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Original Research

Effect of *N*-Acetylcysteine on Blood and Tissue Lipid Peroxidation in Lipopolysaccharide-Induced Obstructive Jaundice

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ABSTRACT In obstructive jaundice, free radical production is increased and antioxidative activity is reduced. *N*-Acetylcysteine (NAC) has a beneficial effect with anti-inflammatory and antioxidant activity, acting as a free radical scavenger. NAC inhibits inducible nitric oxide synthase, suppresses cytokine expression/release, and inhibits adhesion molecule expression and nuclear factor kappa B. The aim of this study was to investigate the effects of NAC on liver/renal tissue and serum lipid peroxidation in lipopolysaccharide (LPS)-induced obstructive jaundice. We randomized 60 rats into 6 groups: group 1, Sham; group 2, obstructive jaundice (OJ) induced after bile-duct ligation; group 3, OJ + NAC (100 mg kg^{-1} subcutaneously); group 4, OJ + LPS (10 mg kg-1); group 5, OJ + NAC + LPS; and group 6, OJ + LPS + NAC. For each group, the biochemical markers of lipid peroxidation and the antioxidant products were measured in serum and liver/renal tissue after sacrifice. Almost all lipid peroxidation products levels were increased and antioxidant products levels were decreased in groups who received LPS (groups 4, 5, and 6), but the effect was less remarkable when NAC was administered before LPS (group 5). The same trend was seen for groups with OJ ± LPS who did not received NAC or received it after induced toxemia (groups 2, 4, and 6) as compared to groups 1 and 3. Moreover, in the case of OJ + LPS, rats treated with NAC before LPS (group 5) had lower lipid peroxidation products levels and higher antioxidant products levels as compared to those who did not received NAC (group 4). This phenomenon was not reproducible with NAC administered after LPS (group 6). Thus, results of this study showed that NAC prevents the deleterious effects of LPS in obstructive jaundice by reducing lipid

peroxidation in serum and liver/renal tissue if administered before LPS. Nonetheless, NAC failed to prevent the lipid peroxidation in the case of established endotoxemia in obstructive jaundice.

KEYWORDS animal, kidney, lipid peroxidation, lipopolysaccharide, liver, LPS, NAC, *N*-acetylcysteine, obstructive jaundice

Obstructive jaundice (OJ) is associated with an increased incidence of endotoxemia resulting from defective host immune response. Lipid peroxidation, increased free radical production, and decreased antioxidative activity are important problems related to OJ [1], with subsequent nitric oxide (NO) formation and increased expression of inducible nitric oxide synthase (iNOS). NO, triggered by the iNOS, reacts with free oxygen radicals, leading to the formation of the most harmful peroxynitrite anion ($ONOO^-$) reponsible for cellular damage and apoptosis [2]. Oxidative stress, defined as an imbalance between radical generating and scavenging systems, creates free radicals that induce tissue damage. Bile acids and toxic bile salts cause oxidative damage to cell membranes by stimulating the oxygen free radicals from mitochondria [3]. The antioxidant defense system is impaired by a decrease in the reduced glutathione and the activity of glutathione peroxidase (GSH). Moreover, the severity of jaundice correlates with high lipid peroxide levels and low antioxidant levels [4]. *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]. It has been demonstrated in some studies that NAC increases GSH pool and has antioxidant effects in cells. In addition, NAC is an effective inhibitor of synthesis of inducible nitric oxide synthase at high doses [6]. Beside its antioxidant properties, NAC also elicits other beneficial effects by demonstrating anti-inflammatory activity such as suppression of cytokine expression/release, inhibition of adhesion molecules expression, and nuclear factor kappa B [7]. The aim of this study was to investigate the role of oxidative injury and to determine whether NAC exerts any beneficial effect on lipid peroxidation in LPS induced obstructive jaundice.

MATERIAL AND METHODS

Animals

This experimental study was conducted in adherence with National Institutes of Health guidelines on the use of experimental animals and was carried out in accordance with institutional policies and guidelines for the care and use of laboratory animals. Adult male Wistar albino rats weighing 150–200 g were kept in a controlled environment with constant temperature (21°C) and light/dark cycles (7 p.m. to 7 a.m. darkness). Animals were given diet and tap water prior to the experiments. All rats were anaesthetized with intramuscular ketamine HCl (50 mg kg^{-1}) (Ketalar, Eczacbas1, Warner Lambert Ilac AS, Istanbul, Turkey) and xylazine (5 mg kg^{-1}) (Rompun, Bayer AG, Leverkusen, Germany). The animals were kept on a warm-water mattress during the procedure and were kept warm postoperatively until regaining consciousness. We randomized 60 rats into 6 groups: group 1, sham; group 2, obstructive jaundice (OJ), group 3; OJ + NAC (Asist 10%, 300 mg/3 mL); group 4, OJ + LPS (*Escherichia coli* LPS serotype L-2630, 100 mg, Sigma); group 5, OJ + NAC + LPS; and group 6, OJ + LPS + NAC.

Surgical Procedures

Bile-Duct Ligation

The chest and abdomen were shaved and each animal was fixed in supine position on the operating table. The abdomen was cleaned with 1% polyvinyl iodine and the operating field was covered with a sterile drape. The abdomen was opened through a midline incision and experimental jaundice was created by ligation of the common bile duct according to the technique described in details by Lee [8]. The duodenum was retracted; the common bile duct was identified then ligated with 5/0 sutures and cut

to prevent recanalization. The abdominal wall was closed with interrupted silk sutures and the skin was approximated with a subcuticular stitch.

Jaundice was observed at the end of postoperative day 5. In group 3, subcutaneous NAC (100 mg/kg) was administered daily for 5 days, starting on day 5. In group 4, we first injected lipopolysaccharide (LPS, 10 mg kg^{-1}) intraperitoneally on day 5 and rats were sacrificed after 6 h. In group 5, NAC was administrated at the end of day 5 and repeated daily for 5 days. LPS was injected at the end of day 10, and rats were sacrificed 6 h later. In group 6, LPS was injected at the end of day 5, and NAC was administered after 6 h and daily for 5 days. Rats were sacrificed on postoperative day 5 for groups 2 and 4 and on postoperative day 10 for groups 3, 5, and 6. The sham operation consisted of mobilization of the bile duct only. The rats in this group were sacrificed immediately after the procedure. In group 2, rats were sacrificed on day 5 in order to reveal the prime changes on lipid peroxidation in the jaundiced rats. The rats in groups 3, 5, and 6 were sacrificed on day 10 in order to find out and demonstrate clearly the biochemical changes developed when NAC was administrated in the jaundiced rats before or after the establishment of endotoxemia. These groups were designed to evaluate the protective and therapeutic effect of the drug in conditions like OJ and LPS-induced OJ. After laparatomy, liver and kidneys were resected. Tissue malondialdehyde (MDA) and ATPase levels, serum MDA, serum myeloperoxidase (MPO), serum ATPase, erythrocyte reduced glutathione (GSH), and vitamin E levels were detected by the following biochemical methods.

Biochemical Measures

MDA

The malondialdehyde (MDA) level, as an index of lipid peroxidation, was determined by thiobarbituric acid (TBA) reaction according to the Hiroshi and Yagi method [9]. That method relies 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 of 1, 1, 3, 3-tetraethoxypropane was used as the primary standard.

Erythrocyte Reduced Glutathione

Virtually all of the nonprotein sulfhydryl compounds of red cells are in the form of GSH. 5,5′-Dithiobis(2-nitrobenzoic acid) (DTND) is a disulfide compound that is reduced by sulfhydryl compounds, forming a highly colored yellow anion. The optical density of this yellow substance is measured at 412 nm.

Vitamin C

The determination of serum vitamin C depends on the reduction of the dye and the change of 2,6-dichlorophenol indophenol into a colorless compound.

Myeloperoxidase

The determination of serum myeloperoxidase activity depends on the reduction of *o* dianozidine. Reduced *o*-diazidine was measured at 410 nm by spectrophotometer.

Na^+ /K^+ ATPase Activities

After the animals were sacrificed, liver and kidneys were resected as quickly as possible. Ten percent homogenates of the tissue were prepared in 0.3 M sucrose containing 1 mM magnesium and homogenize for 90 s using a Teflon pestle with clearance of 0.25–0.38 mm at 1000 rpm. It was then centrifuged at 1000 rpm for 15 min in order to remove fragments. ATPase activities were determined on the resulting supernatants by measuring the rate of liberation of inorganic phosphate (Pi) from disodium ATP (Na_2ATP, Sigma Chemical Co. Ltd.). Incubation media were made up as described previously [10, 11]. The composition of the incubation medium for NA^+/K^+-adenosine 5′-triphosphatases (Na^+/K^+ATP ase) was as follows (mM): $MgCl_2$ 6, KCl 5, NaCl 100, EDTA 0.1, Tris-HCl buffer pH 7.4, 135.

The blank (containing no enzyme) standard and unknowns samples 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 molybdate solution followed by vortexing, and standing at 340 nm was measured. Samples were compared for phosphate content with standards of KH_2PO_4. Specific activities were calculated as nanomoles Pi per milligram protein per hour.

All reagents were of analytical grade unless otherwise stated. Protein content was determined according to the method described by Lowry [12]. Bovine serum albumin was used as a standard.

Statistics

Data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to analyze biochemical data and the Tukey HSD test was used for significant results. Correlation between groups was determined using the Pearson correlation test. In all instances, $p < .05$ was assumed as the lowest level of significance.

RESULTS

The descriptive statistics of serum, liver, and renal MDA, liver and renal ATPase, serum MPO, erythrocyte GSH, ATPase, and vitamin C levels are presented in Figures 1 to 9. Biochemical values were described as mean ± standard deviation (Table 1). The significance levels of pairwise comparisons of groups are also presented according to the Tukey post hoc test. Overall, there was a significantly increased lipid peroxidation products level and a decreased antioxidant products level after LPS injection (groups 4, 5 and 6), but these variations were less remarkable when NAC was administered before LPS (group 5). There was a trend to higher serum MDA, MPO, and tissue MDA levels and lower tissue and serum ATPase levels in jaundiced groups that did not received NAC or received it after induced toxemia (groups 2, 4, and 6) as compared to groups 1 (sham) and 3 (OJ/NAC). Significant differences were observed in most of the biochemical parameters, with increased lipid peroxidation products levels and decreased antioxidant products levels for groups 2 (OJ) and 4 (OJ + LPS) as compared to group 1 (sham) and for group 4 (OJ + LPS) as compared to group 3 (OJ + NAC). Differences were also significant for all but renal ATPase level between group 4 (OJ + LPS) and group 5 (OJ + NAC + LPS), but not between groups 4 and 6 (OJ/LPS/NAC) (except serum MPO and renal MDA). All biochemical parameters (except serum MDA and erythrocyte ATPase/vitamin C level) were different between groups 3 (OJ + NAC) and 6 (OJ + LPS + NAC) and (except serum MPO and erythrocyte vitamin C level) between groups 2

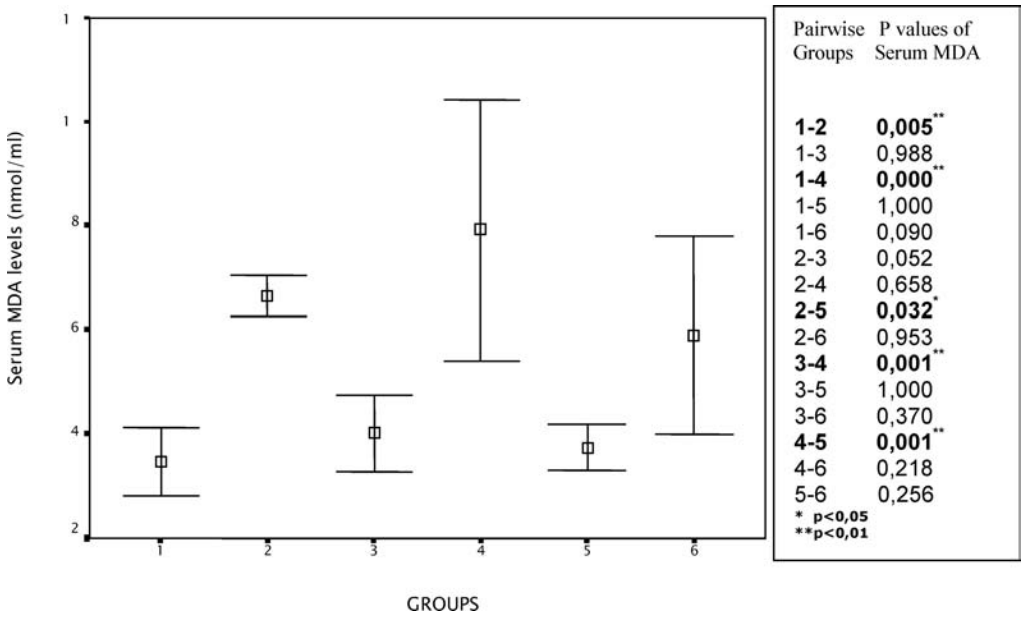

FIGURE 1 Serum MDA levels.

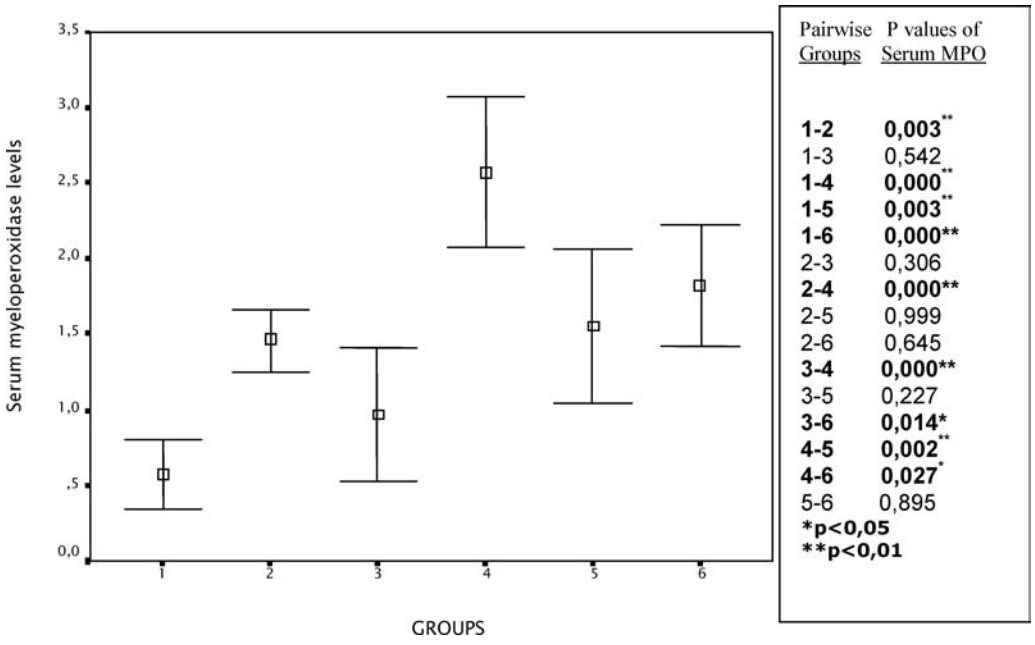

FIGURE 2 Serum myeloperoxidase levels.

(OJ) and 3 (OJ + NAC), with better results in groups treated with NAC.

DISCUSSION

Oxidative stress results from antioxidants depletion and oxidants overproduction. Free oxygen radicals have been implicated as mediators of tissue injury in a variety of diseases. Most free radical reactions involve the reduction of molecular oxygen, leading to the formation of highly reactive oxygen species such as superoxide anion (O_2^-), hydroxyl radical (^-OH), hydrogen peroxide (H_2O_2), and single oxygen ($^1O_2$) [13].

In OJ, the free radicals production is increased and the antioxidative activity is reduced, as shown in this

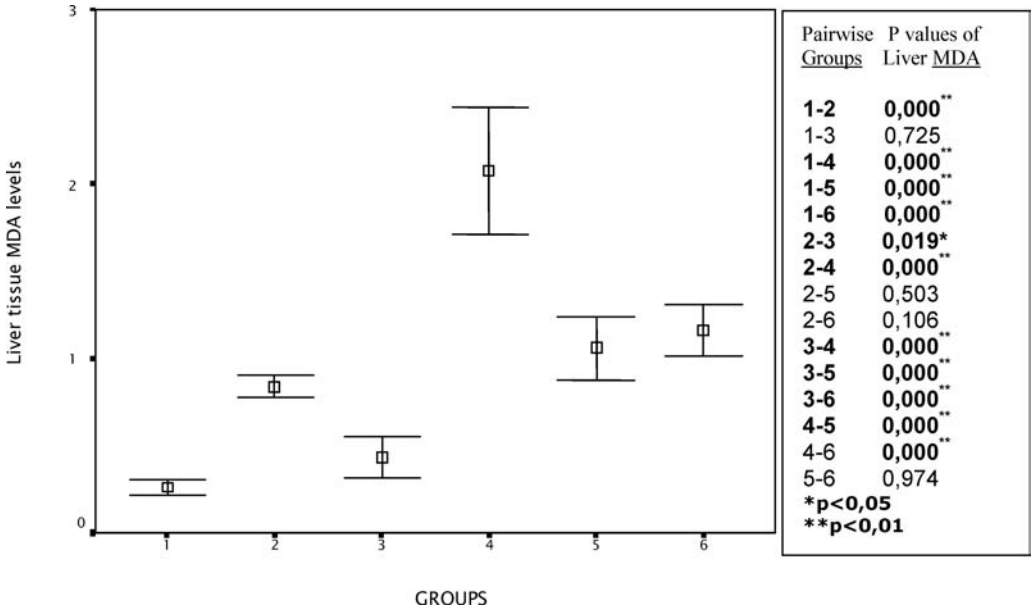

FIGURE 3 Liver tissue MDA levels.

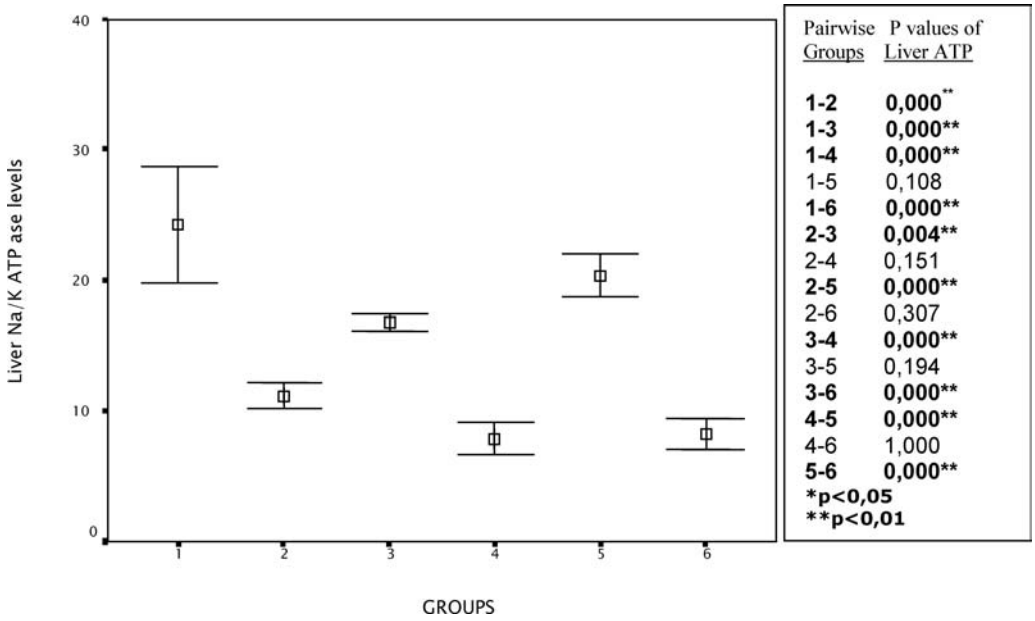

FIGURE 4 Liver Na^+/K^+-ATPase levels.

study. This phenomenon combined with lipid peroxidation at the site of inflammation leads to tissue damage. Cholestatic patients have substantial morbidity related to an increased sensitivity to LPS endotoxin [14]. Moreover, increased secretion of LPS-induced cytokines from Kupffer cells has been described [15]. Combined portal endotoxemia resulting from impaired intestinal barrier and systemic endotoxemia resulting from dysfunctional Kupffer cells lead to sepsis [16].

In endotoxemia, iNOS is activated (independently of Ca) and leads to excessive production of NO. In OJ, it is thought that iNOS is synthesized in tissue from macrophages, leukocytes, and endothelial cells as a result of endotoxemia with LPS [17]. Activation of macrophages, endothelial

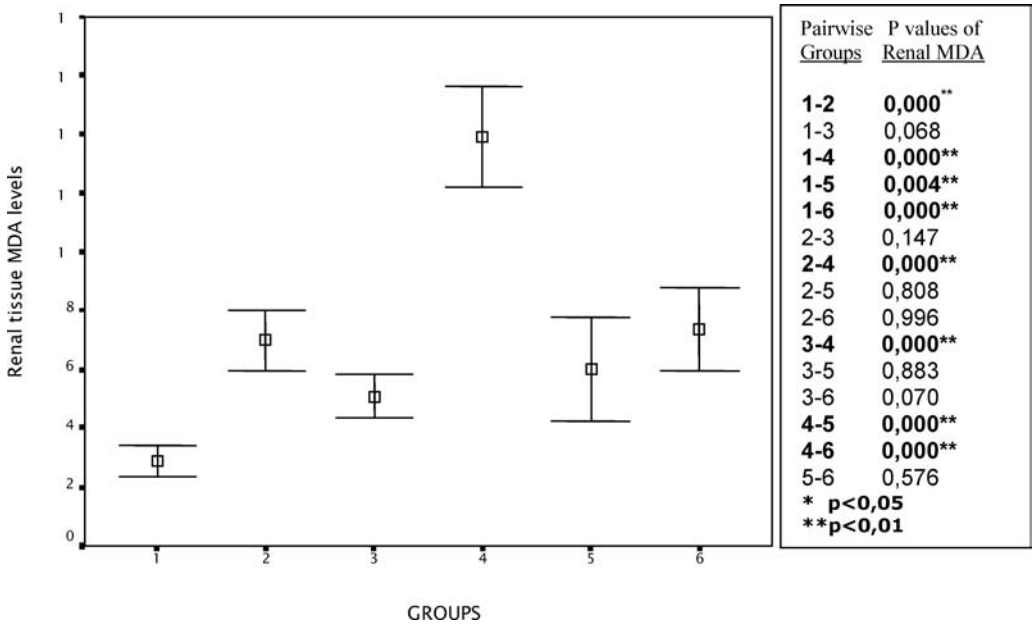

FIGURE 5 Renal tissue MDA levels.

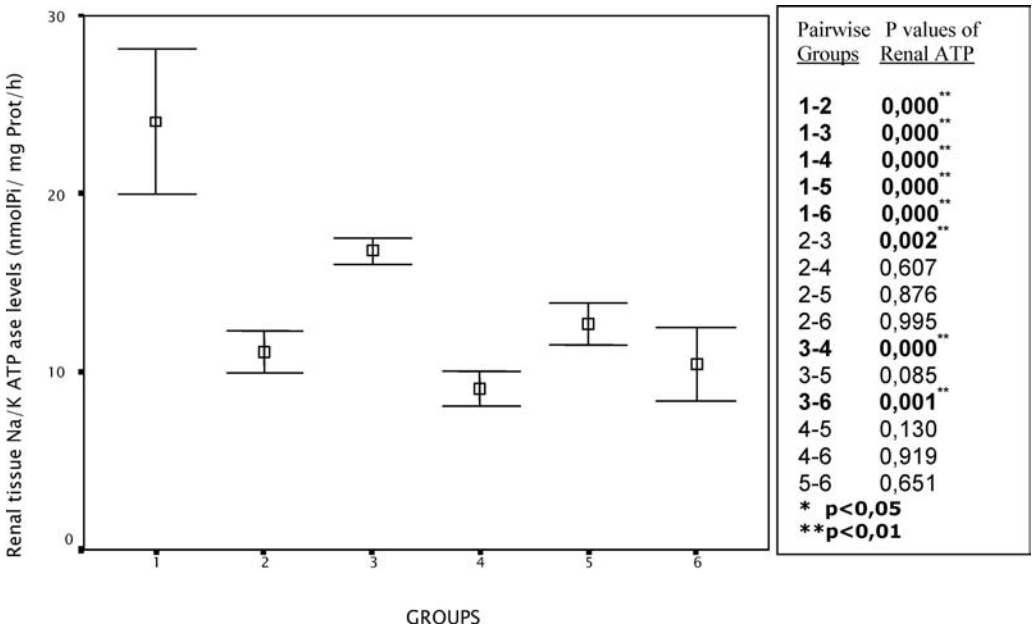

FIGURE 6 Renal tissue Na^+/K^+-ATPase levels.

cells, and leukocytes results in the overexpression of interleukin (IL)-1, tumor necrosis factor (TNF)-α, platelet activating factor (PAF), and interferon (INF)-γ, and synthesis of adhesion molecules like ICAM, VICAM, E-selectin, leukotrienes, lipid mediators such as arachidonic acid metabolites, and mediators originating from endothelial cells. Thereafter, proteases and free radicals increase and a coagulation cascade is activated simultaneously [18, 19]. Moreover, these mediators bind to their respective cell surface receptors and activate tyrosine kinase and nuclear factor kappa B (NFκB). This leads to transcription of iNOS protein in different organs and NO overproduction, which can react with superoxide anion. Subsequent formation of peroxynitrite anion ($ONOO^-$) and increased lipid peroxidation are

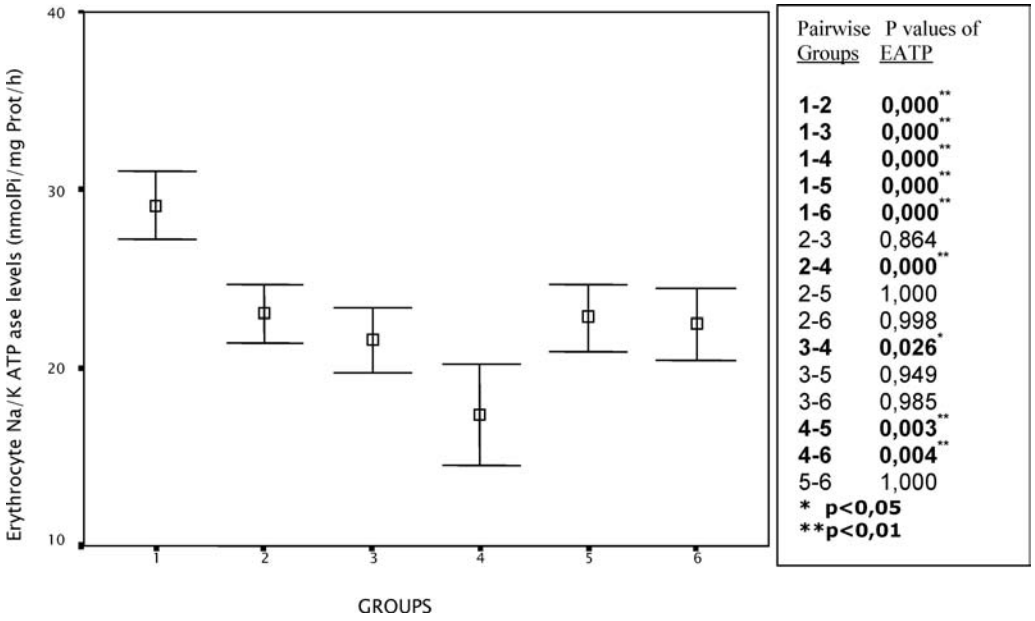

FIGURE 7 Erythrocyte Na^+/K^+-ATPase levels.

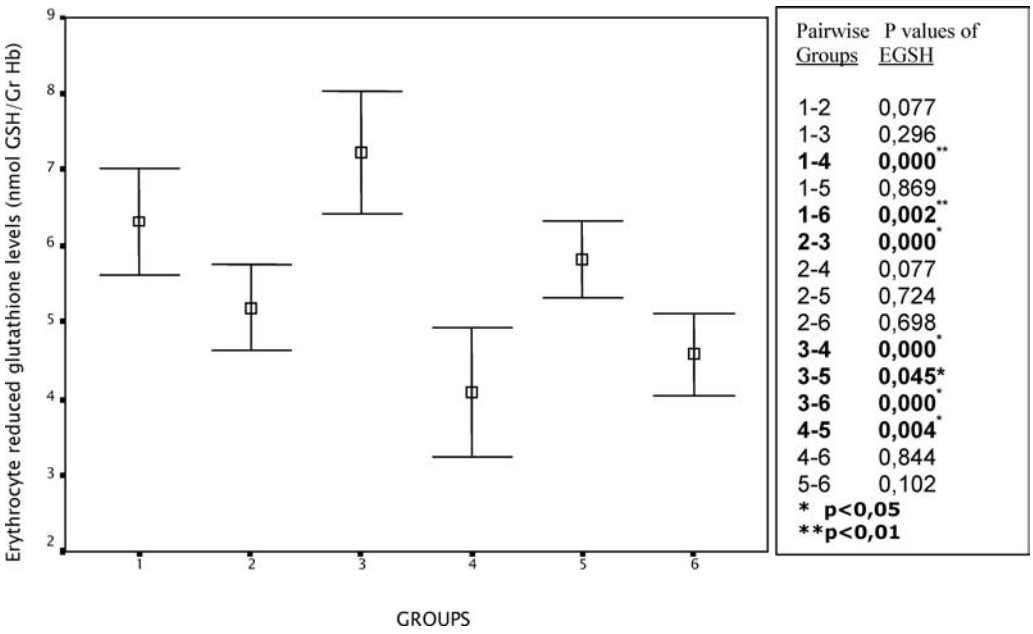

FIGURE 8 Erythrocyte reduced glutathione levels.

responsible for oxidative damage in lungs, kidneys, and liver tissue [20]. Peroxynitrite, free oxygen radicals, environment factors, and DNA strand breakage trigger the activation of the energy-requiring enzyme poly(ADP-ribose) synthetase (PARS) [21].

NAC has been known to act as an antioxidant/free radical scavenger or reducing agent. Pastor et al. [22] reported that DCP and thiobarbituric acid-reactive substances (TBARS) concentrations in liver homogenates were markers of oxidative stress and lipid peroxidation and that bile-duct ligation resulted in their overproduction. In this study, the oxidant/antioxidant imbalance of OJ with induced toxemia was restored by NAC (50 μmol/kg) and cell integrity was maintained, but the two groups (4 and 5) were not sacrificed at the same day. Nonetheless, the

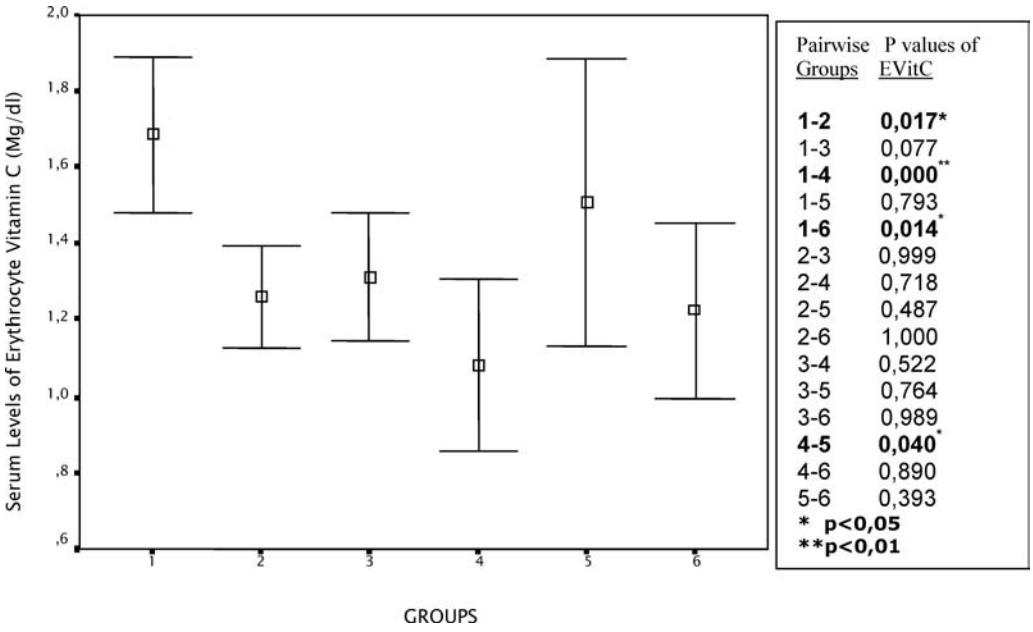

FIGURE 9 Serum levels of erythrocyte vitamin C.

TABLE 1 Blood and tissue (liver and renal) lipid peroxidation and antioxidant products levels in each experimental group

Parameter	*Group 1 (sham)*	*Group 2 (OJ)*	*Group 3 (OJ/NAC)*	*Group 4 (OJ/LPS)*	*Group 5 (OJ/NAC/LPS)*	*Group 6 (OJ/LPS/NAC)*
Serum MDA	3.46 ± 0.90	6.65 ± 0.56	4.03 ± 0.88	7.91 ± 3.5	3.74 ± 0.47	5.88 ± 2.26
Serum MPO	0.57 ± 0.31	1.46 ± 0.29	0.97 ± 0.52	2.57 ± 0.69	1.54 ± 0.54	1.8 ± 0.48
Liver MDA	2.64 ± 0.67	8.37 ± 0.94	4.34 ± 1.38	20.71 ± 5.1	10.57 ± 1.92	12.56 ± 1.69
Liver ATP ase	166.7 ± 41.7	111.8 ± 14.5	167.6 ± 8.8	78.7 ± 16.71	203.6 ± 17.67	82.1 ± 13.82
Renal MDA	2.91 ± 0.71	7.1 ± 1.49	5.1 ± 0.82	13.9 ± 2.37	6 ± 1.91	7.37 ± 1.68
Renal ATP ase	240.3 ± 56.8	111.1 ± 16.2	167.4 ± 9.17	90.7 ± 13.57	126.7 ± 13.1	104.1 ± 24.52
Erythrocyte ATPase	291.1 ± 27.2	230.4 ± 23.7	215.6 ± 21.7	173.6 ± 39.6	228.1 ± 20.21	224.7 ± 45.17
Erythrocyte vitamin C	1.68 ± 0.28	1.26 ± 0.18	1.31 ± 0.22	1.04 ± 0.31	1.54 ± 0.42	1.21 ± 0.27
Erythrocyte reduced glutathione	6.32 ± 0.97	5.24 ± 0.78	7.22 ± 0.95	4.02 ± 1.18	5.81 ± 0.53	4.59 ± 0.64

group treated with NAC had better results with the same acute effect of LPS, even with a longer jaundiced period. In another study, Kawada et al. [23] demonstrated that NAC (5 mmol/L) is an effective antioxidant agent in hepatocyte. This concentration of NAC failed to affect the expression of SMDA and iNOS at any time, although NAC (5 mmol/L) had a potent inhibiting effect on HSC proliferation. Cellular glutathione is the main protective factor against oxidative aggression and reactive species. It plays an important role in T-cell activation, lymphocyte proliferation, and natural killer cell activity [24, 25].

NAC is a thiol-containing compound and a precursor of reduced glutathione (GSH). A recent report has shown that GSH stores are reduced in animals with obstructive jaundice [26]. Thiol antioxidants such as NAC inhibit the release of inflammatory mediators from epithelial cells and macrophages, thus increasing intracellular GSH and decreasing free radicals and NF-κB activation [27]. Kigawa et al. [28] have found that intravenous administration of NAC improves hepatic circulation and function in jaundiced dogs.

The results of this study show that the hepatic and renal tissue lipid peroxidation is increased in OJ. We observed an increase in serum, liver, and renal tissue MDA and MPO levels in groups 2, 4, and 6. Decreased liver/renal tissue and serum ATPase levels were found in the same groups. These data showed an increased lipid peroxidation in the rats with obstructive jaundice and LPS-stimulated OJ. When LPS was administered to the jaundiced rats, we observed that lipid peroxidation and oxidative stress were exaggerated. These data were supported by the significant increase of MDA and MPO levels and by the decreased ATPase activity in serum and tissue. This can be explained by the fact that in conditions like acute pancreatitis and ischemia–reperfusion, there is an increased sensitivity to LPS endotoxin, which can lead to organ dysfunction if endotoxemia is established, as in obstructive jaundice. Another important finding was a protective effect of NAC on lipid peroxidation and oxidative stress in jaundiced rats treated before the establishment of endotoxemia (group 5). Nonetheless, we failed to demonstrate a therapeutic effect of NAC (group 6). After LPS administration, it failed to inhibit lipid peroxidation significantly.

In conclusion, there is an increased sensitivity to LPS endotoxin in OJ that can lead to organ dysfunction if endotoxemia is added. In this study, we aimed to investigate the deleterious effects of LPS in condition of OJ and the beneficial effect of NAC, either preventive or therapeutic. Our study showed that NAC suppressed the adverse effects of endotoxemia in LPS-induced OJ when it is administered before LPS. However, it failed to prevent lipid peroxidation in the case of established endotoxemia. For this reason, we think that NAC could be used as a protective agent in OJ and that clinical applications for jaundiced patients need to be further investigated.

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