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Protective Effect of Pyrrolidine Dithiocarbamate to Liver Injury in a Sepsis Model with Cecum Ligation and Perforation – An Animal Study

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Abstract

Introduction: Pyrrolidine dithiocarbamate is a low molecular thiol antioxidant and is a strong inhibitor of nuclear factor kappa B activation. Recent animal studies have shown a delaying effect of intraperitoneal sepsis on healing after colon anastomosis. Our aim in this study is to use pyrrolidine dithiocarbamate in sepsis treatment to research the place of nuclear factor kappa B inhibition in preventing tissue injury and organ function disorders and the effect on organ failure caused by clinical worsening of sepsis.

Materials and methods: Our study was completed on a total of 28 rats in Istanbul University Faculty of Medicine Hospital. In our study subjects were grouped as control (n=4) liver subjects, "Group A" (n=4) (sham group), "Group B" (n=8) (Sepsis group) (laparotomy and cecal mobilization), "Group C" (n=8) Pyrrolidine dithiocarbamate subjects and "Group D" (n=8) sepsis+ Pyrrolidine dithiocarbamate subjects. Apart from the sham group (control group) rats had the polymicrobial sepsis model described in the literature of cecum ligation and perforation used to induce sepsis. Group A (sham group, n=8) had mini laparatomy performed after anaesthesia and then the abdomen was closed without cecum ligation and perforation. Group B (sepsis group, n=8) had the cecum isolated with mini laparotomy, sepsis was induced with the cecum ligation and perforation method and then the abdomen was closed. Group C (Pyrrolidine dithiocarbamate, n=8) had 100 mg/kg/day Pyrrolidine dithiocarbamate administered by gavage 15 minutes before and 12 hours after mini laparotomy procedure. Group D (sepsis+Pyrrolidine dithiocarbamate, n=8) had pyrrolidine dithiocarbamate administed by gavage 15 minutes before and 12 hours after cecum ligation and perforation to induce sepsis.

Results: When mortality in the groups is assessed, as there was no procedure performed on the sham group (performed cecum ligation and perforation or antioxidant administered) no rat died and all 8 rats were sacrificed for histopathological and immunohistochemical assessment. In the Group B of the 7 rats that died, 5 died from 24-36 hours, and 2 were exitus before 48 hours. In Group D (sepsis+ Pyrrolidine dithiocarbamate) 2 were exitus in the first 48 hours with the other 2 exitus on the 5th day and 3 rats were alive on the 10th day. In Group C (sham +Pyrrolidine dithiocarbamate) 1 rat was exitus in the first 72 hours, while the remaining 6 were still alive on the 10th day. The livers of the cases were investigated for morphology, p65 and inductible nitic oxide synthase activity after hematoxylin-eosin and immunohistochemical staining. The results were statistically assessed.

Conclusion: In these results, pyrrolidine dithiocarbamate appears to be an appropriate medication with potential efficacy for sepsis and septic shock treatment. As the results of clinical studies of antioxidants used for sepsis treatment are still controversial, there is a need for broader and longer duration studies.

Keywords: Sepsis model; Pyrrolidine dithiocarbamate; Nuclear factor kappa B; Liver injury

Introduction

Sepsis is a life-threatening systemic inflammatory response to pathologic infection and is one of the most frequent reasons for intensive care treatment of hospitalized patients worldwide [1]. Despite research advances, the full pathophysiology of sepsis and its role in the development of multi-organ dysfunction (MOD), and the related septic shock, are poorly understood [2].

The Surviving Sepsis Guidelines were first published in 2004, with revisions in 2008 and 2012. In January 2017, the fourth revision of the Surviving Sepsis Guidelines was presented at the 46th annual SCCM meeting and published online jointly in Critical Care Medicine and Intensive Care Medicine [3,4]. A synopsis of the guidelines has also been published [3]. The Third International Consensus Definitions for Sepsis and Septic Shock were published in February 2016. The new definitions

are intended to improve the clarity of the definitions for clinical care, epidemiology, quality improvement and research. The new definitions have been validated retrospectively by using large databases in the USA and Germany [4]. Sepsis is common representing a major public health problem affecting more than 19 million people each year [5]. Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [6]. A recent survey estimated its annual global incidence at 31.5 million cases, resulting in 5.3 million deaths [7]. Sepsis associated hepatic dysfunction is found among septic patients [8]. Hepatic dysfunction is a severe condition causing hepatocyte death and fibrosis, leading to incidence of high mortality rates [9].

Hepatic dysfunction may be manifested by acute cholestasis and increasing serum bilirubin concentrations. Septic hepatic dysfunction is a less common manifestation of severe sepsis, found in only 10%-13% of patients [10,11].

Despite its relative infrequency, acute hepatic dysfunction is an ominous sign that is a strong predictor of mortality in patients with severe sepsis [11,12]. In an observational study that included 29,470 patients with sepsis worldwide, every quarter of participation in the SSC initiative was associated with a significant decrease in the odds of hospital mortality (odds ratio, 0.96; 95% CI, 0.95-0.97; p<0.001) [13].

The most dominant theory for sepsis and multiorgan failure is the formation of an excessive inflammatory response causing clear cellular and organ damage due to the cytokine-mediated defence mechanism in sepsis. The increase in cytokines supports this theory. In studies nuclear factor kappa B (NF-Kβ) has been proposed to increase apoptotic cell death, and additionally if animals are exposed to lipopolysaccharides (LPS) or bacteria, it may cause NF-Kβ activation in multiple organs and cell populations. Free oxygen radicals play an important role in sepsis pathogenesis. Antioxidant agents are effective in reducing cellular injury in sepsis or endotoxemia treatment, shown by measurement of free oxygen radical activities [14]. Pyrrolidine dithiocarbamate (PDTC) is a low molecular thiol antioxidant and is a strong inhibitor of nuclear factor kappa B (NF-kβ) activation [15,16].

In this experimental study the NF-kappa B inhibitor of PDTC was administered to rats with sepsis and acute liver injury induced by cecal ligation and perforation with the aim of preventing oxidative stress and as a result reducing liver injury linked to sepsis.

Materials and Methods

Our study was completed in Istanbul University Faculty of Medicine Experimental Research Centre (DETAM) after receiving permission from Istanbul University Animal Experiments Local Ethic committee. The study used 28 adult Sprague-Dawley rats weighing from 250 to 280 g. The rats were divided into groups of 15. Apart from the sham group (control group) rats had a cecum ligation and perforation (CLP) method used to create a polymicrobial sepsis model as described in the literature [17].

Group A: (Sham group, n=4): Mini laparotomy performed after anaesthesia and then the abdomen was closed without CLP.

Group B: (Sepsis group, n=8): Cecum isolated with mini laparotomy, sepsis was induced with the CLP method and then the abdomen was closed.

Group C: (PDTC, n=8): 100 mg/kg/day PDTC (Ammonium pyrroldine dithiocarbamate, Sigma –Aldrich, CH-9471 Bucha, Switzerland) administered by gavage 15 minutes before and 12 hours after mini laparotomy procedure.

Group D: (Sepsis+PDTC, n=8): PDTC administed by gavage 15 minutes before and 12 hours after cecum ligation and perforation to induce sepsis.

Operative Procedure

Anaesthesia was induced with 50 mg/kg intraperitoneal ketamine hydrochloride and 15 mg/kg xylasine HCL. In the Group B-C and D, after waiting for anaesthesia, rats had laparotomy performed with a 3 cm midline incision. To induce sepsis in rats the CLP model was chosen.

After laparotomy the cecum was isolated, the colon was stroked to fill the cecum with stool and then below the ileocecal valve was tied with 3/0 silk and the anterior of the cecum was punctured twice with a 22 number branule needle.

In the Group A, CLP was not performed but the cecum was explored. After the abdomen was closed with continuous 3/0 silk sutures.

Rats in the all groups were placed in cages and left in rooms with humidity, temperature and light control. From the 12th hour postoperative, they were allowed standard rat feed and drinking water.

Twenty-four hours after the operative procedure, 8 rats chosen at random from each group (2 rats per all group) were sacrificed under anaesthesia and had livers removed for histopathological assessment. The other rats were monitored for mortality and their life durations were recorded. All rats had liver tissue placed in 10% formaline solution for histopathologic assessment with light microscope and stored until time of study. Sections with 5 micron thickness were obtained from tissues prepared in paraffin blocks. After these sections were stained with hematoxylin-eosin, they were investigated at 40 and 100 magnification under a light microscope. Investigations were performed by a pathology expert blind to the groups and which sections came from which groups.

Morphologic scoring

- Normal liver parenchyma and minimal cellular changes
- Swollen hepatocytes, integrity of parenchyma preserved; however portal area of parenchyma irregular with mild or moderate degree of centrolobular damage.
- Piecemeal style necrosis areas in parenchyma, portal structures identifiable. Irregular portal area boundary in

parenchyma observed together with midzonal and centrolobular damage.

- Widespread necrosis findings in liver parenchyma, widespread damage to hepatocytes, portal areas difficult to identify or unidentifiable. Destructive changes to the bilary canalicular structures.

Immunohistochemical study

In this study labelled polyclonal or monoclonal antibodies (NF-kβ/p65 (Rel A), Ab-1 (Neomarkers R-B-1638-R7) and inducible nitric oxide synthase (iNOS) Ab-1 (Neomarkers R-B-1605-R7) developed against the research proteins were used.

Tissue blocks submerged in paraffin after fixation in 10% formaline from all groups had 2 micron sections coated with poly-L-lysine (PLL) and placed on slides. These were left in a 37 centigrade oven overnight and then deparaffinized with zylol twice for 15 minutes each time. Sections were passed through an alcohol series beginning with pure alcohol though 90%-80%-70% and then washed with distilled water. Thus the sections were deparaffinized.

The sections were placed in buffer solution (citrate buffer) and boiled 4 times for 5 minutes each in a microwave oven. They were left to cool for 20 minutes at room temperature. After 20 minutes cooling the sections were washed in distilled water and placed in tris-buffer solution.

To prevent background staining of non-specific endogenous peroxide sources, sections were exposed to 3% H_2O for 20 minutes, then washed with distilled water and placed in tris-buffer solution again. With the aim of preventing non-specific background staining, Ultra V-block (protein blockage) application was performed at room temperature for 5 minutes.

Later 30 minutes incubation with inducible nitric oxide synthase (iNOS) Ab-1 and NF-Kβ/p65 (Rel A) Ab-1 primary antibodies was performed. Sections were washed 3 times in tris buffer and incubated for 20 minutes with biotinized secondary antibodies and for 20 minutes with enzyme-labelled strepatvidin.

Sections placed in tris-buffer were exposed to A EC chromogene for 20 minutes each. After sections were washed with distilled water, they were incubated for 3 minutes with Mayer's hematoxylin for contrast staining. After sections were washed with excess flowing water, they were covered with an aqueous-mount gel. The sections prepared for each case were investigated with a light microscope.

Cases were assessed in terms of the spread and intensity of cytoplasmic staining. The spread and intensity of staining on liver tissue sections was assessed at hepatocyte and kuppfer cell dimensions and scored for the tissue in general as follows;

- No staining = 0
- < 25% staining = +1
- 25-50% staining = +2
- 20-75% staining = +3
- >75% staining = +4

The intensity of staining reflects the staining intensity of cell chromogene showing cytoplasmic staining in tissue sections with scoring of 0 meaning no staining, +1 mild staining, +2 moderate staining, and +3 strong staining and +4 very strong staining.

Statistical evaluation

When assessing the results of the study statistical analyses used the NCSS 2007 & PASS 2008 statistical software (Utah, USA). For comparisons of histopathologic injury, the One way ANOVA (comparison of more than two groups) and Tukey test from Post hoc tests (comparison of two groups) were used. Values $p<0.05$ were accepted as significant.

Results

Our study was completed from 01.05.09 to 15.07.09 at Istanbul University Faculty of Medicine Hospital on a total of 28 rats. In our study subjects were grouped as control (n=4) liver subjects, "Group A" (n=8) sepsis subjects, "Group B" (n=8) sham (laparotomy and cecal mobilization), "Group C" (n=8) PDTC subjects and "Group D" (n=8) sepsis+PDTC subjects and distributions are shown.

When groups are assessed for mortality, as there was no procedure performed on the sham group (CLP performed or antioxidant administered) no rat died and all 8 rats were sacrificed for histopathological and immuno-histochemical assessment. Of the 7 rats that died, 5 died in the first 24-36 hours, and 2 were exitus before 48 hours.

In Group D (Sepsis+PDTC) 2 were exitus in the first 48 hours with the other 2 exitus on the 5th day and 3 rats alive on the 10^{th} day. In Group C (sham+PDTC) 1 rat was exitus in the first 72 hours, while the remaining 6 were still alive on the 10^{th} day **(Table 1)**.

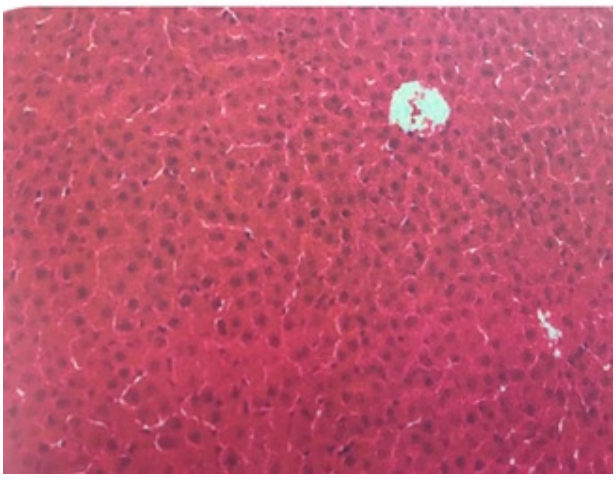

Figure 1 Group A (LC 100x H&E).

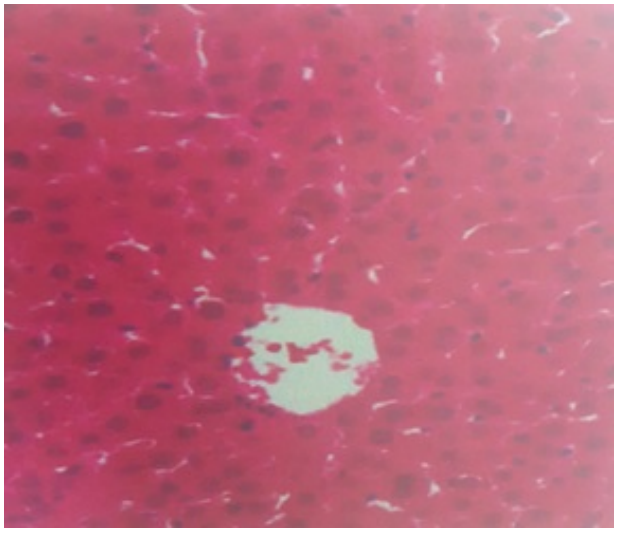

Figure 2 Group A (LC 400x H&E).

According to morphology scores in the groups, there were statistically significant differences present (p<0.01). Two-way comparisons to identify which group was the source of significance found no difference between the Group A and Group C (p>0.05), while the morphology score in the Group A **(Figures 1 and 2)** was low compared to the morphology score in the sepsis **(Figures 3 and 4)** and Group D groups (p<0.01).

The morphology score in the Group C group was higher by a significant level compared to the morphology scores in the Group C **(Figures 5 and 6)** and Group D **(Figures 7 and 8)** groups (p<0.01). The morphology score in the Group C group was identified to be significantly low compared to the Group D group (p<0.01) **(Table 2)**.

iNOS measurements showed statistical differences depending on the group (p<0.01). Two-way comparisons to identify which groups caused the difference found no difference between the sham group and sham+PDTC group (p>0.05) with iNOS measurements in the sham group **(Figure 9)** found to be lower than iNOS measurements in the sepsis **(Figures 10 and 11)** and sepsis+PDTC **(Figures 11-15)** groups (p<0.01). The iNOS measurements in the sepsis group and the sham+PDTC **(Figure 13)** group were identified to be significantly low compared to the sepsis+PDTC group (p<0.05) **(Table 3)**.

Table 1 Staining scores for epithelial component after p65 and iNOS immunohistochemical staining (n).

	Case	iNOS	p65
	1	0	0
	2	0	0
	3	0	0
Group A	4	0	0
	Case	iNOS	p65
	1	3.+++	0.++
	2	3.+++	0.+++
	3	3.+++	0.+++
	4	2.+++	0.+++
	5	3.+++	0.+++
	6	3.++	0.+++
	7	3.+++	0.+++
Group B	8	2.+++	0.++
	Case	iNOS	p65
	1	1.+	0.+/-
	2	2.++	0.+
	3	1.+	0.+
	4	1.+	0.+
	5	2.+	0.+
	6	1.+	0.+
Group D	7	2.+	0.+

	8	1.+/-	0.+
	Case	**iNOS**	**p65**
	1	1.+	0.+
	2	0	0
	3	1.+/-	0
	4	0	0
	5	0	0
	6	1.+	0.+
	7	0	0
Group C	8	0	0

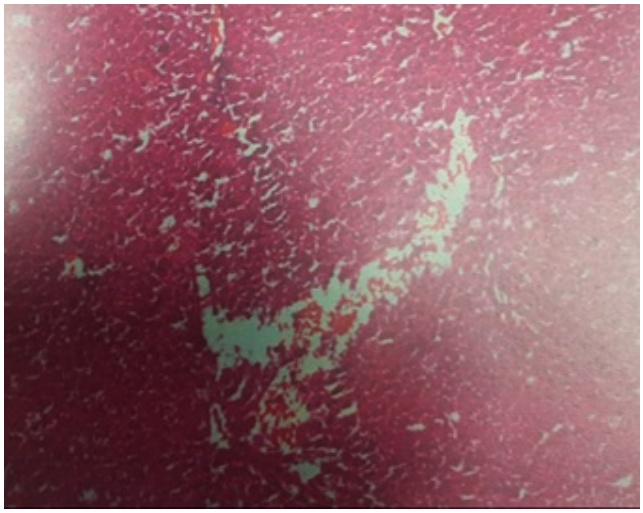

Figure 3 Group B (LC 100x H&E).

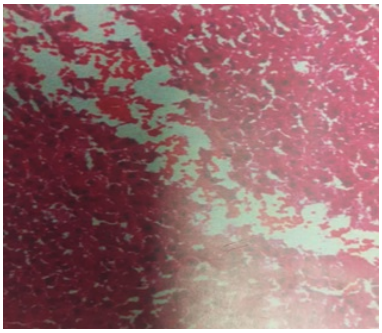

Figure 4 Group B (LC 400x H&E): Widespread necrosis (coagulation necrosis) findings in liver parenchyma, widespread injury to hepatocytes, portal areas difficult or impossible to identify, destructive changes in bilary canalicular structures.

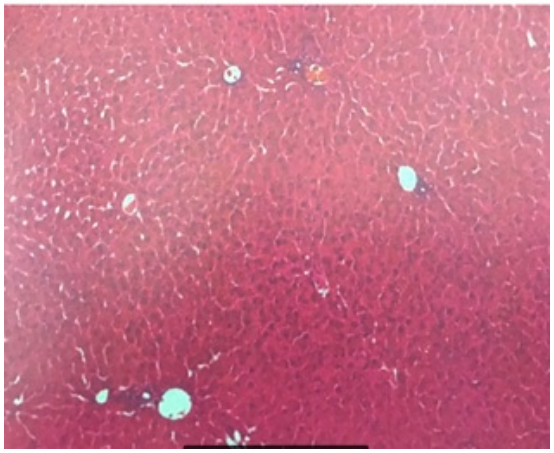

Figure 5 Group C (100x H&E) morphology findings close to normal.

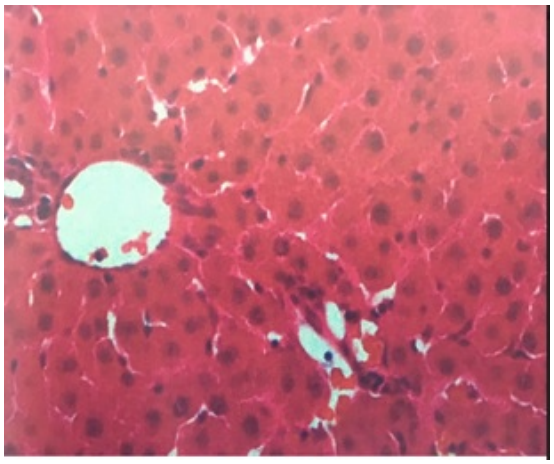

Figure 6 Group C (400x H&E) morphology findings close to normal.

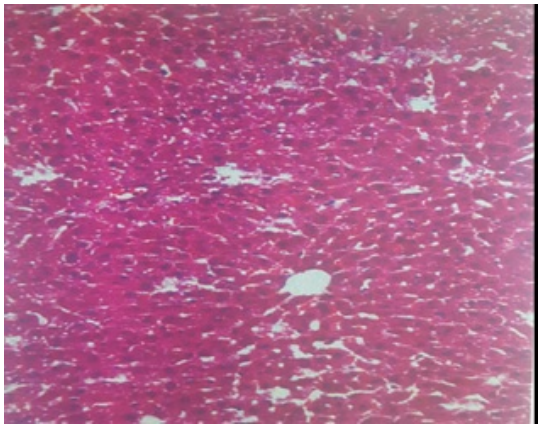

Figure 7 Group D (X100H&E).

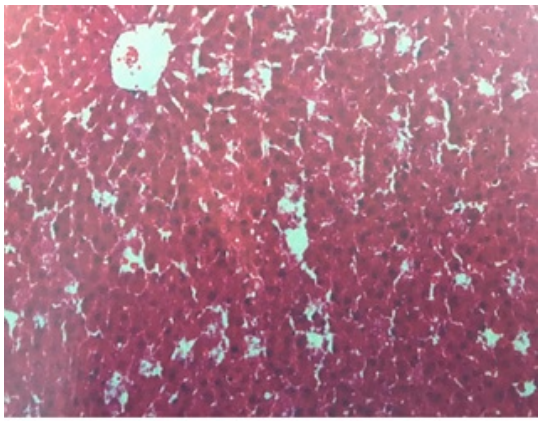

Figure 8 Group D (X100H&E) Swollen hepatocytes, parenchyma preserved however portal area is irregular with mild or moderate degrees of centrolobular injury present.

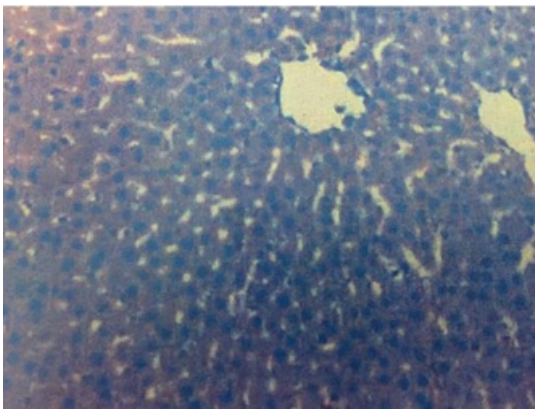

Figure 9 Group A (100x H&E) iNOS negative IHC staining.

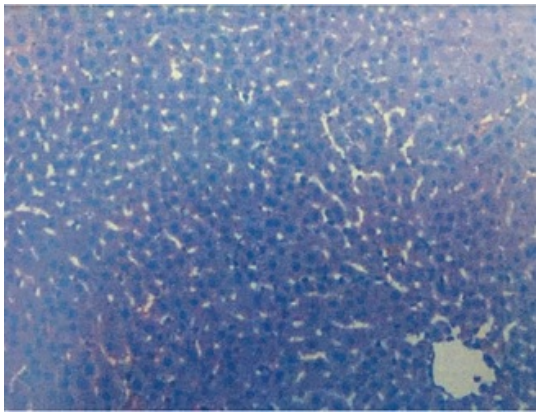

Figure 10 Group A (400x H&E) iNOS negative IHC staining.

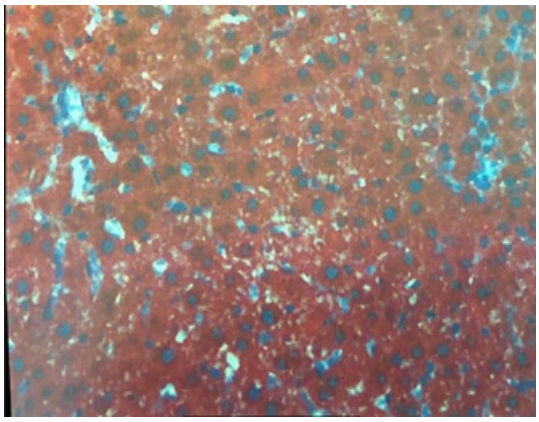

Figure 11 Group B (LC 200x) iNOS strong IHC positive staining.

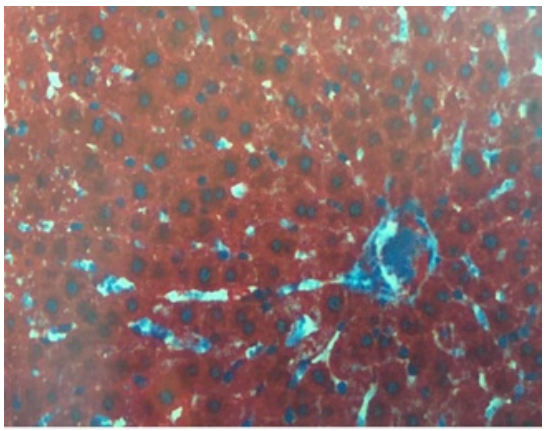

Figure 12 Group B (LC 200x, p65) strongly positive IHC staining.

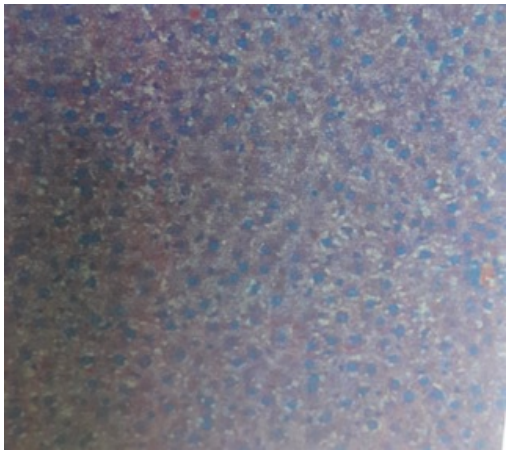

Figure 13 Group C (100x) iNOS weak, IHC positive staining.

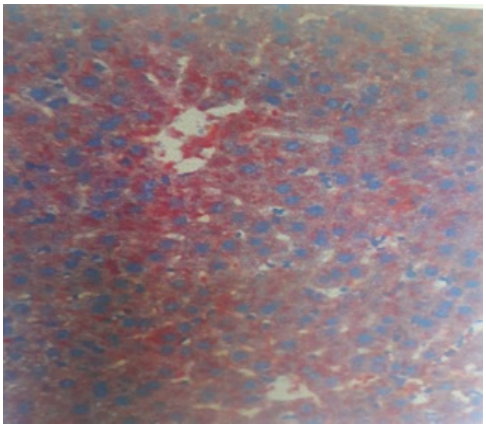

Figure 15 Group D (LC 200x) iNOS weak, IHC positive staining.

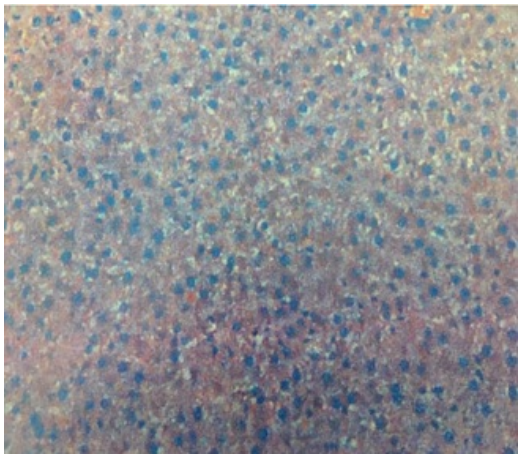

Figure 14 Group C (100x) p65 weak, IHC positive staining.

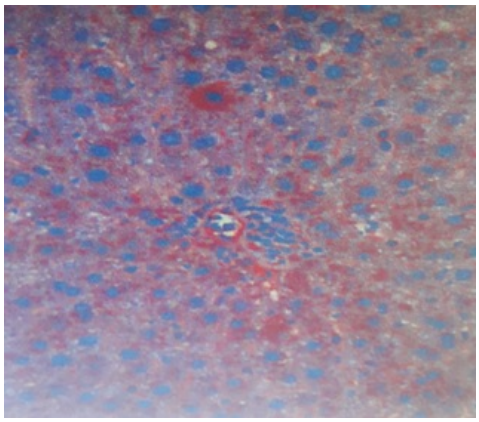

Figure 16 Group D (LC 200x) iNOS weak, IHC positive staining.

Table 2 Assessment of groups according to morphologic investigation (Mean ± SD).

Morphology					
Groups	**n**	**Mean ± SD**	**Median (25%-75%)**	**Groups**	**n**
Group A	4	0.0 ± 0.0	0(0-0)	--	Gr A-B: 0.003**
Group B	8	2.75 ± 0.46	3(2.25-3)	0.001**	Gr A-C: 0.176
					Gr A-D: 0.004**
Group C	8	0.37 ± 0.52	0(0-1)	0.001**	Gr B-C: 0.00i**
					Gr B-D: 0.001**
Group D	8	1.37 ± 0.52	1(1-2)	--	Gr C-D: 0.005**

Table 3 Assessment of groups according to iNOS investigation (Mean ± SD).

Groups	n	Mean ± SD	Median (25-75%)	p	Post -hoc
Group A	4	0.0 ± 0.0	0(0-0)	--	Gr A-B: 0.002**

					Gr A-C: 0.294
					Gr A-D: 0.008**
Group B	8	2.87 ± 0.35	3+(3-3)	--	Gr B-C: 0.001**
Group C	8	0.25 ± 0.46	0(0-0.75)	0001**	Gr B-D: 0.001**
Group D	8	1.00 ± 0.53	1+(1-1)	--	Gr C-D: 0.013**

Assessment of groups according to p65 investigation

p65 measurements showed statistically significant differences depending on the groups (p<0.01). Two-way comparison to identify which groups caused the significance found no difference between the Group A **(Figure 10)** and Group C **(Figure 14)** (p>0.05), with p65 measurements in the Group A lower at p<0.01 level compared to the Group B **(Figure 12)** and at p<0.05 level compared to the Group D **(Figure 16)**. The p65 measurements in the Group B were significantly high compared to the p65 measurements in the Group C and Group D (p<0.01). Group C p65 scores were not significantly different from Group D, p 65 scoring (p>0.05) **(Table 4)**.

Table 4 Assessment of groups according to p65 investigation (Mean ± SD).

Groups	**n**	**Mean±SD**	**Mediyan** (%25-%75)	**Groups**	**n**
Group A	4	0.0 + 0.0	0 (0-0)	--	<Gr A-B: 0.003**
Group A	4	0.0 + 0.0	0 (0-0)	--	Gr A-C: 0.294
Group B	8	2.75 + 0.46	3 (2.25-3)	0.001**	Gr A-D: 0.019*
					Gr B-C: 0.001**
Group C	8	0.25 + 0.46	0 (0-0.75)	--	Gr B-D: 0.001**
Group D	8	0.75 + 0.46	1 (0.25-1)	--	Gr C-D: 0.0 53

Discussion

Sepsis is a serious clinical syndrome that initiates over-response of host defence, leading to septic shock and multi-organ dysfunction syndrome (MODS), which is a major cause of mortality in intensive care units [18].

Organ failures common in the septic patient involve the circulatory, renal, pulmonary, gastrointestinal and hepatic, hematologic, and central nervous systems. Multiple organ system dysfunctions are more common than single system dysfunction. A Spanish study reported that 78% of patients with septic shock in the intensive care setting have more than one organ system deranged, with only 22% of patients with severe sepsis manifesting single organ failure. The most commonly affected organ systems were the respiratory and vascular systems, with acute hepatic failure and neurologic dysfunction being the least common [10].

However, the progression of inflammation from a local to a systemic response involves activation of circulating leukocytes; these release proinflammatory cytokines such as interleukin (IL)-1, -6, -8, and tumor necrosis factor-alpha (TNFα) into the blood [19]. Oxidative stress markers were reported to be higher in patients with sepsis than in healthy subjects [20]. There is growing evidence that the oxidant/anti-oxidant imbalance arising from overproduction of free radicals plays a fundamental role in MOD [19]. Free oxygen radicals (FOR) have pro-inflammatory effects that cause endothelial damage, production of chemotactic factors, leucocyte infiltration, cytokine release, mitochondrial dysfunction, lipid peroxidation, and DNA damage [21]. Lipid and protein oxidation, and the overwhelming production of FOR, have been well reported during sepsis; many studies have documented that oxidative stress and MOD were correlated with pathogenesis and mortality in sepsis [22].

There is a traditional consideration that sepsis-associated hepatic dysfunction is a late incident. However, hepatic dysfunction is presented recently in several studies as an early incident in sepsis [23].

Previous studies showed that sepsis is a severe systemic inflammation and resulted in inflammatory and immune responses represented in the activation of the toll-like receptor-4 (TLR-4)/nuclear factor kappa-B (NF-kβ) pathway [24]. In addition, activation of NF-kβ results in increased gene expression and biosynthesis of pro-inflammatory mediators in sepsis [25]. Sepsis-induced inflammation is characterized by activation and over expression of TLR-4 and release of inflammatory mediators. TLR-4 triggers activation of inducible transcription factors, such as NF-kβ. Activation of NF-kβ allows nuclear translocation of the p65 subunit of NF-kβ leading to the release and expression of cytokines including IL-6 and IL-10 and other inflammatory mediators [26]. Hence, repressing expressions of these inflammatory cytokine genes and production of oxidative and nitrosative stress would be helpful for treating sepsis. In the host response, the liver is a key tissue

that contributes to clearing infectious agents and products during sepsis [27]. PDTC is the most potent NF-kβ inhibitor. It is used as an antioxidant compound against the toxic effects of FOR and enters reactions with the production of pro-inflammatory cytokines. The NF-kβ inhibition property of PDTC is independent of its antioxidant property [28]. Lee et al. reported PDTC prevented the elevation of liver serum enzymes and lipid peroxidase and reduction in glutathione content in hepatic ischemia-reperfusion injury in rats, displaying hepataoprotective effect [29]. Liu et al. reported NF-kβ activation, activated by lipopolysaccharides, was important in sepsis formation linked to multiple organ injury and administration of PDTC may prevent this [30].

NF-kβ activation with sepsis in addition to apoptosis causes induction of NO synthase and cyclooxygenase-2 in cytokines, chemokines, adhesion molecules and coagulation factors [31]. Though NF-kβ activation inhibitors like n-acetyl cysteine and pyrrolidine dithiocarbamate are reported to have therapeutic effect [32], the relationship between sepsis and suppression of NF-kβ activation has ensured recommendation for use of NF-kβ as a target in sepsis treatment [33]. In recent times exogenous HSP70 was shown to be effective in reducing the mortality rate of septic rats [34]. Heat shock proteins (HSPs) protect cells against developing mortal situations [35]. Many authors have proposed that the anti-inflammatory effects of HSPs may be due to the suppression of NF-kβ activation [36]. Heat shock protein-70 (HSP70) is known to prevent cellular damage linked to lipopolysaccharide caused by nitric oxide (NO) synthesis as a result of modulation of inducible nitric oxide synthase (iNOS) activation [37]. With increased iNOS expression-activity and NO production acute pulmonary injury is observed linked to sepsis [38]. NF-kβ affects the target genes for proinflammatory cytokines, chemokines, immunoreceptors, cell adhesion molecules, acute phase proteins and iNOS [39]. Activation of NF-kβ occurs in coordination with the increase in genetic transcription of many products affecting the inflammatory response. The adhesion molecules formed as a result of NF-kβ activation (VCAM, 1CAM-1 and P-selectin) accumulate in the kidneys causing structural damage [40]. To prevent this damage, NF-kβ inhibitors reducing the effect of FOR, in other words antioxidants, are used in treatment [41]. Among these antioxidants red wine, green tea, gingseng, vitamin C, vitamin E, taurin, PDTC, curcumin (diferuloylmethane) and pomegranate juice may be listed.

There is a positive feedback mechanism between these products and NF-kβ and thus local inflammatory response increases and becomes continuous. NF-kβ has a critical role in the inflammatory process. Activation of NF-kβ has been shown to cause damage to many organs like the liver, heart, lungs and kidneys [42].

As activation of NF-kβ is biphasic regulated, in our experimental study we administered 100 mg/kg/day PDTC 15 minutes before and 12 hours via gavage.

In many surgical branches the relevance of FOR in pathologies, the usefulness of known materials with antioxidant effects in treatment or prophylaxis and treatment strategies like ischemic preconditioning, controlled reperfusion and neutrophil therapy are researched and debated [43].

Activation of transcription factors in sepsis results in tissue necrosis and apoptosis. The NF-Kβ inhibitor of PTDC is induced by iNOS expression, adhesion molecule formation and FOR and is shown to prevent apoptosis [44]. Free oxygen radicals play an important role in sepsis pathogenesis. Antioxidants are effective in reducing cellular injury in treatment of sepsis or endotoxemia as shown by measuring FOR activity.

In this study the NF-Kβ inhibitor of PDTC was used and it was identified that in subjects administered PDTC iNOS and eNOS expression reduced. As a result, we think PDTC reduces apoptosis activated in epithelium by oxidative stress linked liver damage.

In these results, PDTC appears to be an appropriate medication with potential efficacy in treatment of sepsis and septic shock. However, it is clear there is still a need for very serious study on this topic. Antioxidant treatment in sepsis increases the efficacy of endogenous antioxidants and speeds up clinical amelioration [45]. In the sepsis model induced in rats with the CLP method, administration of antioxidants lengthens the duration of survival [46].

In our study when groups are assessed for mortality, of 7 rats 5 were exitus in the first 24-36 hours, while the other 2 died before 48 hours. In the Group D, 2 animals were exitus in the first 48 hours and 2 were exitus on the 5th day with the remaining 3 rats surviving on the 10th day. In the Group C, 1 rat was exitus in the first 72 hours, while the remaining 6 were still alive on the 10th day. In our study we believe PDTC increased the efficacy of treatment for sepsis and had positive effects on the survival duration of rats.

In our study in the group treated with PDTC for a sepsis model induced with the CLP method in rats, iNOS and NF-KBβ p65 were identified to reduce in the liver and this was shown histochemically. We believe antioxidants like PDTC targeting NF-Kβ inhibition in oxidative stress caused by sepsis may reduce oxidative stress.

Conclusion

Sepsis, and the commonly accompanying septic shock, is a clinical tableau with difficult treatment, high mortality, increasing incidence and affected by complicated pathophysiologic events. In recent times, due to advances in understanding cellular biology the pathophysiology of sepsis has become better understood with the mediators (TNF, interleukins, FOR) and cytokines playing roles in the event defined and the effect mechanisms and pathophysiologic and metabolic changes developing in the body determined. The results of clinical studies about antioxidants for sepsis treatment are still controversial and there is a need for broader and longer term studies. In conclusion, the NF-Kβ inhibitor of PDTC was effective in reducing liver damage and lengthening survival due to sepsis induced in rats with the cecum ligation and perforation method; however we believe

there is a need for more comprehensive and controlled studies before clinical use.

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