

Histopathologically, when the rats were treated with celecoxib, the lung (Fig. 6a) and spleen (Fig. 6c) architectures was well preserved and the liver (Fig. 6b) and kidney (Fig. 6d) were completely prevented from the histopathological injuries induced by SCLP.

Discussion

Mesenteric hypoperfusion occurs in early septic shock, which leads to impaired intestinal barrier and translocation of bacteria, leading to massive and uncontrolled release of inflammatory mediators such as TNF-α, IL-1β, and IL-6 and overexpressions of the inducible isoforms of nitric oxide synthase (iNOS) and COX (COX-2) enzymes. The massive rapid release of mediators and free radicals are responsible for vascular injury and multiple organ failure.

Administration of celecoxib increases the survival rate in the SCLP+celecoxib group as a consequence of reduced dysfunction/injury of multiple organs. MABF obtained from anesthetized rats was increased. Contractile response of aortic smooth muscle to phenylephrine showed increased activity.

Celecoxib prevents the increase of the serum levels of TNF-α, IL-1β, and IL-6 as well as AST, ALT, LDH, BUN, and Cr in the blood of rats after 20 h after SCLP.

Treatment with celecoxib prevented the SCLP-induced elevations of MDA levels in all of the tissues. The celecoxib treatment prevented the CLP-induced reductions of GSH levels in lung and kidney but failed to prevent the GSH reductions in the septic liver and spleen tissues.

Histopathologically, when the rats were treated with celecoxib, the lung and spleen architectures were well preserved and the liver and kidney were completely prevented from the histopathological injuries induced by SCLP.

Vasopressors such as norepinephrine, phenylephrine, and dopamine are used to counteract the inappropriate vasodilation during septic shock. The refractory vasoplegia and mesenteric hypoperfusion are correlated with poor prognosis in sepsis. Therefore, increasing the blood flow to the mesenteric circulation and restoration of the vasoplegia are the main target for the sepsis therapy [23–25].

Nitric oxide and prostanoids are responsible for vascular and intestinal homeostasis which are constitutively produced by endothelium COX-1 and basal COX-2 activity. However, iNOS and COX-2 overexpression leads to excessive NO and prostanoid (PGI2 and PGE2) production, which are associated with sepsis. So, prevention of iNOS and COX-2 overexpression and treatment of the vascular dysfunction are vital for sepsis management [22–25]. Previous studies have found that COX-2 inhibitors do not protect but rather aggravate the barrier damage induced by experimental colitis and necrotizing enterocolitis [26,27]. Furthermore, specific COX-2 inhibitors have been found to impair the gastrointestinal homeostasis and exhibit gut toxicity, albeit not to the extent of nonspecific COX inhibitors [28,29]. These results are in agreement that both constitutive COX-1 and basal COX-2 activities are necessary for barrier protection [30]. Therefore, COX-2 inhibition must be selective and partial without affecting the constitutive COX-1 and basal COX-2 activity in sepsis therapy. Short *et al.* [16] showed that celecoxib selectively and partially inhibits the COX-2 activity when was given to the rodents at a dose of 0.5 mg/kg/o.g. We used celecoxib at this dose in our study. Celecoxib has been also reported to ameliorate the kidney ischemia/reperfusion induced by oxidants [31]. This antioxidant is very important in sepsis-induced oxidative organ damage because there is correlation between MDA levels and severity of sepsis [32].

This study provides a novel line of therapy with treatment with celecoxib, which seems to improve the survival in SCLP-induced septic rats as a consequence of reduced multiple organ failure. This can be owing to attenuation of IL-1β, IL-6, and TNF-α production, suppression of oxidative stress, and inhibition of COX-2 overexpression; moreover, celecoxib prevents mesenteric hypoperfusion in animals with SCLP-induced sepsis.

Conclusion

Our study showed that celecoxib has protective effects on sepsis and MODS owing to preservation of mesenteric perfusion, contractile function of vascular smooth muscles, and anti-inflammatory and antioxidative effects in septic rats. This study is primarily focused on demonstrating the beneficial and protective effects of celecoxib in an experimental sepsis model.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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