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Research Paper

Effects of pentoxifylline on oxidative stress in rats with abdominal compartment syndrome model

Seracettin Eğin [a,*], Kurtuluş Açıksarı [b], Gülçin Ercan [c], A. Fatih Aydın [d], Esra Aycan Üstyol [d], Mediha Eser [e], Gamze Tanrıverdi [e], Hakan Teoman Yanar [f]

[a] Department of General Surgery, Okmeydanı Training and Research Hospital, İstanbul, Turkey
[b] Department of Emergency Medicine, İstanbul Medeniyet University, Faculty of Medicine, İstanbul, Turkey
[c] Department of General Surgery, Bağcılar Training and Research Hospital, İstanbul, Turkey
[d] Department of Biochemistry, İstanbul University, İstanbul Faculty of Medicine, İstanbul, Turkey
[e] Department of Histology and Embryology, İstanbul University, Cerrahpaşa Faculty of Medicine, İstanbul, Turkey
[f] Department of General Surgery, İstanbul University, İstanbul Faculty of Medicine, İstanbul, Turkey

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ABSTRACT

Background: Abdominal compartment syndrome (ACS) causes severe pathology in the cardiovascular, renal and pulmonary systems. Recent studies showed that pentoxifylline (PTX) has effects on increasing tissue oxygenation, healing capillary refill and reducing superoxides and hydroxyl radicals by inhibiting xanthine oxidase. In this study, our aim was to study the effects of PTX on free oxygen radicals and oxidative damage in rats with ACS model.

Materials and methods: ACS model was created in 32 male Wistar-Albino-rats, which were randomized into one of the four study groups: Group A (n:8), having ACS; Group B (n:8), having ACS and receiving PTX (50 mg/kg/day) intraperitoneal for 10 days; Group C (n:8), receiving PTX (50 mg/kg/day) intraperitoneal for 10 days without having ACS; Group D (n:8), having no ACS and not receiving PTX. On the 11th day blood samples were collected to measure alanine-amino-acid-transferase (ALT) and aspartate-amino-acid-transferase (AST) in the heart, malondialdehyde (MDA), myeloperoxidase (MPO) and glutathione (GSH) in the liver, lung and small bowel. Histopathologic injury scoring was done.

Results: Groups were compared in pairs. Group A compared to Group B: ALT increase, liver MDA, lung GSH and MPO decrease were statistically meaningful in Group B. Group A compared to Group C: ALT and liver MPO decrease and liver MDA increase were statistically meaningful in Group A. Group B compared to Group C: ALT increase, MDA and GSH decrease in the lung were statistically meaningful in Group B. Group B compared to Group D: ALT and MPO increase in the small bowel and MPO decrease in the lung were statistically meaningful in Group B. Group A had the highest histopathologic injury scoring.

Conclusion: Histopathologically confirmed pentoxifylline was effective in the treatment of ACS in these rat models.

1. Introduction

Abdominal compartment syndrome (ACS) is an independent marker for mortality and is associated with an increased rate of multiple organ dysfunction. ACS is defined as increased intra-abdominal pressure equal to or more than 20 mmHg [1]. The pathophysiology of ACS is directly related to the increased intraabdominal pressure (IAP). Increased IAP compresses the thoracic cavity through the diaphragm and reduces ventilation. Increased IAP compresses the vena cava and decreases the cardiac output by decreasing venous return. Decrease in cardiac output and venous return cause decreased kidney, splenic and liver perfusion. This multi-organ hypoperfusion causes pathologies as systemic inflammatory response and adult respiratory distress syndrome [2–6]. IAP may increase with various reasons like trauma, abdominal surgery, laparoscopic surgery, over fluid replacement, severe retroperitoneal hemorrhage, acute renal failure, acute pancreatitis, retroperitoneal malignancies, and massive ascites [6,7].

Ischemia is defined as insufficient arrive or interruption of blood flow to certain tissues or organs [8]. Hypoxia-related tissue damage occurs. Prolonged ischemia impairs the structural integrity of cells and cell death may occur [8,9]. Reperfusion is tissue restoration of blood flow [8,10]. Previous studies reported that severity of the tissue

* Corresponding author. Department of General Surgery, Okmeydanı Training and Research Hospital, İstanbul, Turkey. Fax: +90 212 2217748.
E-mail address: seracettin_egin@hotmail.com (S. Eğin).

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injury varies according to the duration and grade of the ischemia [11,12]. Tissue injury occurs in the ischemic period and also in the reperfusion period. When ischemic tissue blood flow is resupplied, free oxygen radicals are released from polymorphonuclear leukocytes in the tissue, and tissue damage is exacerbated [8,10,13]. Ischemia/ reperfusion damage is not only seen in the affected tissue, but also in other organs due to systemic inflammatory response [14,15]. To limit the potential damage of free oxygen radicals, enzymatic and non-enzymatic antioxidants are released from the cells.

Pentoxifylline is a methlxanthine derivative drug, similar to theophylline. Pentoxifylline shows its effect by inhibiting phosphodiesterase enzymes and this increases the cyclic AMP on polymorph nuclear leukocytes and decreases the free oxygen radical production [16]. It is commonly used to improve microvascular circulation in patients with vascular insufficiency, because it reduces platelet aggregation [17]. Recent studies showed that pentoxifylline inhibits xanthine oxidase, thus reduces superoxide (SO) and hydroxyl (OH) radicals, heals capillary circulation and tissue oxygenation. Besides it also inhibits free oxygen radicals and Phospholipase A2, which increases prostacyclin release [17,18].

Harmful physiological changes that occur as a result of ACS clinically affect most cardiovascular, renal and pulmonary systems. Reduction in cardiac output, increase in peripheral resistance, oliguria, anuria and hypoxia may occur. Fatal organ failure may occur in untreated cases. ACS working model experimentally carried out is not possible except for animal.

We designed an animal model of ACS to study the effects of a vasodilating agent, pentoxifylline, on the treatment of oxidative damage caused by ACS.

2. Methods

This study was approved by the Animal Experiments Local Ethics Committee of İstanbul University (Process number 2014/101). The experiments were performed in adherence with the international guidelines for the care and use of laboratory animals at the Laboratory of Surgical Physiopathology.

The test subjects were randomly enrolled in one of the 4 study groups. The rats were fasted before the surgery night. Rats were subsequently anesthetized with ketamine hydrochloride 50 mg/ml and Xylazine hydrochloride 20 mg/ml given intraperitoneally in a dosage of 0.1 ml/100 g. Following this, the rats were fixed in the supine position on a regularly disinfected and surgically draped operating table. Model of ACS was stimulated by insertion of a 16 Gauge cannula under sterile conditions into the peritoneal cavity and insufflation of 20 mmHg CO2 for two hours. Ten days after this procedure the rats were sacrificed. Blood samples were taken from the heart apex for ALT and AST, and tissue samples were taken from the lung, liver and small bowel.

Group A (n:8), having ACS. The rats were fasted before the surgery night. Rats were subsequently anesthetized with ketamine hydrochloride 50 mg/ml and Xylazine hydrochloride 20 mg/ml given intraperitoneally in a dosage of 0.1 ml/100 g. Following this, the rats were fixed in the supine position on a regularly disinfected and surgically draped operating table. Model of ACS was stimulated by insertion of a 16 Gauge cannula under sterile conditions into the peritoneal cavity and insufflation of 20 mmHg CO2 for two hours.

Group B (n:8), having ACS and receiving pentoxifylline (50 mg/kg/day) intraperitoneal for 10 days. The rats were fasted overnight prior to surgery, and were subsequently anesthetized with ketamine hydrochloride 50 mg/ml and xylazine hydrochloride 20 mg/ml given intraperitoneally in a dosage of 0.1 ml/100 g. Following this, the rats were fixed in the supine position on a regularly disinfected and surgically draped operating table. Model of ACS was stimulated by insertion of a 16 Gauge cannula under sterile conditions into the peritoneal cavity and insufflation of 20 mmHg CO2 for two hours. After desufflation of the peritoneal cavity, pentoxifylline was given (50 mg/kg/day) in one cc of 9% NaCl intraperitoneal for 10 days.

Group C (n:8), receiving pentoxifylline (50 mg/kg/day) intraperitoneal for 10 days without having ACS. The rats were fasted overnight prior to surgery, and were subsequently anesthetized with ketamine hydrochloride 50 mg/ml and xylazine hydrochloride 20 mg/ml given intraperitoneally in a dosage of 0.1 ml/100 g. Following this, pentoxifylline was given (50 mg/kg/day) in one cc of 9% NaCl intraperitoneal for 10 days.

Group D (n:8) having no ACS and not receiving pentoxifylline was the sham group. The rats were fasted overnight prior to surgery, and were subsequently anesthetized with ketamine hydrochloride 50 mg/ml and xylazine hydrochloride 20 mg/ml given intraperitoneally in a dosage of 0.1 ml/100 g.

32 Male Wistar rats (250–300 g) were purchased from the İstanbul University, Institute of Experimental Medicine. Male rats were preferred, because menstrual cycle may have affected the blood test results.

All rats were stored in metal cages and maintained in 12-hour dark/light cycle at a controlled temperature of 22 °C (±1). All rats were fed with 21% protein food and fresh tap water, only rats to get IAP will be unfed one day before. All cages were cleaned daily, 4 rats were put in a cage and the rats that were in the same group were stored together.

All rats were sacrificed on the 11th day with high dose ketamine and blood samples were collected to measure alanine-amino-acid-transferase (ALT) and aspartate-amino-acid-transferase (AST) in the heart, malondialdehyde (MDA) and glutathione (GSH) in the liver, lung and small bowel. Histopathologic injury scoring was done in the liver, lung and small bowel. All tissues were put in a cryotube into the deep freezer at –80 °C after being washed at +4 °C phosphate buffer saline (PBS) and stored until the biochemical analysis. Blood samples were taken from the heart apex while the heart rate is presented and centrifuged at 3000–35,000 rpm for 15 minutes in the dry yellow cap biochemistry tube. All samples of serum obtained from each animal were put into three separate eppendorf tubes and kept into the deep freezer at –80 °C again until the biochemical analysis. MDA and GSH levels were used as oxidative stress and MPO activity was used as inflammatory parameters.

2.1. ALT and AST analyses

ALT and AST analyses were measured with cobas 8000 e602 modular automatic analyzer (Roche Diagnostics, Mannheim, Germany).

2.2. Glutathione (GSH) analysis

Dithiobisnitrobenzoic acid and GSH sulfhydryl (-SH) group makes a reaction. The product of this reaction is yellow. For one mole -SH group, one mole dithiobisnitrobenzoic acid forms and this product has an absorbance at 412 nm at UV spectrophotometer. After ten minutes of waiting at room temperature GSH activity was calculated according to the formula described in the manufacturer's protocol.

2.3. Myeloperoxidase (MPO) analysis

Tissue homogenites were made with 1 ml of cold hexdecyltrimethly ammonium bromide buffer (50 mM KPO+ and 0.5% hexdecyltrimethly ammonium bromide [pH 6.0]). Homogenites were sonicated on ice for 10 seconds, and centrifuged at 10,000 rpm for 15 min at 4 °C. Twenty ml of supernatant solution was transferred into well plate, and 200 µl of O-dianisidine hydrochloride solution at 37 °C (16.7 mg O-dianisidine, 100 ml: 90% water and 10% 50 mM KPO4 buffer + 0.0005% H^2O^2) was added. Immediately after

this, solution optical density was read at 450 nm for three minutes. Absorbance changes were calculated as (ΔA/min) and the results were read nmol/mg protein/min.

2.4. Malondialdehyde (MDA) analysis

MDA is one of the end products of lipid peroxidation measured by spectrophotometry with its complex thiobarbituric acid. This red product has a peak absorption at 532 nm at UV spectrophotometer. 0.2 ml of 10% of tissue homogenate was taken. 0.2 ml of 8.1% sodium dodecyl sulfate, 5 ml 20% acetic acid, 1.5 ml of 0.8% thiobarbituric acid and 0.6 ml distilled water were added on this homogenate. This mixture was kept in boiling water bath for one hour. After cooling, 1 ml of distilled water and 5 ml of butanol/pyridine (15/1) mixture were added and organic phase was separated by centrifuging. The homogenate free materials absorption was read in a spectrophotometer at 532 nm wavelength. Calculation was done with 1,1,3,3-tetraethoxypropan. Results expressed as nmol/g tissue were identified.

2.5. Protein analysis

The amount of protein was determined by bicinchoninic acid method in tissue homogenates prepared for MPO analysis. The absorption was read in well-plates in a spectrophotometer at 562 nm wavelength at 37 °C for 30 minutes to the blind tube.

2.6. Histopathologic injury scoring of liver, lung and small bowel

For small bowel histopathologic injury score, one cm sample was taken from 3rd, 5th and 7th 10-cm segment. These samples were fixed in formalin (10%, neutral buffered) for at least 24 hours. 3 mm cross-sections were stained from paraffin bloc. Samples were examined and photographed under light microscope (Olympus BX61, Japan). Histopathologic changes were evaluated in blinded manner (blinded assessment of outcomes by one independent assessor). Histopathologic injury scoring of small intestine was scored on a scale of 0–8 as follows [19]: Grade 0, normal mucosa; grade 1, subepithelial Gruenhagen space, capillary congestion; grade 2, extension of the subepithelial space with a moderate epithelial lifting; grade 3, massive epithelial lifting down the sides of villi, few tips denuded; grade 4, denuded villi; grade 5, loss (destruction) of villi, hemorrhage; grade 6, crypt layer injury; grade 7, transmucosal infarction; grade 8, transmural infarction.

Histopathologic injury scoring of liver samples was scored on a scale of 0–4 as follows [20]: Grade 0, minimal or no evidence of injury; grade 1, mild injury characterized by cytoplasmic vacuolization and focal nuclear pyknosis; grade 2, moderate injury exhibiting cytoplasmic vacuolization, confluent areas of hepatocyte ballooning but no frank necrosis, sinusoidal dilatation and congestion, and blurring of intercellular borders; grade 3, moderate to severe injury with areas of coagulative necrosis, cytoplasmic hypereosinophilia, extensive sinusoidal dilatation and congestion; grade 4, severe injury consisting of severe confluent coagulative necrosis, and disintegration of and hemorrhage into hepatic chords leading to loss of tissue architecture.

Lung tissue injuries in blinded fashion are in accordance with a scoring system modified by Kandilci et al. and Pirat et al. [21,22]. The scoring system is summarized in Table 1. Five parameters (interstitial edema/infiltration, intra-alveolar edema/infiltration, intra-alveolar hemorrhage, capillary congestion and airway epithelial-cell damage) were evaluated for lung injury and the total lung injury score was calculated.

2.7. Statistical analysis

Data were analyzed with IBM SPSS Statistics 15. Two group comparisons were performed by using Mann–Whitney U test and for comparisons involving three or more groups, Kruskal–Wallis H test was used. The correlation between the variables was calculated with Fisher's Exact Test. The value for significance was considered $p \leq 0.05$.

3. Results

Each group included in each analysis consists of eight rats except for sham group. One rat in sham group perished before the end of the experiment. The number of rats in each group included in each analysis is respectively 8/32, 8/32, 8/32, 7/32.

During the study period one rat died in Group D. Laboratory and histological findings for all Groups are shown in Table 2. As you may see in Table 2, there were significant differences for ALT, liver tissue MDA, liver tissue MPO, lung tissue MPO and small bowel tissue MPO ($p < 0.05$). Groups were compared in pairs in order to understand which groups have differences with Mann–Whitney U test. Group A compared to Group B: ALT increase, liver MDA, lung GSH and MPO decrease were statistically meaningful in Group B (Table 3a). Group A compared to Group C: ALT and liver MPO decrease and liver MDA increase were statistically meaningful in Group A (Table 3b). Group B compared to Group C: ALT increase, MDA and GSH decrease in the lung were statistically meaningful in Group B (Table 3c). Group B compared to Group D: ALT and small bowel MPO increase and lung MPO decrease were statistically meaningful in Group B (Table 3d).

Histopathologic injury scoring for all groups and for all rats is shown in Table 4. There are significant differences in all groups. Groups were compared in pairs in order to understand which groups have differences with Pearson Chi-Square test. In Group A histopathologic injury score for liver, lung and small bowel was significantly higher than the other three groups ($p < 0.05$).

Any important adverse event did not happen on any experiment group.

4. Discussion

Recent studies showed that pentoxifylline has effects on increasing tissue oxygenation, healing capillary refill and reducing superoxides and hydroxyl radicals by inhibiting xanthine oxidase in different clinical presentations like strangulated closed loop small

Table 1
Histological scoring system.

Parameters	Scores				
	4	3	2	1	0
Interstitial edema/infiltration	%75–100 involvement	%51–75 involvement	%26–50 involvement	%1–25 involvement	%0 involvement
Intra-alveolar edema/infiltration	%75–100 involvement	%51–75 involvement	%26–50 involvement	%1–25 involvement	%0 involvement
Intra-alveolar hemorrhage	%75–100 involvement	%51–75 involvement	%26–50 involvement	%1–25 involvement	%0 involvement
Capillary congestion	%75–100 involvement	%51–75 involvement	%26–50 involvement	%1–25 involvement	%0 involvement
Airway epithelial-cell damage	%75–100 involvement	%51–75 involvement	%26–50 involvement	%1–25 involvement	%0 involvement

Table 2
Laboratory findings and P value for all groups.

Group		Group A (ACS)	Group B (ACS + pentoxifylline)	Group C (pentoxifylline without ACS)	Group D (sham)	Total
ALT	**(p < 0,0001)**	48,66 ± 4,19 Min: 44,3 Max: 53,4	76,96 ± 16,97 Min: 50,3 Max: 101,7	63,4 ± 10,93 Min: 48,2 Max: 81,5	58,41 ± 8,68 Min: 42,9 Max: 68,9	61,97 ± 14,99 Min: 42,9 Max: 102
AST	(p: 0,135)	108,37 ± 31,67 Min: 82,6 Max: 166	134,47 ± 35,41 Min: 88,7 Max: 182,9	105,8 ± 19,34 Min: 80,6 Max: 138,2	107,5 ± 15,02 Min: 85,5 Max: 132	114,24 ± 28,41 Min: 80,6 Max: 183
Liver tissue MDA nmol/g	**(p < 0,0001)**	150,48 ± 36,04 Min: 108,4 Max: 211	90,39 ± 29,85 Min: 46,8 Max: 134,9	88,54 ± 18,55 Min: 55,8 Max: 115,4	78,98 ± 12,75 Min: 55,2 Max: 91,7	102,84 ± 38,18 Min: 46,8 Max: 211
Liver GSH micromolg/tissue	(p: 0,293)	5,32 ± 3,15 Min: 2,66 Max: 12.7	6,47 ± 2,16 Min: 4,11 Max: 10,83	8,86 ± 4,71 Min: 3,96 Max: 17,41	7,76 ± 4,65 Min: 2,36 Max: 15	7,08 ± 3,84 Min: 2,36 Max: 17,4
Liver MPO nmol/mg/protmin	**(p: 0,008)**	15,1 ± 8,03 Min: 5,89 Max: 29,8	28,82 ± 14,96 Min: 10,2 Max: 48,33	40,12 ± 17,41 Min: 19,9 Max: 66,37	31,03 ± 10,48 Min: 13 Max: 45	28,69 ± 15,66 Min: 5,89 Max: 66,4
Lung MDA_ nmolg/tissue	(p: 0,219)	33,46 ± 14,57 Min: 23,57 Max: 68,3	30,87 ± 8,38 Min: 15,4 Max: 42,16	41,85 ± 11,07 Min: 29,8 Max: 64,77	36,52 ± 6,04 Min: 28,1 Max: 43,5	35,65 ± 10,93 Min: 15,4 Max: 68,3
Lung GSH micromol/g/tissue	(p: 0,074)	0,37 ± 0,15 Min: 0,1 Max: 0,63	0,28 ± 0,02 Min: 0,23 Max: 0,31	0,48 ± 0,21 Min: 0,23 Max: 0,9	0,58 ± 0,38 Min: 0,2 Max: 1,13	0,42 ± 0,24 Min: 0,1 Max: 1,13
Lung MPO/nmolmg/protmin	**(p: 0,033)**	25,70 ± 21,78 Min: 5,38 Max: 68,5	6,46 ± 3,17 Min: 2,32 Max: 11,59	14,51 ± 9,02 Min: 4,3 Max: 28,88	13,14 ± 4,3 Min: 5,76 Max: 18,5	15,01 ± 13,66 Min: 2,32 Max: 68,5
Small bowel MDA nmolg/tissue	(p: 0,272)	25,75 ± 6,52 Min: 17,64 Max: 37,8	21,85 ± 6,04 Min: 13,8 Max: 29,18	25,17 ± 8,71 Min: 13,2 Max: 37,84	19,75 ± 4,06 Min: 15,4 Max: 25,3	23,24 ± 6,74 Min: 13,2 Max: 37,8
Small bowel GSH micromolg/tissue	(p: 0,245)	0,88 ± 0,31 Min: 0,35 Max: 1,3	1,42 ± 0,59 Min: 0,6 Max: 2,46	1,68 ± 1,46 Min: 0,35 Max: 4,21	0,97 ± 0,61 Min: 0,25 Max: 2,01	1,25 ± 0,89 Min: 0,25 Max: 4,21
Small bowel MPO nmolmg/protmin	**(p: 0,007)**	20,90 ± 10,69 Min: 11,64 Max: 35,9	13,86 ± 4,45 Min: 5,98 Max: 17,54	15,42 ± 10,06 Min: 4,33 Max: 36,79	5,38 ± 2,34 Min: 2 Max: 8	14,16 ± 9,29 Min: 2 Max: 36,8

Results are in Mean ± Standard Deviation, **Std.D:** Standard Deviation, **Min:** Minimum, **Max:** Maximum, **ALT:** Alanine-amino-acid-transferase, **AST:** aspartate-amino-acid-transferase. (Kruskal–Wallis-H p < 0,05).
Numbers (p < 0,05) were written in bold.

bowel, ischemic colitis or intestinal ischemia/reperfusion injury [23–25]. According to our search for the literature, we have found no study about the effect of pentoxifylline on ACS. In this study, our aim was to study the effects of pentoxifylline on free oxygen radicals and oxidative damage in rats with ACS model. Our study has two components; first we collected blood samples for serum levels of ALT and AST and tissue samples from liver, lung and small bowel for MDA, MPO and GSH levels, second we performed histopathologic injury scoring for liver, lung and small bowel.

In Group A (ACS model without PTX), ALT increase, liver MDA, lung GSH and MPO decrease were statistically meaningful to Group B (ACS model with receiving PTX). ALT and AST are normally found in serum at low levels [26]. Elevated transaminases mostly indicate liver damage [27]. In our study ALT increase was meaningful in Group A to Group B and AST levels were not. In recent studies we know that if increase in serum AST levels was higher than serum ALT levels, this should be due to occult muscle disease [28].

MDA is a significant indicator of lipid peroxidation level and MDA levels were higher in any injury [29]. In Group A, MDA levels were statistically meaningfully higher than Group B. In ischemic reperfusion model the effect of PTX was inhibition of platelet-activating factor (PAF) and we believe that PTX had its protective effect by this way [30]. GSH is a tripeptide antioxidant molecule that helps in reduction of oxidative stress and can be used as oxidative injury indicator [31]. In our study lung GSH levels were higher in Group A than in Group B (p < 0.05). Tissue MPO activities are considered to indicate oxidative stress. In our study lung tissue MPO activity was significantly lower in Group A than in Group B and MPO activity in liver or small bowel was not meaningful. These results show us that PTX has a protective effect on oxidative stress or inflammatory process in lung but has minimal effect in liver and small bowel by laboratory findings. When we look at the histopathologic injury score, this conclusion may change.

In Group A histopathologic injury score for liver, lung and small bowel was significant higher than the other three groups and these results show us that PTX has a real effect on preventing oxidative stress. We believe that this difference was due to the small sample size (n:8), and as sample size increases, the statistics may be more meaningful.

All funding was provided by the surgeons.

5. Conclusion

In conclusion ACS causes oxidative injury on liver, lung and small bowel. PTX prevents/overcomes this injury with its antioxidant properties according to the histopathologic injury score and protects from oxidative stress injury. According to our results we may say that larger sample sizes are necessary for a clean conclusion.

Ethical approval

Ethical Approval was given by local ethics committee of animal experiments of İstanbul University.

Judgment's reference number: 2014/101

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Table 3
(a) Difference between Group A (ACS without receiving PTX) and Group B (ACS with receiving PTX). (b) Difference between Group A (ACS without receiving PTX) and Group C (without ACS, receiving PTX). (c) Difference between Group B (ACS with receiving PTX) and Group C (without ACS, receiving PTX). (d) Difference between Group B (ACS with receiving PTX) and Group D (sham).

Groups A and B	Mann–Whitney U	P value
(a)		
ALT	4,00	**0.002**
AST	15,00	0,830
Liver tissue MDA nmol/g	3,50	**0,001**
Liver GSH micromolg/tissue	17,00	0,130
Liver MPO nmol/mg/protmin	14,00	0,065
Lung MDA_ nmolg/tissue	29,00	0,798
Lung GSH micromol/g/tissue	10,00	**0,021**
Lung MPO/nmolmg/protmin	6,00	**0,021**
Small bowel MDA nmolg/tissue	22,00	0,328
Small bowel GSH micromolg/tissue	15,00	0,83
Small bowel MPO nmolmg/protmin	27,00	0,645
(b)		
ALT	4,00	**0.002**
AST	30,00	0,878
Liver tissue MDA nmol/g	2,00	**0,01**
Liver GSH micromolg/tissue	14,00	0,65
Liver MPO nmol/mg/protmin	3,00	**0,001**
Lung MDA_ nmolg/tissue	14,00	0,065
Lung GSH micromol/g/tissue	22,00	0,328
Lung MPO/nmolmg/protmin	24,00	0,442
Small bowel MDA nmolg /tissue	30,50	0,878
Small bowel GSH micromolg/tissue	20,50	0,234
Small bowel MPO nmolmg/protmin	25,00	0,505
(c)		
ALT	4,00	**0.002**
AST	30,00	0,878
Liver tissue MDA nmol/g	2,00	**0,01**
Liver GSH micromolg/tissue	14,00	0,65
Liver MPO nmol/mg/protmin	3,00	**0,001**
Lung MDA_ nmolg/tissue	14,00	0,065
Lung GSH micromol/g/tissue	22,00	0,328
Lung MPO/nmolmg/protmin	24,00	0,442
Small bowel MDA nmolg /tissue	30,50	0,878
Small bowel GSH micromolg/tissue	20,50	0,234
Small bowel MPO nmolmg/protmin	25,00	0,505
(d)		
ALT	9,50	**0,029**
AST	15,00	0,152
Liver tissue MDA nmol/g	18,00	0,281
Liver GSH micromolg/tissue	27,00	0,955
Liver MPO nmol/mg/protmin	26,00	0,867
Lung MDA_ nmolg/tissue	16,50	0,189
Lung GSH micromol/g/tissue	13,00	0,094
Lung MPO/nmolmg/protmin	6,00	**0,009**
Small bowel MDA nmolg/tissue	23,00	0,613
Small bowel GSH micromolg/tissue	15,50	0,152
Small bowel MPO nmolmg/protmin	4,00	**0,004**

Numbers ($p < 0,05$) were written in bold.

Table 4
Histopathologic injury scoring for all groups.

Group		Small bowel injury score	Liver injury score	Lung injury score
A	1	5,00	1,00	4,00
	2	6,00	3,00	4,00
	3	6,00	2,00	3,00
	4	7,00	3,00	3,00
	5	6,00	3,00	3,00
	6	4,00	2,00	3,00
	7	7,00	2,50	3,00
	8	7,00	3,00	3,00
B	1,00	1,00	1,50	2,00
	2,00	2,00	1,00	3,00
	3,00	1,50	0,50	2,50
	4,00	1,00	0,50	2,75
	5,00	0,50	1,00	2,50
	6,00	0,50	1,00	1,00
	7,00	1,00	0,50	1,50
	8,00	3,00	1,00	2,50
C	1,00	1,00	0	1,50
	2,00	0	0	1,50
	3,00	1,00	0	2,00
	4,00	0	0,50	1,00
	5,00	0	0,00	1,50
	6,00	1,00	0,50	1,00
	7,00	0	0,50	1,25
	8,00	0	0	2,00
	Total	8	8	8
D	1,00	0	0	1,00
	2,00	1,00	0	2,00
	3,00	0	0	1,00
	4,00	0	0	0
	5,00	0	0,50	2,00
	6,00	1,00	0	1,00
	7,00	0	0	0

Author contribution

Seracettin Eğin: Design of the study; Performs operations on rats; Interpretation of data; Drafting the article and revising it critically for important intellectual content; Writing the paper; Related literature review; Final approval of the version to be submitted.

Kurtuluş Açıksarı: Performs operations on rats; Data collections; Interpretation of data.

Gülçin Ercan: Performs operations on rats; Conception of the study.

A.Fatih Aydın: Acquisition of data; Analysis of data.

Esra Aycan Üstyol: Acquisition of data; Analysis of data.

Mediha Eser: Acquisition of data; Analysis of data.

Gamze Tanrıverdi: Acquisition of data; Analysis of data.

Conflict of interest statement

All authors have not any financial and personal relationships with other people or organizations.

Guarantor

Seracettin Eğin

Research registration UIN

This research is not human study.

Appendix: Supplementary material

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