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The effect of PARS inhibition on ileal histopathology, apoptosis and lipid peroxidation in LPS-induced obstructive jaundice

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Abstract

In our experimental study, we investigated the protective effect of 3-aminobenzamide (3-AB), the poly (ADP-ribose) synthetase (PARS inhibitor), on the ileal histopathology and the apoptosis in lipopolysaccharide (LPS)-induced inflammation in rats with obstructive jaundice (OJ). We randomized 40 rats into five groups. Group 1: sham group; Group 2: OJ group; Group 3: OJ + LPS; Group 4: OJ + 3-AB + LPS; Group 5: OJ + LPS + 3-AB. At the fifth day; the rats were jaundiced. In Group 3; 10 mg kg^{-1} LPS was injected intraperitoneally at the fifth day and then after 6 h the rats were sacrificed. In Group 4; 10 mg kg^{-1} 3-AB was administrated intraperitoneally at the fifth day and repeated daily for 3 days and at the eighth day, 10 mg kg^{-1} LPS was injected intraperitoneally. In Group 5, 10 mg kg^{-1} LPS was injected intraperitoneally at the fifth day and after 6 h 10 mg kg^{-1} 3-AB was administrated intraperitoneally and repeated daily for 3 days. At the eighth day, rats were sacrificed. Blood samples were taken for detection of serum MDA levels. Ileum samples were taken after relaparotomy for histopathological examination to evaluate the endotoxin-related intestinal injury and Caspase-3 apoptosis and for detection of tissue MDA and ATPase activities. There was marked destruction of villous and crypt epithelial cells and extensive apoptosis in Groups 3 and 5 in histopathological examination. In Group 4, the scores of intestinal mucosal damage and apoptotic cells were reduced significantly ($P < 0.05$). On the other hand, the scores of intestinal mucosal damage and apoptotic cells were not improved in Group 5. After the administration of 3-AB (Group 4), serum and ileal MDA levels decreased, ileal ATPase increased as compared to Groups 1 and 2. Our study showed that 3-AB prevented the mucosal damage and apoptotic loss of intestinal epithelial cells significantly if it was administrated before LPS. However, 3-AB failed to prevent the mucosal damage and apoptotic loss of intestinal epithelial cells significantly if there was established endotoxemia in OJ.
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Keywords: Obstructive jaundice; LPS; 3-Aminobenzamide; Apoptosis; Caspase-3

1. Introduction

Systemic endotoxemia developed in obstructive jaundiced (OJ) is the result of the depression in the clearance of lipopolysaccharide (LPS) and other endotoxins in the portal circulation by Kuppfer cells in the liver, which are originated because of bacterial translocation. In OJ, hepatic Kuppfer cells' endocytosis is impaired and their endotoxin clearance ability is decreased. Portal endotoxemia, which is the result of impaired intestinal barrier in OJ, and the systemic endotoxemia, which is developed as a result of impaired function of Kuppfer cells, lead to sepsis [1–3]. The sensitivity of the host to LPS and other endotoxins is increased in OJ and endotoxins become much more harmful in obstructive jaundiced patients than in healthy ones. Free radical production is increased and antioxidative activity is reduced in OJ. Nitric oxide (NO) formation and increased expression of iNOS also take place in OJ [4]. Reactions

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between NO and free oxygen radicals lead to the formation of the most harmful peroxynitrite anion ($ONOO^-$) and this anion leads to lipid peroxidation, cellular damage and apoptosis.

It is thought that oxidative and nitrosative stress developed in OJ and the formation of peroxynitrite can lead to cellular damage. In recent years, positive results were reported with the inhibition of PARS enzyme in experimental sepsis models [5,6]. However, in our literature investigation, we have not found any study about poly (ADP-ribose) synthetase (PARS) enzyme inhibition in LPS-induced experimental obstructive jaundice model and its effects in organ functions. For this reason, in our experimental study, we investigated the effects of inhibition of PARS with 3-aminobenzamide (3-AB) (PARS inhibitor) on small intestine morphology which is an important barrier for micro organisms and which is very frequently effected in OJ and we also investigated its effects on serum and tissue malondialdehyde (MDA) levels and on tissue ATPase activities which show lipid peroxidation.

2. Materials and methods

The experiments described in this article were performed in adherence to National Institutes of Health guidelines on the use of experimental animals. Male, Wistar rats, weighing 180–220 g, were housed at constant temperature with 14:10 h periods of light and dark exposure, respectively. Animals were allowed access to standard rat chow and water ad libitum acclimation period of at least 5 days prior to use in these experiments. Approval of our faculty's ethics committee was taken.

We randomized 40 rats into five groups.

Group 1: sham group ($n = 8$).
Group 2: obstructive jaundice group ($n = 8$).
Group 3: OJ + lipopolysaccharide ($10\,mg\,kg^{-1}$) (*Escherichia coli* LPS serotype L-2630, 100 mg, Sigma) intraperitoneally ($n = 8$).
Group 4: OJ + 3-aminobenzamide ($10\,mg\,kg^{-1}$) (E.C. No. 222-586-9) intraperitoneally; +LPS ($10\,mg\,kg^{-1}$) intraperitoneally ($n = 8$).
Group 5: OJ + LPS ($10\,mg\,kg^{-1}$) intraperitoneally + 3-AB ($10\,mg\,kg^{-1}$) intraperitoneally ($n = 8$).

Rats were anaesthetized with intramuscular ketamine $50\,mg\,kg^{-1}$ and xylazine $7\,mg\,kg^{-1}$ and median laparotomy was performed. Sham operation was performed on the rats in Group 1. The sham operation consisted of mobilization of the bile duct only. The rats in this group were sacrificed just after the procedure.

In Groups 2, 3, 4, and 5; common bile duct (CBD) of the rats was found after retracting liver and ligated twice with 5/0 pyrolen. It was divided to forbid recanalization.

At the fifth day, all rats in Groups 2, 3, 4, and 5 were jaundiced.

In Group 2, we anaesthetized and sacrificed the rats at the fifth day to reveal the prime changes on ileal histopathology, apoptosis and lipid peroxidation in the jaundiced rats.

In Group 3, at the fifth day, we injected LPS intraperitoneally to the jaundiced rats and after 6 h the rats were sacrificed to reveal the prime changes on ileal histopathology, apoptosis and lipid peroxidation in LPS-induced jaundiced rats.

We made Groups 4 and 5 to show the therapeutic and preventive effect of 3-AB in the jaundiced rats before or after the establishment of endotoxemia. In Group 4, 3-AB was administrated before the established endotoxemia to reveal the protective effect of the drug. In Group 5, LPS was administrated before 3-AB to reveal the therapeutical effect of the drug in conditions with endotoxemia.

In Group 4, at the fifth day, we administrated 3-AB intraperitoneally to the jaundiced rats and repeated daily for 3 days and at the eighth day, LPS was injected intraperitoneally.

In Group 5, at the fifth day, we administrated LPS intraperitoneally to the jaundiced rats, after 6 h 3-AB were administrated intraperitoneally, and repeated daily for 3 days.

At the eighth day, all rats in Groups 4 and 5 were anaesthetized and sacrificed.

The rats in Group 2 were sacrificed at the fifth day because lack of bile in small bowel could lead to deleterious morphological changes on intestinal mucosa within 5 days. The rats in Group 3 were sacrificed 6 h after LPS injection to reveal the prime changes on ileal histopathology, apoptosis and lipid peroxidation in LPS-induced jaundiced rats. But the rats in Groups 4 and 5 were sacrificed at the eighth day because we aimed to find out and demonstrate clearly the histopathological and biochemical changes developed when 3-AB was used in jaundiced rats before or after the establishment of endotoxemia.

Table 1 shows the groups and interventions performed with motivation of dosage and timing of the administered substances (LPS and 3-AB).

After the relaparotomy, ileum was resected for histopathological examination to evaluate the endotoxin-related intestinal injury and apoptosis and for detection of tissue MDA and ATPase activities. Blood samples were taken by cardiac puncture for detection of serum MDA levels.

2.1. Conventional light microscopy

The ileal specimens were fixed in 10% formaldehyde. Hematoxylin and eosin-stained slides were prepared by using standard methods. Using a well-established grading scheme described by Chui et al. [7], histopathological assessments were performed according to mucosal damage, inflammation and hyperemia/hemorrhage ranging from 0 to 5.

Table 1
The groups and interventions performed with motivation of dosage and timing of the administered substances (LPS and 3-AB)

	Day	Group 1 (sham)	Group 2 (OJ)	Group 3 (OJ + LPS)	Group 4 (OJ + 3-AB + LPS)	Group 5 (OJ + LPS + 3-AB)
Sham operation	1	x	–	–	–	–
CBD ligation	1		x	x	x	x
OJ	5	–	x	x	x	x
LPS (10 mg kg^{-1})	5	–	–	x	–	x
	6	–	–	–	–	–
	7	–	–	–	–	–
	8	–	–	–	x	–
3-AB (10 mg kg^{-1})	5	–	–	–	x	x[a]
	6	–	–	–	x	x
	7	–	–	–	x	x
	8	–	–	–	–	–
Sacrificed	1	x	–	–	–	–
	5	–	x	x[b]	–	–
	8	–	–	–	x[b]	x

[a] 3-AB was administered 6 h after the administration of LPS.
[b] Six hours after the administration of LPS the rats were sacrificed.

Grade 1: represented normal mucosal villi (Fig. 1a).
Grade 2: represented development of mucosal slough at villous tips (Fig. 1b).
Grade 3: represented extension of the subepithelial space with the epithelial layer lifting up in sheets, presence of a few denuded villous tips, and mild capillary congestion (Fig. 1c).
Grade 4: represented denuded villi with exposed lamina propria; dilated, exposed capillaries with evidence of hemorrhage; and increased cellularity of the lamina propria (Fig. 1d).
Grade 5: represented digestion and disintegration of the lamina propria in villi and presence of hemorrhage and ulceration (Fig. 1e).

Apoptotic cells in the crypt epithelium were counted in 10 adjacent crypts in four separate regions and their mean was calculated based on characteristic cellular morphologic changes, including cell shrinkage with compacted/condensed nuclei (pyknosis) and/or nuclear fragmentation (Fig. 2).

2.2. Immunohistochemistry

Immunohistochemistry for Caspase-3 (Inactive Rabbit polyclonal antibody, CPP 32, Ab-4, dilution 1:50, Lab Vision, 47790 Westinghouse, Dr. Fremont, CA, USA) was performed using a combination streptavidin–biotin–peroxidase method and microwave antigen retrieval on formalin-fixed paraffin-embedded tissues. After deparaffinization, sections were treated with 10% hydrogen peroxidase in filtered water to block endogenous peroxidase activity. To retrieve antigen, slides were boiled with 10 mmol l^{-1} citrate buffer (pH 6.8) for 10 min. After preincubation with Ultra V block (Lab Vision) for 20 min, sections were incubated with primary antibody (1 h at room temperature) followed sequentially with biotinylated goat anti-polyvalent (Lab Vision) for 20 min, and streptavidin–peroxidase complex (Lab Vision) for 30 min. 3-Amino-9-ethylcarbazole (AEC) (Lab Vision) was used as the chromagen, and hematoxylin for nuclear counter stain. Omitting the primary antibody performed the negative control. The positive control was tonsil. The slides were evaluated in a blinded fashion by the author (LC). The positively stained cells were counted with the same technique as mentioned above used for the hematoxylin and eosin-stained specimens (Fig. 3).

2.2.1. Lipid peroxides assay

The levels of serum and tissue lipid peroxidation products as thiobarbituric acid (TBA)–malondialdehyde adducts were measured spectrophotometrically by the method described by Yagi [8]. The results were expressed as nanomoles of MDA formed per milliliter for serum and nanomoles of MDA per gram for tissue.

2.2.2. Determination of ATPase activities

After the animals were killed, the tissues (ileum) were removed as rapidly as possible. Ten percent homogenates of the tissue was prepared in 0.3 M sucrose containing 1 mM magnesium for homogenising 90 s by using Teflon pestle clearance 0.25–0.38 mm at 1000 rpm. To remove the debris, it was then centrifuged at 1000 rpm for 15 min. Measurement of ATPase specific activity is based on the principal of the inorganic phosphate released in 1 h for each milligram protein at the presence of 3 mM disodium ATP, added to the incubation medium. The inorganic phosphate released from the ATP to the incubation medium, and then measured according to the method suggested by Reading and ispir [9,10].

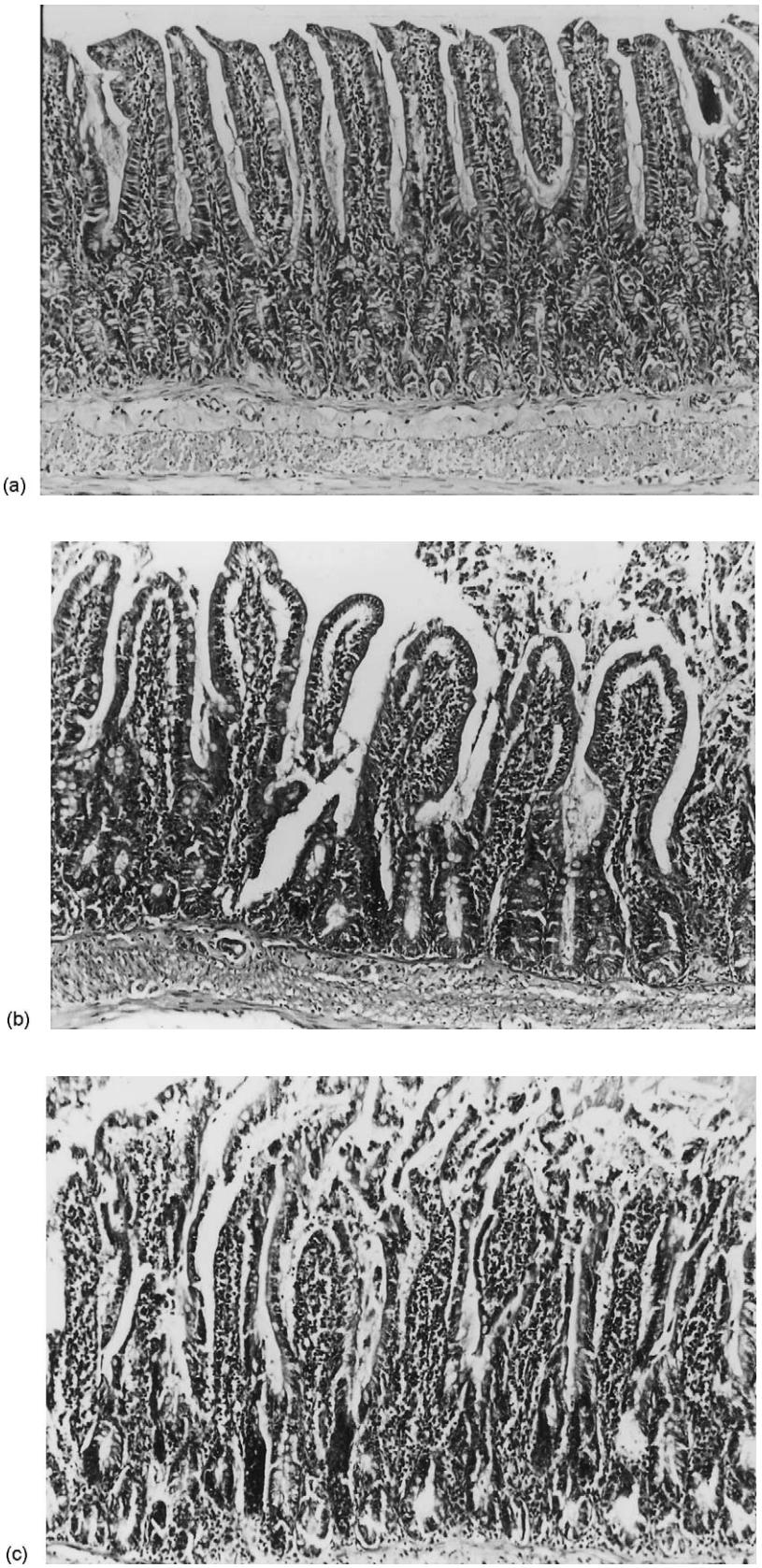
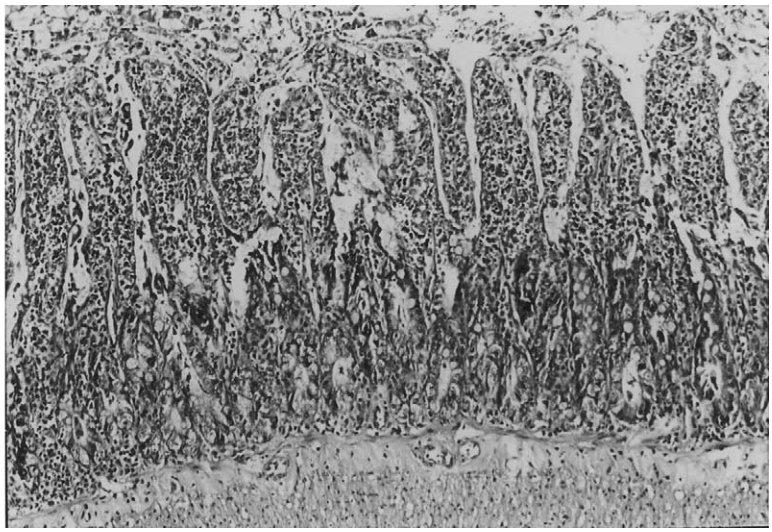


Fig. 1. Representative hematoxylin–eosin-stained sections of small bowel showing histological intestinal injury grading. Grade 1 (a), grade 2 (b), grade 3 (c), grade 4 (d), grade 5 (e), 100×.

(d)



(e)

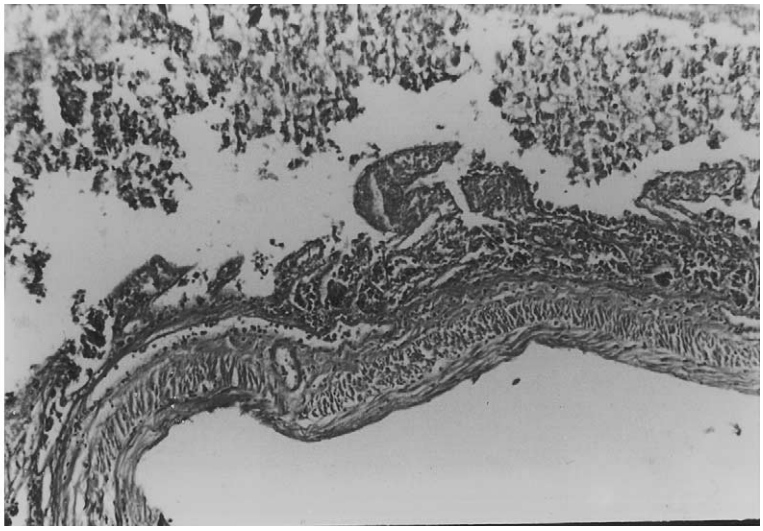


Fig. 1. (*Continued*).

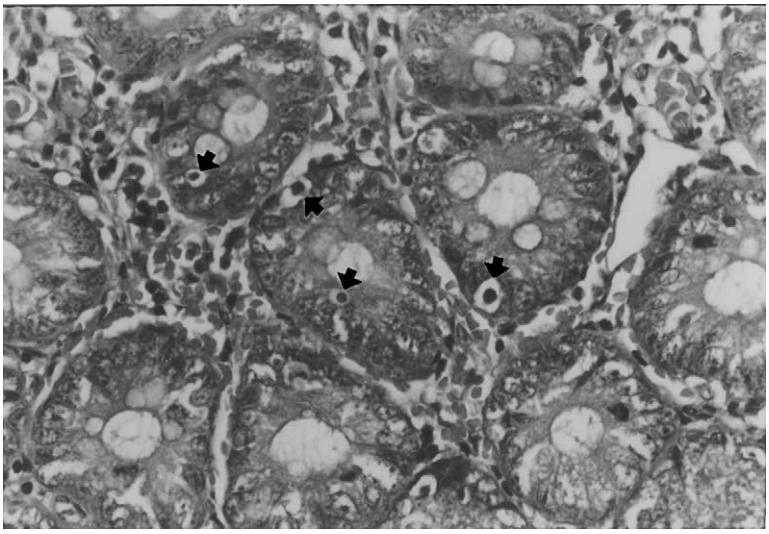


Fig. 2. Representative apoptotic cells (arrows) in crypt. Hematoxylin–eosin 400×.

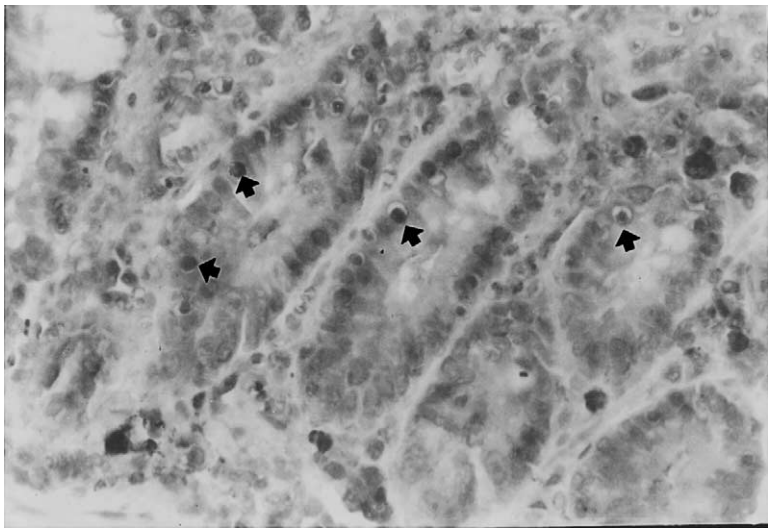


Fig. 3. Caspase-3 positive apoptotic cells (arrows) in immunohistochemical staining of representative small bowel sections 400×.

The protein quantity contained sample was determined according to the method developed by Lowry et al. [11].

The results of the ATPase enzyme systems were stated in nanomoles of P_i per milligram of protein per hour.

All reagents were of Analar grade unless otherwise stated. Disodium adenosine-5′-triphosphate (Na_2ATP) was obtained from Sigma Chemical Co. Ltd.

2.3. Statistical analysis

Biochemical values were described as mean ± S.E.M. values. Statistical differences for serum MDA, ileal MDA and ileal ATPase values were evaluated using one-way ANOVA followed by Tukey HSD post hoc test. Histopathological examinations were presented as median and 25–75% percentiles in parenthesis. Comparisons of hematoxylin–eosin-stained sections for Chui, apoptosis and Caspase-3 scores were analyzed by using Kruskall–Wallis test and Mann–Whitney *U* test. *P* values less than 0.05 were considered statistical significant. Box-plot graphics were used to show medians of Chui, apoptosis and Caspase-3 scores.

3. Results

3.1. Histopathological examination

The histopathological examination of hematoxylin–eosin-stained sections of small bowel in Group 1 (sham group) revealed normal mucosal villi (grade 1) (Fig. 1). In this group, there were no apoptotic cells either in immunohistochemical examination with Caspase-3.

In Group 2 (OJ group), the histopathological examination of hematoxylin–eosin-stained sections of small bowel showed extended subepithelial space with the epithelial layer lifting up in sheets, presence of a few denuded villous tips, and mild capillary congestion (grade 3) (Fig. 1c). There were significant numbers of apoptotic cells in hematoxylin–eosin-stained sections and in immunohistochemically examined slides with Caspase-3.

In Group 3 (OJ + LPS group), the histopathological examination of hematoxylin–eosin-stained sections showed denuded villi and crypt with exposed lamina propria; dilated, exposed capillaries with evidence of hemorrhage and ulceration; and increased cellularity of the lamina propria (grade 5) (Fig. 1e). There was extensive apoptosis in hematoxylin–eosin-stained sections and in immunohistochemically examined slides with Caspase-3.

In Group 4 (OJ + 3-AB + LPS group), the histopathological examination of hematoxylin–eosin-stained sections showed almost normal mucosal villi with developed mucosal slough at villous tips (grade 2) (Fig. 1b). There was very few apoptosis in hematoxylin–eosin-stained sections and in immunohistochemically examined slides with Caspase-3.

In Group 5 (OJ + LPS + 3-AB group), the histopathological examination of hematoxylin–eosin-stained sections showed denuded villi and crypt with exposed lamina propria; dilated, exposed capillaries with evidence of hemorrhage; and increased cellularity of the lamina propria (grade 4) as seen in the histopathological examination of Group 3 (Fig. 1d). There was extensive apoptosis both in the hematoxylin–eosin-stained sections and in the immunohistochemically examined slides with Caspase-3.

In the statistical analysis of intestinal damage (Chui scores), medians were found to be 0 (0–1.5) in Group 1; 3 (2.0–4.0) in Group 2; 4.0 (4.0–4.5) in Group 3; 0 (0–2.0) in Group 4; and 0 (0–0) in Group 5. There were significant differences between Groups 1 and 2, Groups 1 and 3, Groups 1 and 5, Groups 2 and 3, Groups 2 and 4, Groups 3 and 4, and Groups 4 and 5 ($P < 0.05$). But the difference between Groups 1 and 4, Groups 2 and 5, and Groups 3 and 5 were

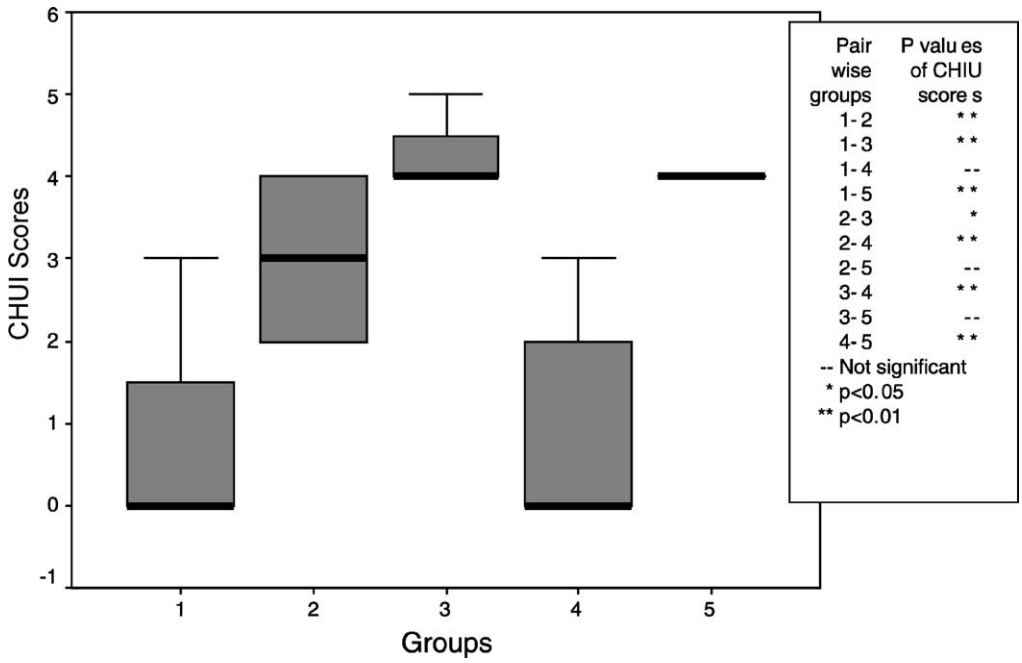


Fig. 4. Box-plot graphic showing the differences between medians and the significance levels of pair wise comparisons of groups in hematoxylin–eosin-stained sections of small bowel according to Chui scores.

not significant. There was marked destruction of villous and crypt epithelial cells in Groups 3 and 5. However, in Group 4 histology of villous was prevented with 3-AB (Fig. 4).

In the statistical analysis of apoptotic loss of intestinal epithelial cells, medians were found to be 1.0 (0–1.5) in Group 1; 14.0 (7.0–18.0) in Group 2; 30.0 (28.0–32.0) in Group 3; 1.0 (1.0–8.0) in Group 4; and 32.0 (31.0–32.5) in Group 5. There was significant difference between Groups 1 and 2, Groups 1 and 3, Groups 1 and 5, Groups 2 and 3, Groups 2 and 4, Groups 2 and 5, Groups 3 and 4, and Groups 4 and 5 ($P < 0.05$). But the difference between Groups 1 and 4 and Groups 3 and 5 were not significant. Morphological evaluation of hematoxylin–eosin-stained sections of Groups 2, 3 and 5 showed extensive apoptosis, however in Group

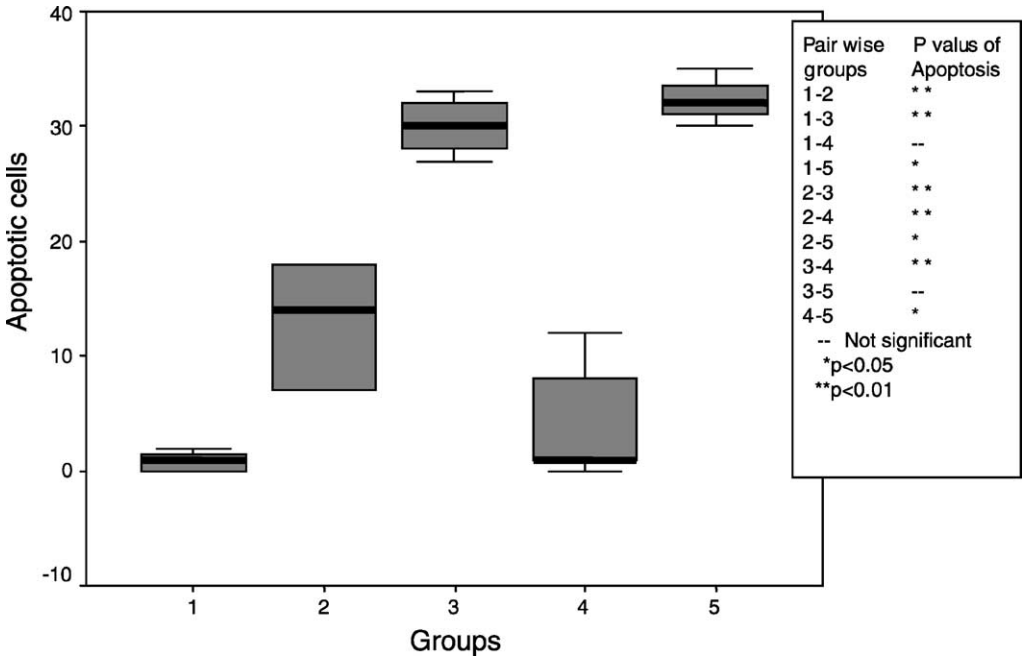


Fig. 5. Box-plot graphic showing the differences between medians and the significance levels of pair wise comparisons of groups in hematoxylin–eosin-stained sections of small bowel according to apoptotic loss of intestinal epithelial cells.

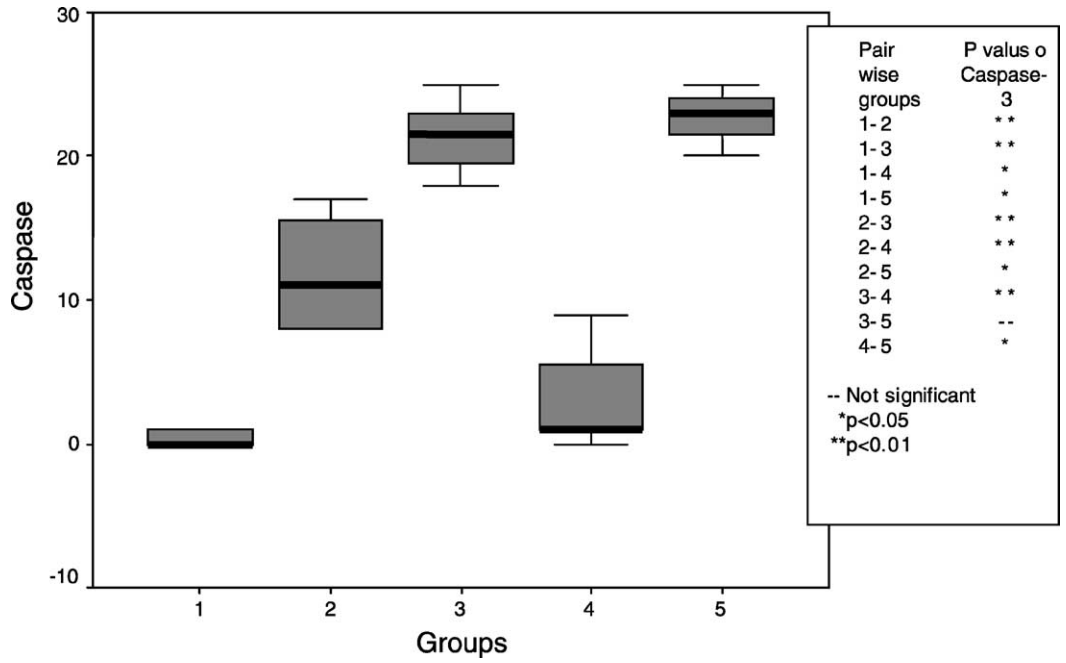


Fig. 6. Box-plot graphic showing the differences between medians and the significance levels of pair wise comparisons of groups in immunohistochemical examination with Caspase-3.

4, the scores of apoptotic cells were reduced significantly to the level of the scores of Group 1 (Fig. 5).

On the other hand, in the statistical analysis of apoptotic loss of intestinal epithelial cells stained with Caspase-3 immunohistochemically, medians were found to be 0 (0–1.0) in Group 1; 11.0 (8.0–15.5) in Group 2; 21.5 (19.5–23.0) in Group 3; 1.0 (1.0–5.5) in Group 4; and 23.0 (21.0–24.0) in Group 5. There was significant difference between Groups 1 and 2, Groups 1 and 3, Groups 1 and 4, Groups 1 and 5, Groups 2 and 3, Groups 2 and 4, Groups 2 and 5, Groups 3

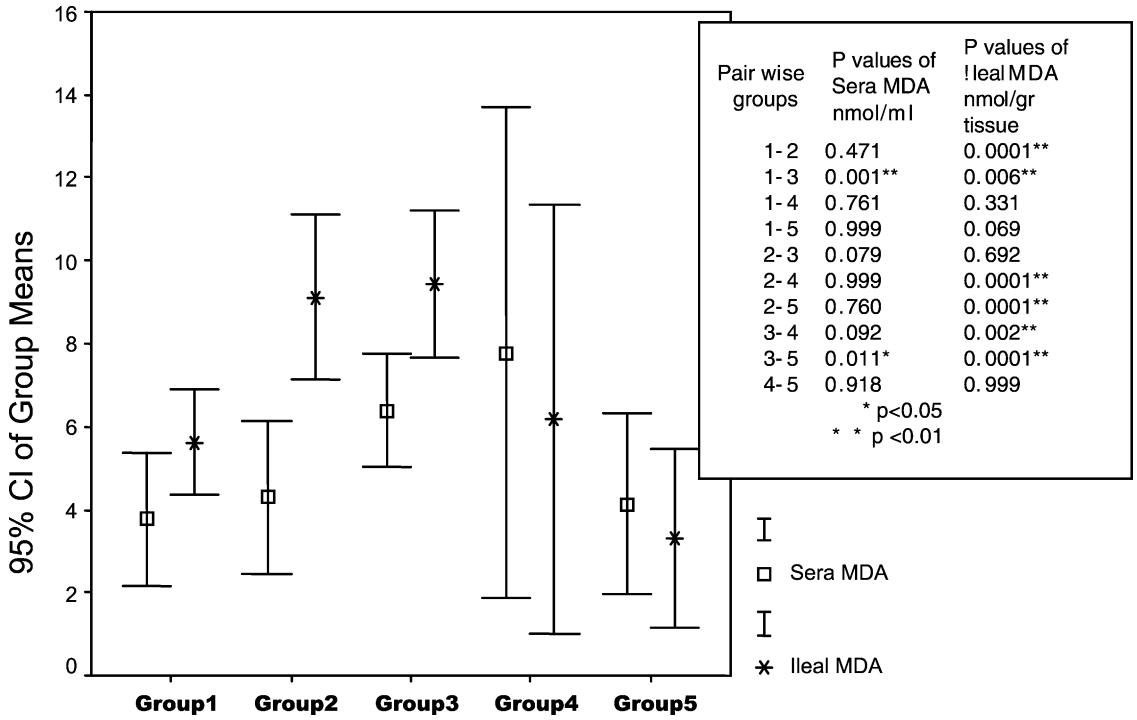


Fig. 7. Graphic showing the differences between mean ± S.E.M. and the significance levels of pair wise comparisons of groups in sera MDA and ileal MDA levels.

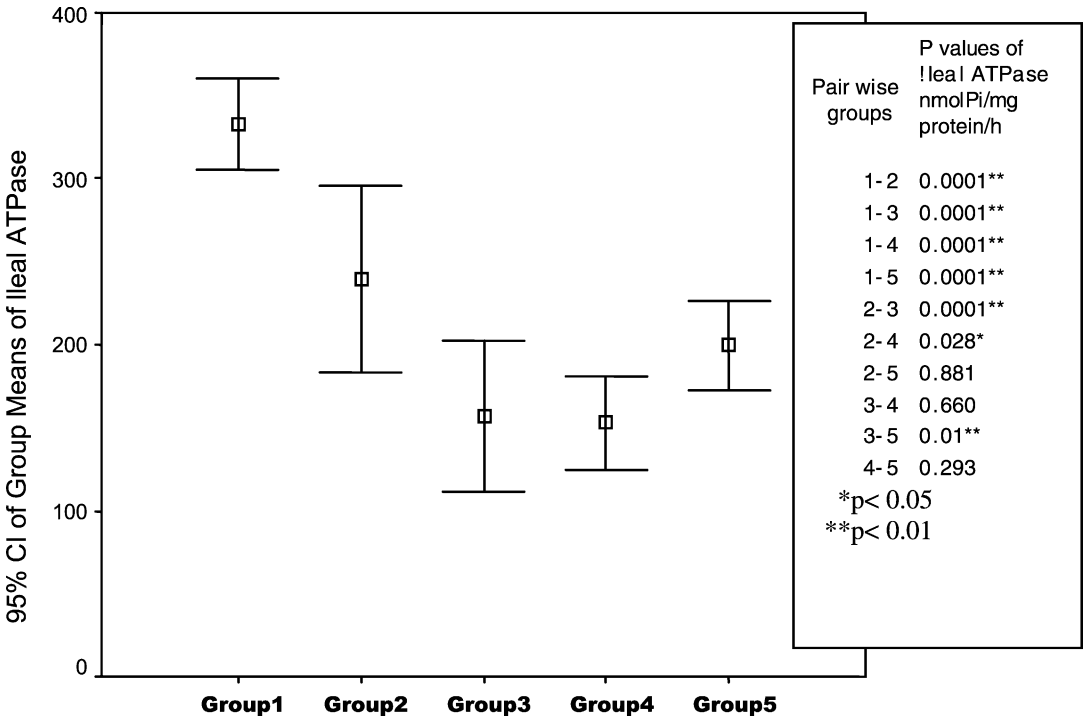


Fig. 8. Graphic showing the differences between mean ± S.E.M. and the significance levels of pair wise comparisons of groups in ileal ATPase.

and 4, and Groups 4 and 5 ($P < 0.05$). But the difference between Groups 3 and 5 was not significant (Fig. 6). This data demonstrated that apoptosis of intestinal cells in Group 4 reduced when 3-AB administrated and the score of apoptotic cells in this group was almost equal to sham-operated control group. On the other hand, apoptosis of intestinal cells in Group 5 was almost equal to LPS-induced jaundiced rats even 3-AB administrated later for therapy.

3.2. Biochemical examination

The descriptive statistics of serum MDA, ileal MDA and ileal ATPase are presented in Figs. 7 and 8, respectively. The significance levels of pair wise comparisons of groups according to Tukey post hoc test are presented in each figure. mean±S.E.M. levels of sera MDA was 3.4±0.6 in Group 1; 5.2±0.6 in Group 2; 8.1±1.2 in Group 3; 4.9±0.9 in Group 4; and 3.7 ± 0.7 in Group 5. Mean ±S.E.M. levels of ileal MDA was 5.9±0.5 in Group 1; 10.3±0.9 in Group 2; 9.1± 0.6 in Group 3; 3.3±0.3 in Group 4; and 3.3±0.5 in Group 5. Mean ±S.E.M. levels of ileal ATPase was 330.4 ± 10.6 in Group 1; 216.4±14.5 in Group 2; 130.9±12.8 in Group 3; 156.6 ± 11.2 in Group 4; and 198.6 ± 6.5 in Group 5. There were increase in serum and ileal MDA and decrease in ileal ATPase in Group 2 when compared to Group 1. These differences were significant in ileal MDA and ileal ATPase, except serum MDA ($P < 0.001$). After LPS injection when the levels of serum MDA and ileal MDA increased, ileal ATPase levels decreased. There were significant differences in serum MDA, ileal MDA and ileal ATPase levels between Groups 1 (sham) and 3 (jaundiced rats with LPS-induced endotoxemia) ($P < 0.001$). As it is seen in Fig. 8, after the administration of 3-AB (Group 4), ileal ATPase levels increased as compared to Groups 1 and 2 ($P < 0.05$).

4. Discussion

Sepsis is one of the major complications related to OJ and it is also the reason of high morbidity and mortality in OJ [12]. In the lack of bile in the intestinal lumen, the intestinal mucosa cannot get the benefit of bile salts, bile pigments and phospholipids and the neutralization and elimination of the intestinal endotoxin is reduced [13]. Impaired gut barrier function leads to bacterial translocation [14]. Polynuclear leukocytes, monocytes and macrophages are activated by endotoxin in OJ and this activation leads to release of many mediators, which contribute to the pathophysiology of the systemic inflammatory response syndrome, sepsis, and multiple organ failure [15–17]. At present, it is thought that in endotoxemia, increases in cytokine levels and especially mediators such as NO play a major role in multiorgan failure [18,4].

Nitric oxide is synthesized by the enzyme nitric oxide synthase (NOS). iNOS is not expressed under normal conditions but is induced by cytokines and endotoxin [19]. In endotoxemia, iNOS is activated (independently of Ca) and leads to excessive production of NO [20,21]. Shiomi et al. have shown that there is excessive production of NO leading to impairment in mitochondrial function in rats with OJ [22]. Recently, oxidative stress is recognized as an important initiator of apoptosis [23]. Peroxynitrite ($ONOO^-$),

as an oxidative and nitrosative stress mediator, can cause DNA strand breakage, which triggers the activation of PARS. PARS plays a role in the repair of strand breaks in DNA and, its activation results in a substantial depletion of NAD, thus leading cell dysfunction [24]. Recent studies have demonstrated that in endotoxemia, PARS inhibitors, such as 3-aminobenzamide, have beneficial effects against oxidative and nitrosative stress-induced organ dysfunctions.

The mechanism of the NO mediated intestinal damage may involve the NO derived peroxynitrite, which activates a nuclear enzyme, PARS. Cuzzocrea et al. have reported the effects of reactive species and PARS inhibitor in intestinal tight junction permeability [25]. On the other hand, there is a general assumption that the gastrointestinal tract is regarded as a major target organ in endotoxic shock [26]. Intestinal epithelial cell apoptosis is a new concept in sepsis, which appears to be the key phenomenon in gut barrier dysfunction because apoptotic loss of intestinal epithelial cells may compromise bowel wall integrity [27–29].

In our study, we observed that there was increase in serum and ileal MDA levels in Group 2. We found decreased ileal ATPase levels. This data showed that there were increased lipid peroxidation in the rats with OJ. Enhanced oxidative stress leads to increase in NO and peroxynitrite ($ONOO^-$) formation. With regard to the histopathological examination of the ileal specimens in Group 2, we observed that median of the scores of ileal mucosal damage, median of the scores of apoptotic cells and median of the scores of apoptosis with Caspase-3 pathway were increased significantly (Figs. 4–6). When LPS was administrated to the rats with OJ, we observed that lipid peroxidation and oxidative stress were exaggerated. These data were supported with the significant increase of serum MDA and ileal MDA levels, and with the decreased ileal ATPase activity. In the histopathological examination of the ileal specimens in Group 3, we found that median of the scores of ileal mucosal damage, median of the scores of apoptotic cells and median of the scores of apoptosis with Caspase-3 pathway increased significantly as compared to sham and OJ groups (Figs. 4–6). This can be explained by the fact that in conditions like acute pancreatitis (AP) and OJ, there is increased sensitivity to LPS endotoxin and this sensitivity can lead to organ dysfunction if any kind of endotoxemia is established. In Group 4 (OJ + 3-AB + LPS), we observed that serum MDA, ileal MDA levels were decreased and ileal ATPase activity was increased. This data showed that lipid peroxidation and oxidative stress was prevented with the protective effect of 3-AB. In the histopathological examination of the ileal specimens of this group, we observed that median of the scores of ileal mucosal damage, median of the scores of apoptotic cells and median of the scores of apoptosis with Caspase-3 pathway were decreased significantly as compared to Groups 2 and 3 ($P < 0.05$) (Figs. 4–6). 3-AB prevented the mucosal damage and apoptotic loss of intestinal epithelial cells significantly. However, in Group 5 (OJ + LPS + 3-AB), serum MDA, ileal MDA levels were decreased and ileal ATPase activity was increased. This data showed that the enhanced sensitivity of the host to LPS and other endotoxins in OJ led to increased apoptotic loss of intestinal epithelial cells because of increased formation of peroxynitrite ($ONOO^-$) and 3-AB could not prevent the apoptotic loss of intestinal epithelial cells if it was administrated after LPS. Extremely high oxidant concentrations, overwhelming PARS-independent mechanisms of cytotoxicity become activated. PARS-independent cytotoxic mechanisms include a direct inhibition of mitochondrial respiration, inhibition of membrane pumps, lipid peroxidation, and protein oxidation and nitration [6]. Perhaps for this reason in Group 5; serum MDA, ileal MDA levels would be decreased and ileal ATPase activity would be increased. With regard to the histopathological examination, there were no significant differences between Groups 3 and 5. So, in conditions with exaggerated lipid peroxidation as established endotoxemia in OJ (Group 5), 3-AB failed to diminish the deleterious effects of LPS on intestinal morphology and apoptosis. This shows that there is no therapeutical effect of 3-AB if organ dysfunction in any kind of endotoxemia is established. But if 3-AB was administrated before LPS as in Group 4, establishment of organ dysfunction was prevented by reducing sensitivity of the host to LPS and other endotoxins.

In conclusion, in conditions like AP and OJ, there is increased sensitivity to LPS endotoxin and this sensitivity can lead to organ dysfunction if endotoxemia is added. In this study, we aimed to investigate the deleterious effects of LPS on intestinal morphology in condition of OJ and the protective effect of 3-AB, PARS inhibitor, to show whether it is effective when administrated before LPS or after LPS. Our study showed that it prevented the mucosal damage and apoptotic loss of intestinal epithelial cells significantly if it was administrated before LPS. However, it failed to prevent the mucosal damage and apoptotic loss of intestinal epithelial cells significantly if there was established endotoxemia in OJ.

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