ACADEMIC PRESS

Pharmacological Research 49 (2004) 227–238

Pharmacological research

www.elsevier.com/locate/yphrs

The effect of *N*-acetylcysteine (NAC) on liver and renal tissue inducible nitric oxide synthase (iNOS) and tissue lipid peroxidation in obstructive jaundice stimulated by lipopolysaccharide (LPS)

Mehmet Çağlıkülekci [a,*], Cengiz Pata [b], Duygu Dusmez Apa [c], Musa Dirlik [a], Lulufer Tamer [d], Faik Yaylak [a], Arzu Kanik [e], Suha Aydin [a]

[a] *Department of General Surgery, Mersin University Medical School, Mersin, Turkey*
[b] *Department of Internal medicine, Mersin University Medical School, Mersin, Turkey*
[c] *Department of Pathology, Mersin University Medical School, Mersin, Turkey*
[d] *Department of Biochemistry, Mersin University Medical School, Mersin, Turkey*
[e] *Department of Biostatistic, Mersin University Medical School, Mersin, Turkey*

Accepted 1 September 2003

Abstract

Morbidity and mortality rates are very high in obstructive jaundice when it is associated with sepsis and multiple organ failure. Nitric oxide (NO) formation and increased expression of inducible nitric oxide synthase (iNOS) also take place in obstructive jaundice (OJ). *N*-Acetylcysteine (NAC) has a beneficial effect by demonstrating anti-inflammatory activity such as inhibits cytokine expression/release, inhibiting the adhesion molecule expression and inhibiting nuclear factor kappa B (NFκB). The aim of this study was to investigate the effects of NAC on liver and renal tissue iNOS, and liver tissue lipid peroxidation in lipopolysaccharide (LPS) induced obstructive jaundice. We randomized 48 rats into six groups. Group A: Sham group; group B: OJ group; group C: OJ + NAC; group D: OJ + LPS (*Escherichia coli* LPS serotype L-2630, 100 mg, Sigma) group E: OJ + NAC + LPS; group F: OJ + LPS + NAC. NAC was started subcutaneously 100 mg/kg. LPS was injected intraperitoneally and then at the tenth day we sacrificed the rats.

Liver malondialdehyde (MDA) increased and liver ATPase decreased in groups B–D when compared to group A. After the administration of NAC (groups C–E), liver MDA levels decreased, tissue ATPase levels increased as compared to other groups. The liver and renal tissue iNOS expression was increased in groups B, D, and F. After the administration of NAC (groups C–E) the liver and renal tissue iNOS expression were decreased. Our results indicated that NAC prevented the deleterious effects of LPS in OJ by reducing iNOS expression via lipid peroxidation in liver and renal tissue; if it was administrated before LPS. But NAC failed to prevent the iNOS expression and lipid peroxidation if there was established endotoxemia in OJ.

Keywords: Obstructive jaundice; *N*-Acetylcysteine; LPS; iNOS expression; Tissue MDA; Tissue ATPase

1. Introduction

Gram negative sepsis is a very serious complication of obstructive jaundice. Morbidity and mortality rates are very high in obstructive jaundice when it is associated with sepsis and multiple organ failure. One of the important problem in obstructive jaundice (OJ) is the increased incidence of endotoxaemia that results from defective host immune response [1,2]. Lipid peroxidation is an important problem which takes place in OJ. Free radical production is increased and antioxidative activity is reduced in OJ [3]. Nitric oxide (NO) formation and increased expression of inducible nitric oxide synthase (iNOS) also take place in OJ [4]. NO that trigged by the iNOS is reacted with free oxygen radicals and this lead to the formation of the most harmful peroxynitrite anion ($ONOO^-$) and this anion leads to lipid peroxidation, cellular damage and apoptosis. *N*-Acetylcysteine (NAC) is probably one of the most widely investigated agents that serves as a precursor of glutathione and also acts as a direct scavenging agent [5]. The glutathione concentration was significantly reduced in obstructive jaundice. It has been noted in many studies that NAC increases glutathione pool in hepatic cells and in turn has antioxidant effects in cells and thus

* Corresponding author. Present address: Palma 2, Sitesi A1, Blok 75.yıl Mah, Kat: 6 No: 17, 33320 Mezitli-Mersin, Turkey. Tel.: +90-324-481-5427; mobile: +90-532-325-6376; fax: +90-324-337-4305.
E-mail address: mcaglikulekci@yahoo.com (M. Çağlıkülekci).

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doi:10.1016/j.phrs.2003.09.013

increases resistance and elasticity of hepatic cell membrane [6]. In addition to its antioxidant properties, NAC has several anti-inflammatory properties such as inhibits cytokine expression/release, inhibiting the adhesion molecule expression and inhibiting NFκB [7,8].

We have found no previous studies about the effect of NAC on liver and kidney tissue iNOS expression and lipid peroxidation in lipopolysaccharide (LPS) induced obstructive jaundice.

The aim of this study was to investigate the effects of NAC on liver and renal tissue iNOS, and tissue lipid peroxidation under unfavorable conditions which are created by LPS induced obstructive jaundice.

2. Material 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 and acclimation period of at least 5 days prior to use in these experiments. All rats were anaesthetized with intramuscular ketamine 50 mg/kg and xylazine 7 mg/kg.

We randomized 48 rats into six groups. Group A: Sham group; group B: OJ group; group C: OJ + NAC; group D: OJ + LPS (*Escherichia coli* LPS serotype L-2630, 100 mg, Sigma); group E: OJ + NAC + LPS; group F: OJ + LPS + NAC.

2.1. Surgical procedures: bile duct ligation

The chest and abdomen were shaved and each animal was fixed in a supine position on the operating table. The abdomen was cleaned with 1% polyvinyl iodine and when dry, the operating field was covered with a sterile drape. The abdomen was opened through a midline laparotomy and experimental jaundice was created by ligation of the common bile duct through a midline laparotomy by the technique described in details by Lee [9]. The bile duct was divided near to confluence (bifurcation of right and left hepatic duct). In addition the bile duct was divided to forbid recanalization. The abdominal wall was closed with interrupted silk sutures and the edges of the skin were approximated with a subcuticular stitch. All the rats that were subjected to bile duct division became evidently jaundiced within 5 days, at which time the mean serum bilirubin levels overreached 8 mg%.

At the end of the fifth day; the rats were jaundiced. In group C at the fifth day NAC was started subcutaneously 100 mg/kg for 5 days. In group D, at the fifth day, LPS was injected intraperitoneally and then at the tenth day we sacrificed the rats. In group E, we administrated NAC at the end of fifth day and repeated daily for 5 days and at the end of the tenth day, LPS was injected and then after 6 h we sacrificed the rats. In group F at the end of the fifth day; we injected LPS, after 6 h NAC administrated daily for 5 days. At the tenth day, whole of the rats were sacrificed.

After the laparotomy, liver and kidney were resected for histopathological examination. During histopathological examination of resected liver and renal tissue iNOS was illustrated immunohistopathologically. The pathologist was blinded as to what group an animal belonged. In addition liver tissue malondialdehyde (MDA) and ATPase levels were detected in biochemical methods.

2.2. Histological evaluation

The extracted liver and renal tissue were fixed in 10% phosphate-buffered formalin and subsequently embedded in paraffin, sectioned, finally stained with hematoxylin and eosin.

2.3. Immunohistochemical assay

Five-micron thick sections were prepared from the paraffin blocks of liver and renal tissue was used to quantify the iNOS expression. The avidin–biotin complex immunoperoxidase staining system was used. Primary antibodies used, anti-iNOS, a synthetic peptide derived from the extreme C-terminus of the human iNOS protein (Zymed Laboratories, San Francisco, CA, USA); with a dilution of 1:1000. The sections were deparaffinized in xylene through ethanol to phosphate-buffered saline (PBS; pH 7.2). To block endogenous peroxidase activity, 3% hydrogen peroxide was applied for 30 min. The slides incubated in citrate buffer were heated in microwave Owen for 5 min. After waiting 20 min, they were removed, and ultra V block (Lab Vision) was added. Primary antibody for iNOS was applied and incubated overnight in a moist chamber at 4 °C. The slides were subsequently incubated in biotinylated goat anti-polyvalent (Lab Vision) for 10 min, and in streptavidin peroxidase (Lab Vision) for 20 min. Finally, AEC substrate system (Lab Vision) was applied for about 3 min. After incubation, the sections were rinsed with distillate water and tap water. The tissue was counterstained with Mayer's hematoxyline. All slides were covered with a coverslip after mounting in buffered glycerin.

2.4. Morphometric determination

iNOS protein was observed in the cytoplasm of parenchymal cells of liver and epithelial cells of kidney. iNOS protein expression in the groups was assessed according to the intensity of staining; score 0: <2% absent; score 1: 2–10% weak; score 2: 10–50%, intermediate; score 3: >50% strong. Evaluating the iNOS staining of the parenchymal cells we selected the most representative areas and then tried to estimate the percentage of the cells which were positively stained. Ten high power field areas were scored and an

average value was given for each case. No reference case stained 100% was used.

2.5. Liver tissue MDA and ATPase levels: the measurement of MDA

The MDA levels, as an index of lipid peroxidation was determined by thiobarbituric acid (TBA) reaction according to the Yagi [10]. The principle of the method depends on measurement of the pink color produced by interaction of the barbituric acid with malondialdehyde elaborated as a result of lipid peroxidation. The colored reaction 1,1,3,3-tetraetoxypropane was used as the primary standard.

2.6. Determination of ATPase activities

After the animals were sacrificed, the tissues were removed as rapidly as possible. Ten percent homogenates of the tissue was prepared in 0.3 M sucrose containing 1 mM magnesium by homogenisated for 90 s. Pestle clearance was used 0.25–0.38 mm at 1000 rpm. To remove the debris, it was then centrifuged at 1000 rpm for 15 min. ATPase activities were determined on the resulting supernatant by measuring rate of liberation of inorganic phosphate (Pi) from disodium ATP incubation media were made up as described previously [11,12].

Adenosine five triphosphates was as follows: Na^+/K^+ ATPase (in mM): $MgCl_2$ 6, KCl 5, NaCl 100, EDTA 0.1, Tris–HCl buffer (pH 7.4), Ca^{2+}/Mg^{2+} ATPase 135, $MgCl_2$ 0.15, EDTA 0.1, HCl buffer (pH 7.4) 135. After pre-incubation for 5 min at 37 °C disodium ATP was added to catch tube to reach a final concentration of 3 mM. The blank sample containing no enzyme, standard and unknowns were incubated at 37 °C for 30 min the reaction was stopped by putting the samples on ice. Inorganic phosphate was determined on 1 ml aliquots of the incubated mixtures by the addition of lubrol-molybadate solution followed by vortexing and standing at 340 nm was measured. All assays were done in triplicate and run with enzyme and reaction blanks. Samples were compared for phosphate content with standards of KH_2PO_4. Specific activities were calculated as nmol Pi/h/mg protein.

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

Protein content was determined according to the method described by Lowry and bovine serum albumin was used as a standard [13].

2.7. Statistical analysis

Biochemical values were described as mean ± S.E.M. values. Statistical differences for serum MDA and nitrate values were evaluated using one-way ANOVA followed by Tukey HSD post-hoc test. Comparisons iNOS staining scores were analyzed using Kruskall–Wallis test followed by Dunn test. Median values were described with interquartile range. *P*-values less than 0.05 were considered statistical significant.

3. Results

3.1. Histology

3.1.1. Liver

Histopathological sections obtained from the liver tissues were examined under a light microscope. The major dilated bile ducts were observed in OJ groups (groups B–F). The portal tracts were enlarged fwith an inflammatory infiltration including mostly lymphocytes and proliferated bile ducts in group B (OJ) (Fig. 1). Different from the other groups, micro abscess was noted in rats of group D (OJ + LPS) (Fig. 2). Minimal inflammatory chances in the portal region were shown in group A (Sham) (200×, H&E).

Moderate enlargement in portal region with minimal inflammatory infiltration and ductile proliferation were shown in group C (OJ + NAC) (200×, H&E).

Enlarged portal region with inflammatory infiltrate and ductile proliferation were shown in group E (OJ + NAC + LPS) (200×, H&E).

Histopathological sections of groups A (Sham), C (OJ + NAC) and E (OJ + NAC + LPS) were shown in Figs. 3–5.

3.1.2. Kidney

Histopathological sections obtained from the liver tissues were examined under a light microscope. In the histopathological sections of the kidneys in groups B–F the changes were noted to be nonspecific. These were; fibrin in glomerular capillaries, endothelial cell swelling, and proteinous in the lumina of some tubules.

3.2. Immunohistochemical iNOS expression

3.2.1. Liver

In the immunohistochemical examination of iNOS Expression in the liver of rats in Sham group (group A); very few iNOS staining was detected around the central venules of the lobules. In the statistical analysis of liver iNOS staining, medians were found to be 0 (0 to 0) in group A.

In group B (OJ group); the number of iNOS stained cells were increased. In the statistical analysis of liver iNOS staining, medians were found to be 1 (1 to 1) in group B.

In group C (OJ + NAC group); there was sparse iNOS staining in group C. In the statistical analysis of liver iNOS staining, medians were found to be 1 (0 to 1) in group C.

In group D (OJ + LPS group); there was an intense iNOS staining in group D. In the statistical analysis of liver iNOS staining, medians were found to be 3 (3 to 3) in group D.

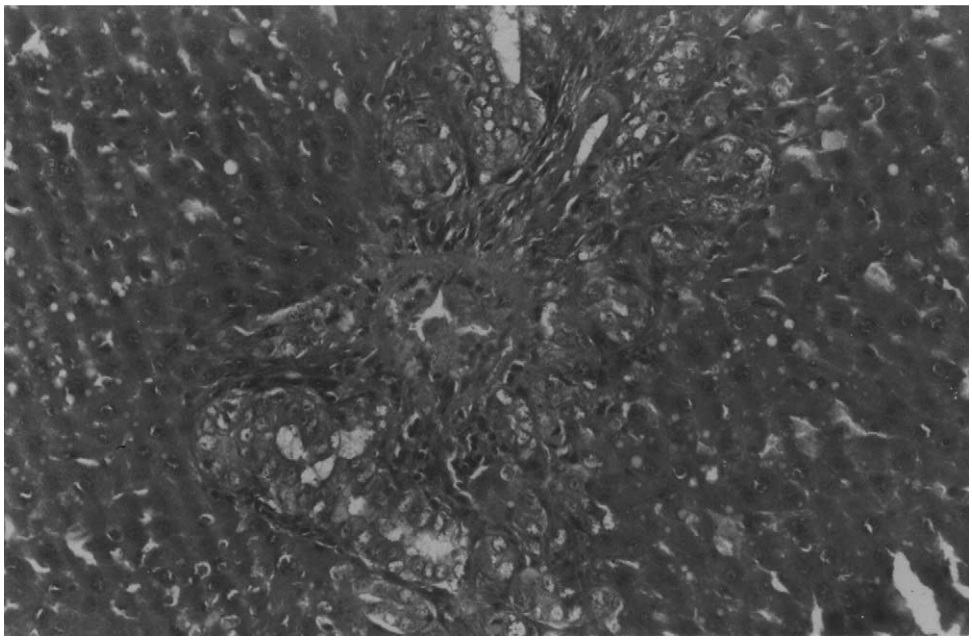

Fig. 1. The portal tracts enlarged with an inflammatory infiltration including mostly lymphocytes and proliferated bile ducts (group B: OJ) (200×, H&E).

In group E (OJ + NAC + LPS); there was sparse iNOS staining in group E. In the statistical analysis of liver iNOS staining, medians were found to be 0.5 (0 to 1) in group E.

In group F (OJ + LPS + NAC group); there was an intense iNOS staining in group F. In the statistical analysis of liver iNOS staining, medians were found to be 2 (2 to 2) in group E.

There was significant difference between groups A and B, groups A and D, groups A and F, groups B and C, groups B and D, groups B and E, groups C and D, and groups C and F, and groups D and E ($P < 0.05$) (Fig. 6).

The immunohistochemical examination with iNOS demonstrated that iNOS staining in group A (Sham-operated control group), group C (OJ + NAC), and group E (OJ + NAC + LPS) were lower than group B (OJ), group D (OJ + LPS) and group F (OJ + LPS + NAC). The results of the iNOS staining in the parenchymal cells of the liver are summarized in Table 1. The iNOS expression of the liver parenchymal cells in group D (OJ + LPS) was statistically

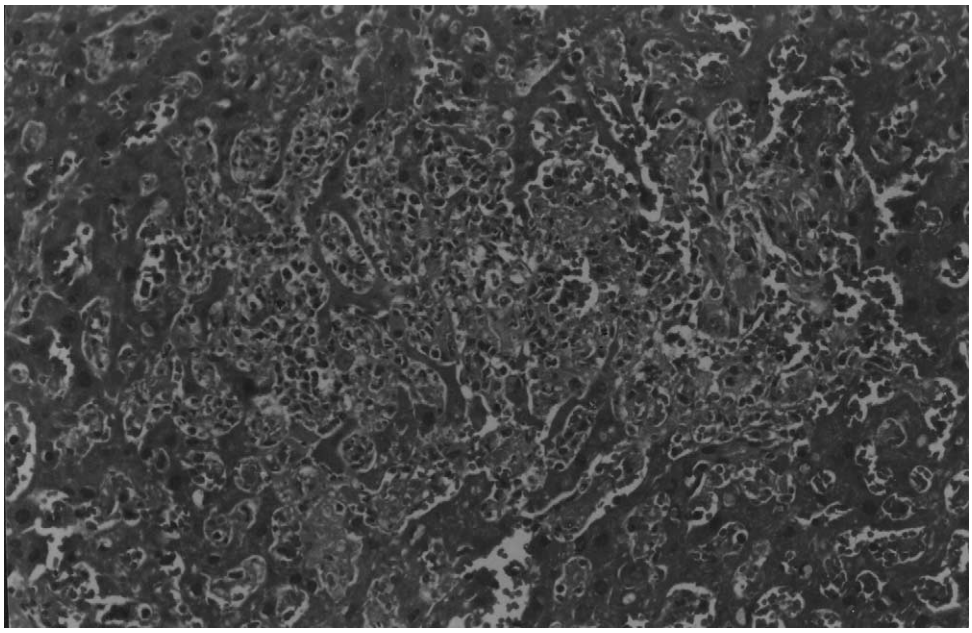

Fig. 2. Micro abscess in the liver parenchyma of group D (OJ + LPS) (200×, H&E).

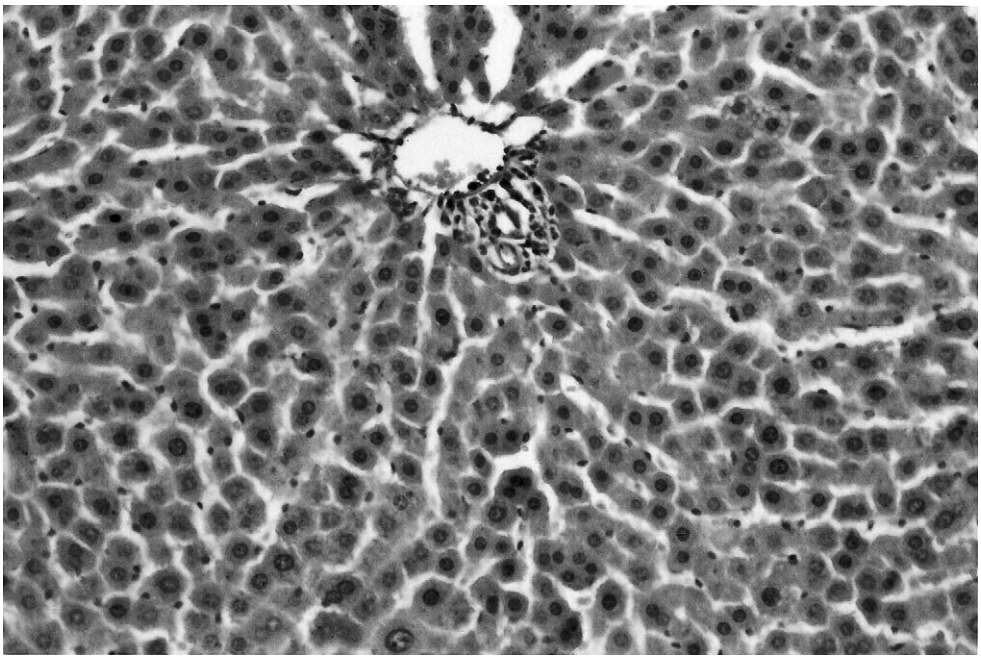

Fig. 3. Mild inflammatory chances in the portal region group A (Sham) (200×, H&E).

significant when compared to group E (OJ + NAC + LPS) (Fig. 7a and b).

3.2.2. Kidney

In the kidney tissue iNOS expression was noted in the tubulary epithelium. There were severe immunostaining in the tubular epithelium, in both cortical and medullar region, being prominent in the outer medulla. The results of the iNOS staining in the epithelial cells of kidney tissue were shown in Table 2. The statistical values of iNOS scores was shown in Fig. 8.

In the immunohistochemical examination of iNOS expression in the kidney of rats in Sham group (group A); very few iNOS staining was detected around the tubuler epitelium. In the statistical analysis of kidney iNOS staining, medians were found to be 0.5 (0 to 1) in group A.

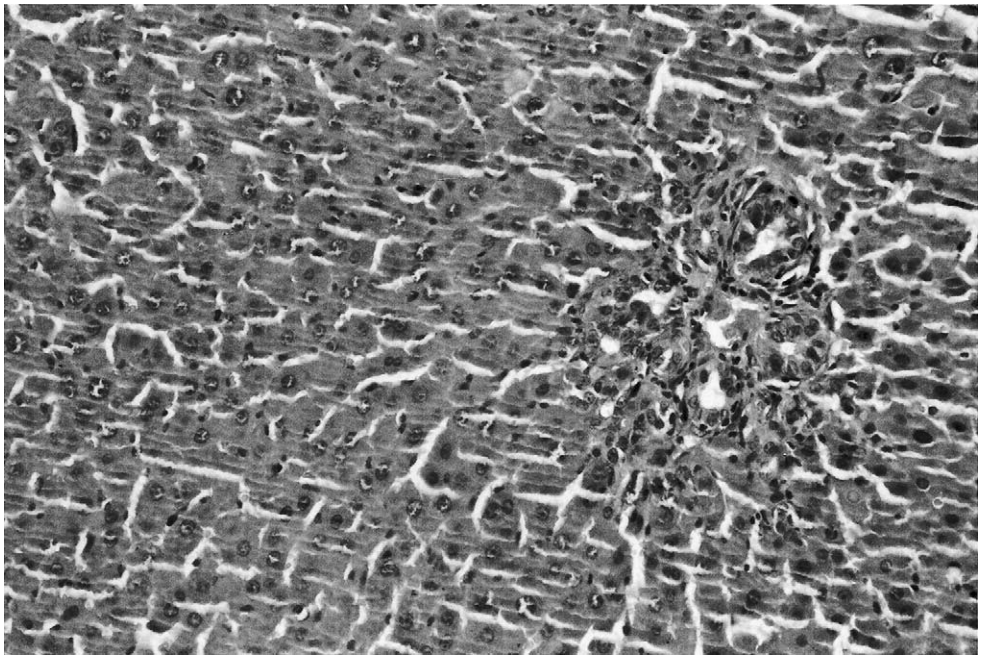

Fig. 4. Moderate enlargement in portal region with minimal inflammatory infiltration and ductile proliferation group C (OJ + NAC) (200×, H&E).

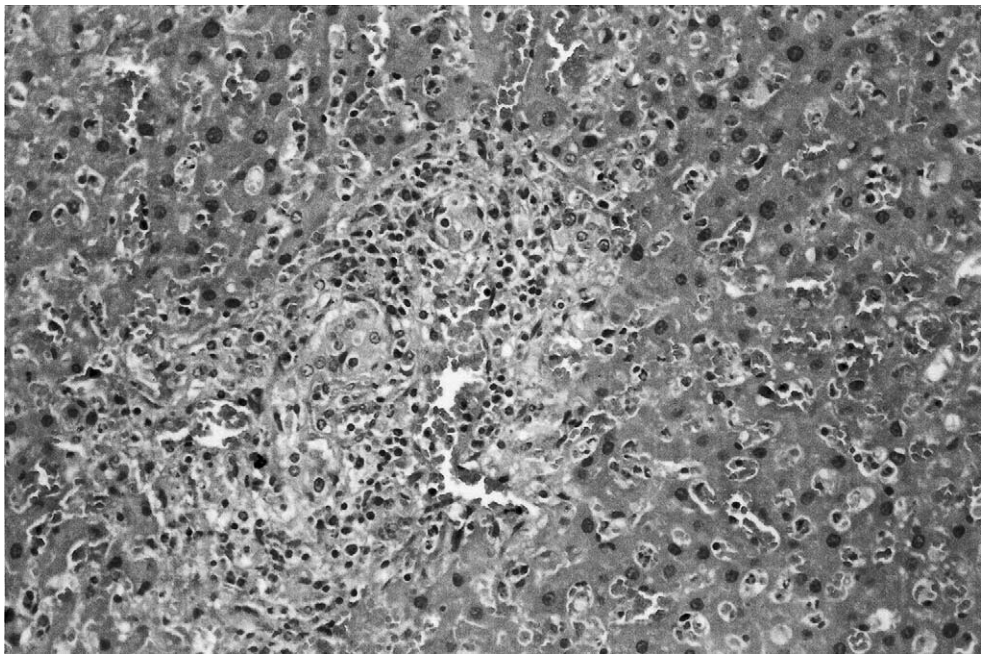

Fig. 5. Enlarged portal region with inflammatory infiltrate and ductile proliferation group E (OJ + NAC + LPS) (200×, H&E).

In group B (OJ group); the number of iNOS stained cells were increased. In the statistical analysis of kidney iNOS staining, medians were found to be 1 (0.5 to 1) in group B.

In group C (OJ + NAC group); there was sparse iNOS staining in group C. In the statistical analysis of renal iNOS staining, medians were found to be 1 (1 to 2) in group C.

In group D (OJ + LPS group); there was an intense iNOS staining in group D. In the statistical analysis of renal iNOS staining, medians were found to be 2 (2 to 3) in group D.

In group E (OJ + NAC + LPS group); there was sparse iNOS staining in group E. In the statistical analysis of renal iNOS staining, medians were found to be 1 (1 to 2) in group E.

In group F (OJ + LPS + NAC group); there was intense iNOS staining in group F. In the statistical analysis of renal iNOS staining, medians were found to be 2 (2 to 2) in

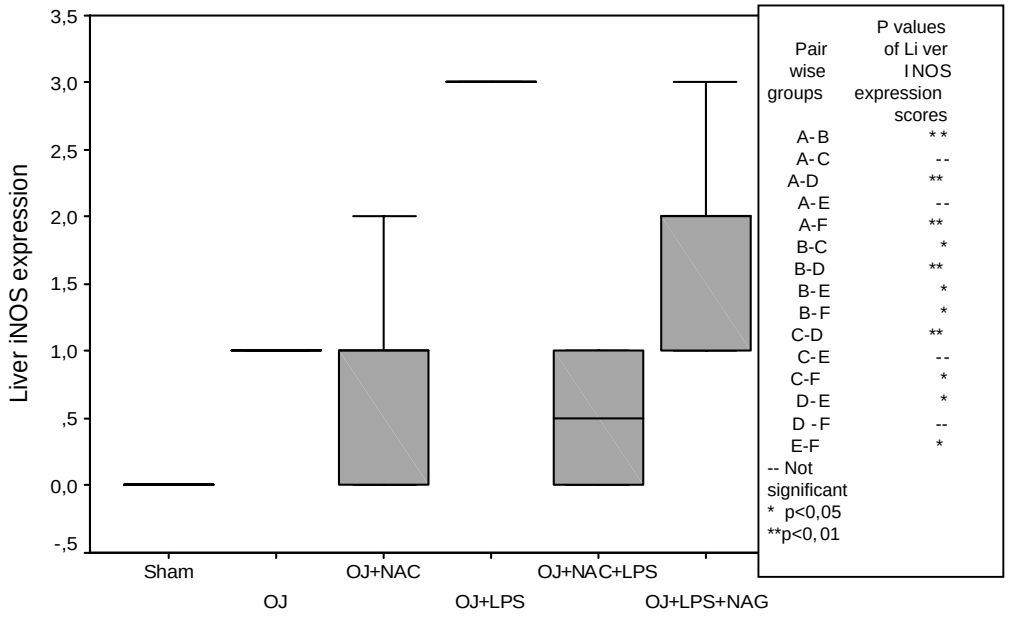

Fig. 6. Levels of liver iNOS expression in the groups.

Table 1
The number of cases scored according to iNOS expression of the liver tissue

Group	Number of rats in groups (*n*)			
	Score 0	Score 1	Score 2	Score 3
A	7	1	0	0
B	1	6	1	0
C	2	4	2	0
D	0	0	1	7
E	3	3	2	0
F	0	1	3	4

Table 2
The number of cases scored according to iNOS expression of the kidney tissue

Group	Number of rats in groups (*n*)			
	Score 0	Score 1	Score 2	Score 3
A	4	3	1	0
B	2	5	1	0
C	0	3	5	0
D	0	1	6	2
E	2	4	2	0
F	0	3	5	0

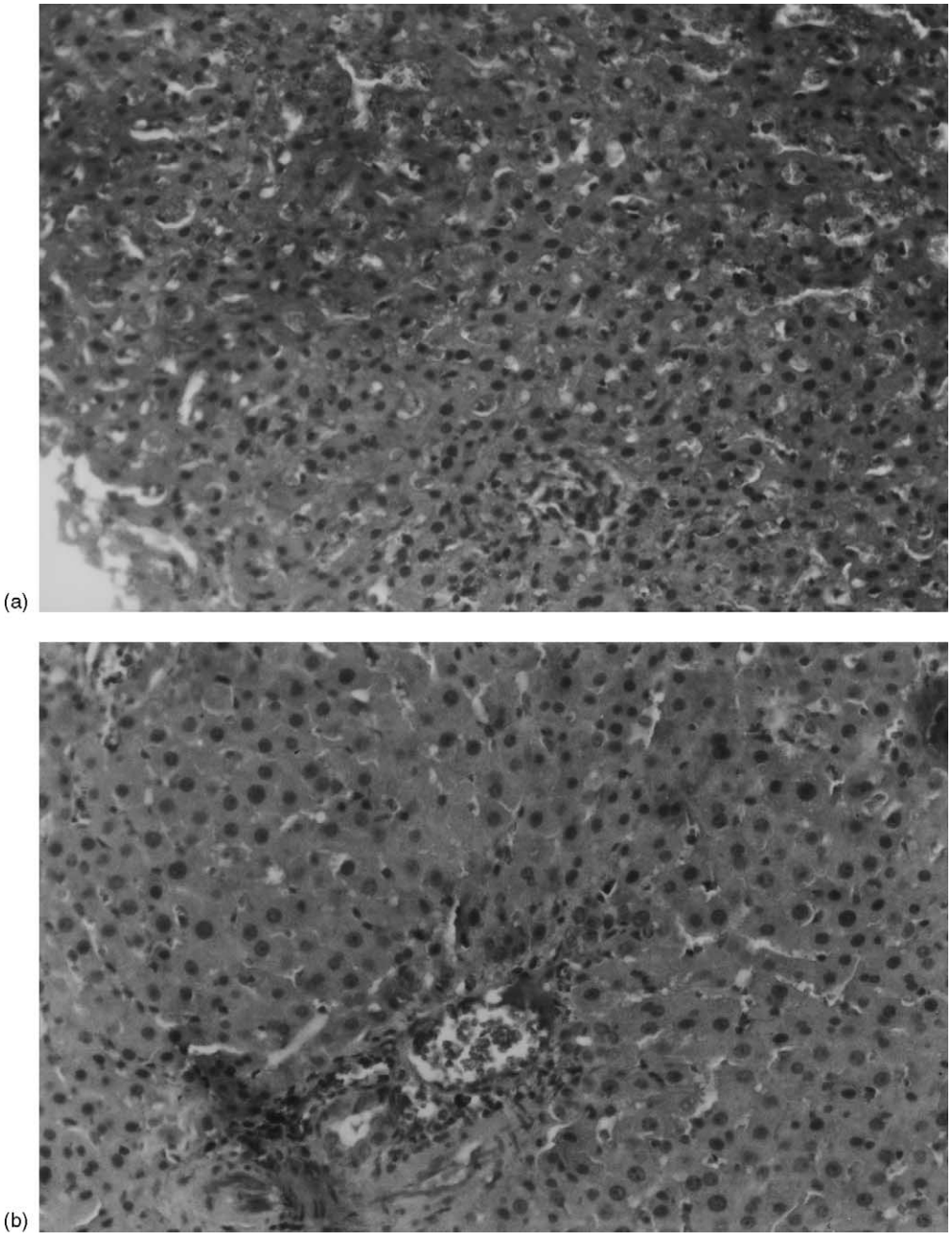

Fig. 7. (a) Increased iNOS expression in the parenchymal cells of the liver tissue in group D (OJ + LPS) (200×). (b) Decreased iNOS expression in the parenchymal cells of the liver tissue in group E (OJ + NAC + LPS) (400×).

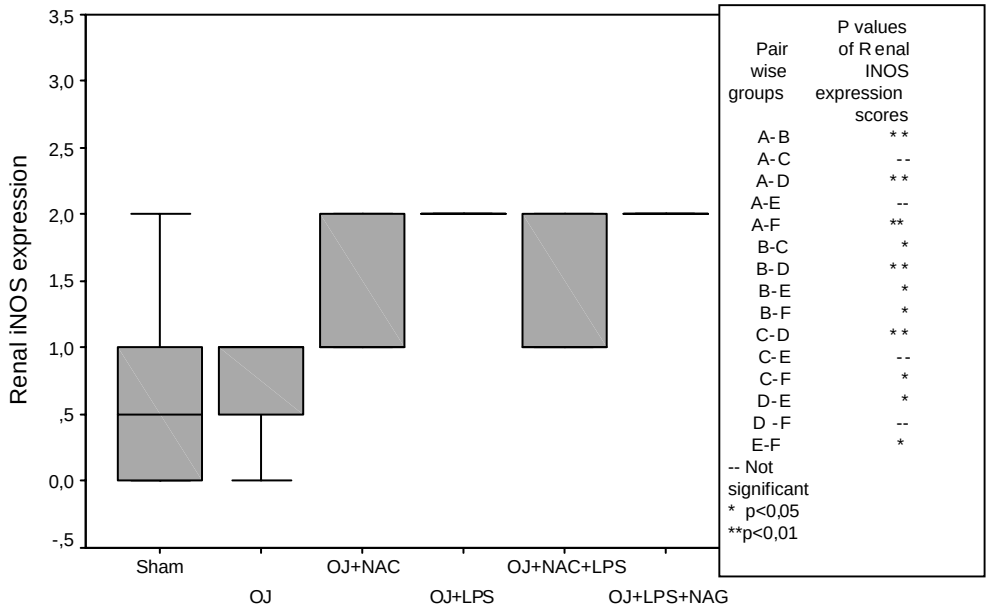

Fig. 8. Levels of renal iNOS expression in the groups.

group F. NAC does not reduce iNOS expression in kidney of jaundiced rats, after LPS injection.

There was significant difference between groups A and B, groups A and D, groups A and F, groups B and C, groups B and D, groups B and E, groups B and F, groups C and D, and groups C and F, and groups D and E ($P < 0.05$) (Fig. 8).

The tubular epithelium showed more intense staining in cases of group D (OJ+LPS) and group F (OJ+LPS+NAC) (Fig. 9a) when compared to group E (OJ + NAC + LPS) (Fig. 9b).

3.3. Biochemical examination

The descriptive statistics of liver MDA and liver ATPase are presented in Figs. 10 and 11, respectively. The significance levels of pairwise comparisons of groups according to Tukey post-hoc test are presented in each figure.

Mean±S.E.M. levels of liver MDA was 2.6±0.6 in group A, 7.9 ± 0.6 in group B, 6.8 ± 0.6 in group C, 20.1 ± 5.5 in group D, 12.6 ± 4.5 in group E and 9.2 ± 1.1.

Mean ± S.E.M. levels of liver ATPase was 166.7 ± 8.6 in group A, 114.7 ± 13.6 in group B, 229.7 ± 80.4 in group C, 76.9 ± 17.3 in group D, 91.7 ± 12.6 in group E and 88.5 ± 18.3 in group F.

When the liver MDA of the groups were compared; there was significant difference between groups A and B, groups A and D, groups A and F, groups B and C, groups B and D, groups B and E, groups C and D, groups C and E, groups C and F, groups D and E, groups D and F, and groups E and F ($P < 0.05$) (Fig. 10).

When the liver ATPase of the groups were compared; there was significant difference between groups A and B, groups A and C, groups A and D, groups A and E, groups A and F, groups B and C, groups B and D, groups B and E, groups C and D, groups C and E, groups C and F, groups D and E, and groups E and F ($P < 0.05$) (Fig. 11).

After LPS injection when the levels of liver MDA increased, liver ATPase levels decreased. There were no significant differences in liver MDA and liver ATPase levels between group A (Sham), group C (OJ + NAC) and group E (OJ + NAC + LPS) ($P > 0.05$). After the administration of NAC (groups C and E), liver MDA levels decreased, liver ATPase levels increased as compared to groups B, D and F.

4. Discussion

The systemic sequelae of hyperbilirubinemia and obstructive jaundice have been well described. OJ can cause problems such as cardiovascular changes, RES depression, coagulation disorders, gastrointestinal bleeding, delayed wound healing, pulmonary insufficiency, secondary biliary cirrhosis, sepsis and renal failure [1].

The sensitivity of the host to LPS and other endotoxins are increased in OJ and therefore endotoxins become much more harmful in obstructive jaundiced patients than in healthy persons. Cholestatic patients have substantial morbidity because of increased sensitivity to LPS endotoxin [3].

In our study, we observed that there was an increase in liver MDA levels in groups B, D and F. In the same groups decreased liver ATPase levels were found. This data showed that there was an increased lipid peroxidation in the rats with obstructive jaundice. As a result of enhanced oxidative stress increased NO formation should lead to increased ($ONOO^-$) formation. The histopathological examination of the liver and renal specimens in groups B, D and F; we

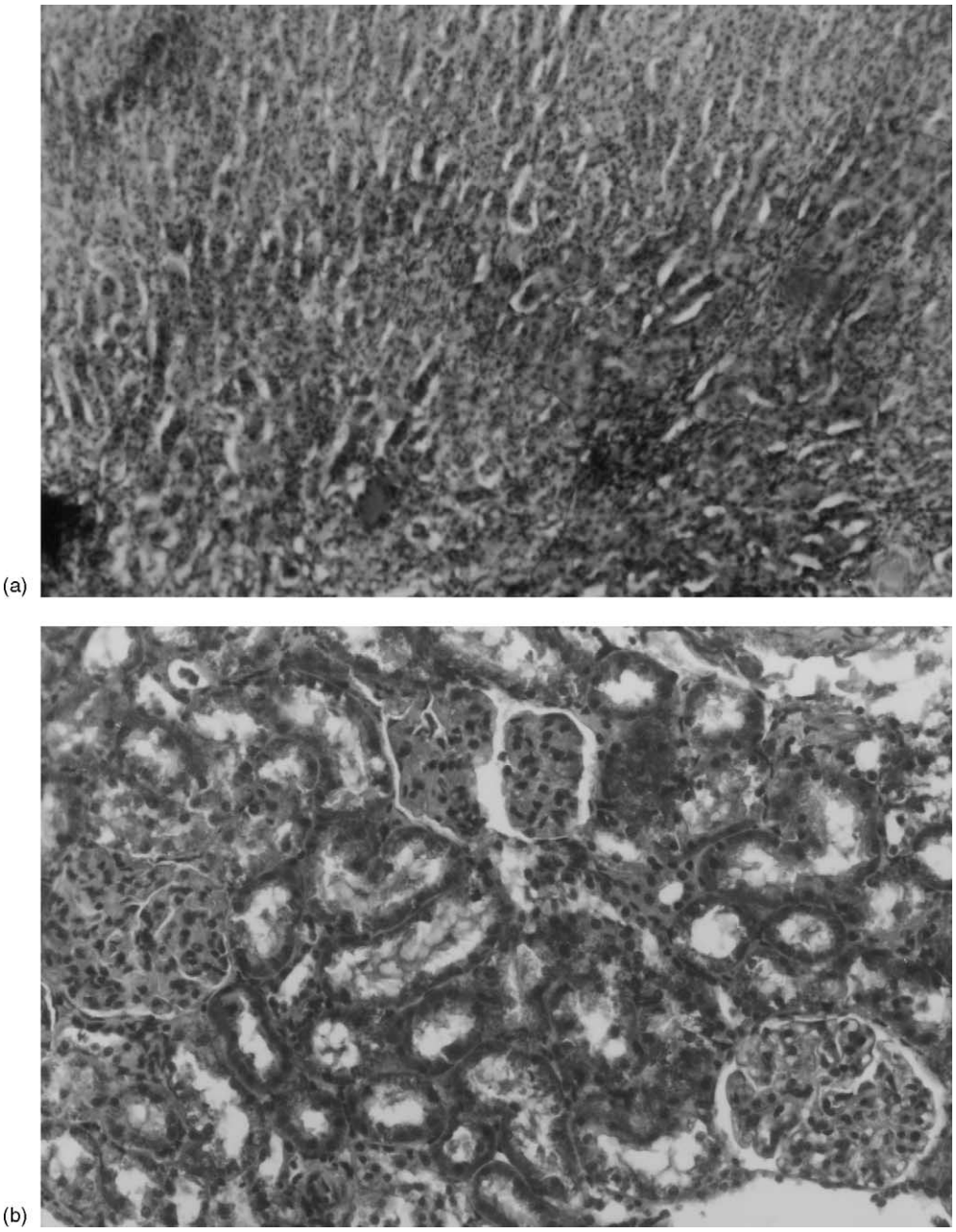

Fig. 9. The tubular epithelium showing: (a) more intense staining in group D (OJ + LPS) (100×) and (b) sparse staining in group E (OJ + NAC + LPS) (200×).

observed the liver and renal tissue damage. When LPS was administrated to the rats with obstructive jaundice (group D), we observed that lipid peroxidation was increased. This data was supported with the significant increase of liver MDA levels, and with the decreased liver ATPase activity.

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 [4]. In endotoxemia, iNOS is activated (independently of Ca) and leads to excessive production of NO. It is thought that, in OJ, iNOS is synthesized in tissue from macrophages, leukocytes and endothelial cells as a result of endotoxemia with LPS. Activation of the macrophage, endothelial cells and leukocytes results in the overexpression of IL-1, TNF-α, platelet activating factor (PAF), INF-γ and synthase of adhesion molecules like ICAM, VICAM, *E*-selectin, leukotrienes, lipid mediators such as arachidonic acid metabolites and mediators originating from endothelial cells. As a result of these mediators and adhesion of cells, proteases and free radicals increase and at the same time, a coagulation cascade is activated [14]. On the other hand; these mediators bind to the respective

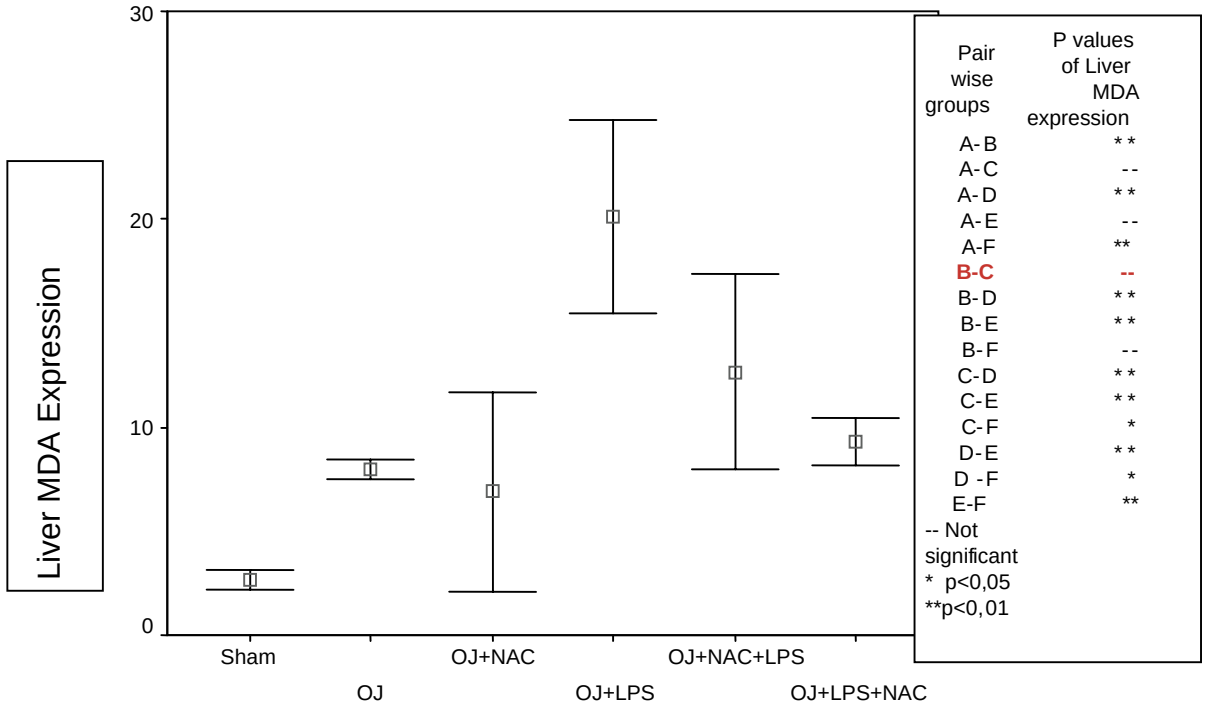

Fig. 10. Levels of liver MDA expression in the groups.

cell surface receptors and activate tyrosine kinase and nuclear factor kappa B (NFκB). This leads to transcription of iNOS protein in different organs and in an overproduction of NO [15].

NO can react with superoxide anion and this leads to formation of peroxynitrite anion and increased lipid peroxidation and leads to oxidative damage in lungs, kidneys and liver tissue [16]. Peroxynitrite ($ONOO^-$), free

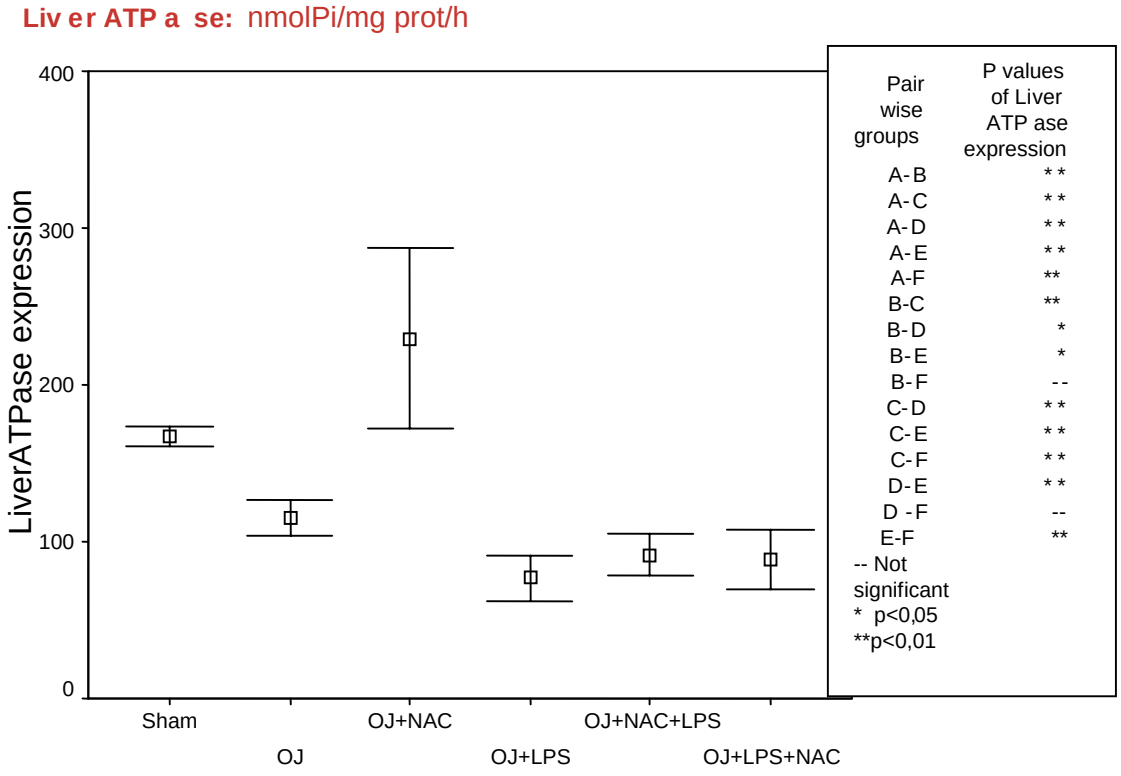

Fig. 11. Levels of liver ATPase expression in the groups.

oxygen radicals, environment factors and DNA strand breakage trigger the activation of energy-requiring enzyme poly(ADP-ribose) synthetase (PARS) [17,18].

Kawamura et al. have shown that increased hepatic lipid peroxidation, hepatocellular mitochondrial dysfunction and decreased glutathione occur in OJ [19]. Shiomi et al. have shown that there is excessive production of NO leading to impairment in mitochondrial function in rats with OJ [20].

Recent literatures indicates that obstructive jaundice leads to increased sensitivity to LPS endotoxin, there is enhanced intestinal epithelial cell apoptosis in jaundiced patients which is the source of Gram negative sepsis and a reason of mortality [21–23].

NAC protects against glutathione-depleted rat liver and acts as a cellular antioxidant. So NAC shows an anti-mutagenic and anti carcinogenic effects [24].

NAC has been known to act as an antioxidant/free radical scavenger or reducing agent. NAC prevents formation of peroxynitrite. NAC inhibits PARS activation [25]. Pastor et al. reported that impaired oxidant/antioxidant balance in OJ could be restored by NAC, and cell integrity could be maintained [26]. NAC reduces oxidative stress produced by LPS. Cholestatic rats that received NAC had less severe hepatic damage. The effects of NAC on hepatic ischemia/reperfusion were shown by Hur et al. [27]. They reported that hepatic I/R in rats induce iNOS gene transcription via activation of NFκB and treatment with NAC before reperfusion inhibits NFκB binding activity and iNOS mRNA expression [27]. In addition, some investigators demonstrated that the decreased antioxidant effect of glutathione pool in OJ was brought to the normal by NAC treatment. Because of this useful effect of NAC in OJ, as a consequence, NAC can be useful in prevention of hepatic damage, but the inhibitory effects of NAC on iNOS function were not elucidated [28]. Protective effects of NAC on lung injury and red blood cell modification induced by carrageenan in the rat were shown by Cuzzocrea et al. [29]. Their results clearly demonstrate that NAC offers a novel therapeutic approach on lung injury [29]. Schmidt et al. [30] reported that NAC attenuated endotoxin-induced leukocyte-endothelial cell adhesion and macromolecular leakage. They proposed that these effects of NAC were attributed to a decrease TNF-α production, inhibition of oxygen radicals induced breakdown of nitric oxide [30]. Verhasselt et al. [31] investigated the effect of NAC on the induction phase of T cell responses on human dendritic cells. They proposed that NAC down-regulated the production of cytokines by dendritic cell as well as their surface expression of HLA-DR [31]. In two recent studies NFκB and iNOS inhibition was shown to be induced by NAC [32,33].

Free oxygen radical production by PNM's after LPS administration is higher in the jaundiced rats compared with the Sham-operated rats. The reason is not clear but mitochondrial dysfunction or hepatocellular damage in the cholestatic liver are probably factors. A decreased mitochondrial GSH pool due to LPS implicated in hepatic injury in the cholestatic liver contributed susceptibility [34].

In conditions as an obstructive jaundice there is increased sensitivity to LPS endotoxin and this sensitivity can lead to organ dysfunction if any kind of endotoxemia is established. In group C (OJ + NAC), and group E (OJ + NAC + LPS) we observed that liver MDA levels were decreased and liver ATPase activity was increased. These results showed that lipid peroxidation and oxidative stress was prevented with the protective effect of NAC. The liver and renal iNOS staining increased in groups B (OJ) and D (OJ + LPS). The iNOS staining strongly increased in liver and renal tissue in group D (OJ + LPS) compared to the other groups. On the other hand, the immunohistochemical examination with iNOS demonstrated that in group A (Sham-operated control group), group C (OJ+NAC), and group E (OJ+NAC+LPS) were lower than group B (OJ), group D (OJ+LPS) and group F (OJ + LPS + NAC). This data showed that the enhanced sensitivity of the host to LPS and other endotoxins in OJ lead to increased tissue iNOS expression and NAC decreased this expression if it was administrated before LPS but NAC did not cause a decrease if it was administrated after LPS.

In this study, we aimed to investigate the deleterious effects of LPS on liver and renal tissue iNOS expression in condition of OJ and the protective effect of NAC, to show whether it is effective when administrated before LPS or after LPS.

Our study indicates that NAC has a protective effect by reducing iNOS expression via diminishing lipid peroxidation when it was administrated before LPS, but it failed to prevent the tissue iNOS expression and lipid peroxidation if there was established endotoxemia in OJ.

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