TÜBİTAK

Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2019) 43: 566-579
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doi:10.3906/zoo-1907-15

All aspects of the toxic effects of lipopolysaccharide on rat liver and the protective effect of vitamin E and sodium selenite

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Received: 07.07.2019 • **Accepted/Published Online:** 02.09.2019 • **Final Version:** 01.11.2019

Abstract: This work was planned to research the effects of lipopolysaccharide (LPS) and/or vitamin E (VE) and sodium selenite (SS), which have antioxidant properties, administered to the liver tissue of male rats. For this purpose, histopathology, immunohistochemistry, antioxidant capacity, terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling assay, liver function, and DNA structure tests were performed. The lipid profile was also evaluated in this study. Rats that were administered LPS were treated with VE and/or SS. The liver function tests and lipid profile parameters showed statistically significant changes and histological alterations in the LPS treated groups. The number of cells entering apoptosis was increased by LPS administration when compared to the control group. LPS treatment increased the DNA damage and decreased the ferric reducing antioxidant power and trolox equivalent antioxidant capacity values when compared to the control group. VE and/or SS provided protective effects against the examined parameters. These results indicated that we can assume that the treatment of VE and SS together may be more efficient than using them individually against LPS.

Key words: Lipopolysaccharide, ferric reducing antioxidant power, vitamin E, toxicity, liver

1. Introduction

Lipopolysaccharide (LPS), a gram (–) bacteria found in the cell wall, is the body's most important natural bacterial product recognized by immunity. An endotoxin called lipid A is responsible for the toxicity, which is substantial for the starting and advance of the septicity (Cohen, 2002). LPS is biologically inactive as long as it remains on the cell wall and initiates a series of events that are released during cell growth or cell disruption. When LPS is administered to experimental animals, adult respiratory fatal syndrome, interstitial pneumonia, acute tubular necrosis, and fatal effects, such as coagulopathy and hypoglycemia, can be observed (Young et al., 1991; Lugtenberg et al., 1983).

LPS obtained from *E. coli* is used more frequently in experimental sepsis studies. Lyophilized LPS is dissolved in water and applied to experimental animals in the peritoneum or vein. The induction of stimulation with some interleukin secretion is arisen by LPS in the sepsis proinflammatory cytokines and the organism responds with antiinflammatory (IL-4) and interleukin-10 (IL-10) from the cytokines (Skirecki et al., 2012; Lewis et al., 2012). The released proinflammatory mediators trigger free radical formation. Oxidative stress is induced by free radicals. Oxidative stress and endotoxic shock cause superior mortality together.

Selenium is an element that dissolves in the air and water, and is also solid in soil and rocks. Thus, it passes through plants, fungi, bacteria, and humans, and then returns to nature (Bal et al., 2015). Humans and animals need trace amounts of selenium for growing. Sodium selenite (SS), previously known for its antitoxic and anticarcinogenic properties, has been evaluated as a significant molecule for live cells as a result of recent studies. SS also inhibits liver necrosis in rats with vitamin E (VE) deficiency. SS, interacting with VE, protects the cell components against free radical attacks caused by the membrane damage (Gebre-Medhin et al., 1984).

VE is a powerful antioxidant for cell membranes. The free radicals formed by lipid peroxidation are thought to be related to membrane and plasma lipoproteins. VE protects

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the cell membrane from hemolysis by preventing it from spreading. The peroxyl radicals react with tocopherol to form the tocopheryl radical (Vit EO*). The tocopheryl radical is reduced by reacting with SS. In this way, the cell membrane is protected from the harmful effects of the peroxyl radical (Traber and Stevens, 2011; Traber and Atkinson, 2007).

The purpose of this study was to examine LPS, an experimental sepsis material that may cause the pathological changes in the antioxidant capacity and DNA structure in rat liver for 6 h, to determine the lipid profile parameters in the blood and differences in the amounts of liver function, and also to research the protective role of VE and SS on hepatotoxicity-induced by LPS.

2. Materials and methods

2.1. Chemicals

The VE (DL-α-tocopherol acetate), SS, and LPS (obtained from *Escherichia coli*), were purchased from Sigma-Aldrich (St. Louis, MO, USA), in addition to the rest of the chemicals, which were all analytical grade. The doses of the chemicals used were determined as previously studied (Blaszcyk et al., 2015; Çelikoğlu et al., 2015; Kalaz et al., 2016; Pandır et al., 2017) LPS (10 mg/kg bw) and SS (0.35 mg/kg b.w.) were dissolved in water, but the VE (200 mg/kg bw) was dissolved in maize oil.

2.2. Animal categorization and treatment

In the current study, 56 male Wistar albino rats were used. Next, 8 groups of 7 rats were formed: the control, VE, SS, VE + SS, LPS, VE + LPS, SS + LPS, and SS + LPS + VE groups. An injection of 1 mL of 0.9% NaCl saline solution was given intraperitoneally (ip) to the control group 30 min apart 2 times. The treatment groups were given a single 10 mg/kg body weight (bw) (n = 7) dose of LPS, ip. VE and SS were given orally to the rats.

The animals were kept for one week before the experiment at 22–24 °C and 50% humidity and a 12:12 sunlight/dark photoperiod during the experiment. All of the animals were housed in plastic cages each containing seven rats. Animal cages were kept clean, and fresh drinking water and standard diet were given to rats as regularly, daily. After 6 h of exposure, the rats were dissected, and the liver and blood were taken for biochemical, hematological, and light microscope examinations. The protocol was approved by Erciyes University (16/133).

2.3. Biochemical evaluation

The alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, triglycerides, and total cholesterol were measured spectrophotometrically with an autoanalyzer in serum using Roche commercial kits (Roche Group, Basel, Switzerland).

Assessment of the antioxidant capacity with the trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) was also performed.

The FRAP assay was applied using the method of Benzie and Strain (1996). The level of the serum samples was determined at 593 nm. The measured compound that was formed appeared colorless in the oxidized Fe^{III}, yet appeared blue in the Fe^{II}-tripyridyltriazine. This reaction occurs with the electron transfer from antioxidants. The FRAP reagent was formulated as 1 volume of ferric chloride in a buffer comprising 1 volume of 2,4,6-tripyridyl-s–triazine, which was added to the fresh sample. A change in absorbance was obtained at 593 nm.

TEAC includes the coupling of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) oxidation, resulting in $ABTS^{\bullet+}$ radical solution. Reduction of the addition radical of the antioxidant-containing sample was observed as blue/green $ABTS^{\bullet+}$. Determination of the radical in color was performed at 600–750 nm and the outcome was expressed as a trolox equivalent (Re et al., 1999).

2.4. Evaluation of the histological changes

The obtained samples were rinsed at pH 7.2 with buffer, and 10% formalin was used for the fixations, which were then passed through a graded ethanol series. Hematoxylin and eosin (H&E) was administered for staining after a routine paraffin embedding technique. An Olympus light microscope equipped with a camera (Olympus Scientific Solutions, Shinjuku, Tokyo, Japan) was used for the histological imaging. The results of each group were gradated as none (–), slight (+), medium (++), and heavy (+++) damage.

2.5. Evaluation of the immunohistochemistry

Proliferating cell nuclear antigen (PCNA) is a nuclear protein associated with cell cycle, which congregates in the cell proliferation condition and reacts with the rat monoclonal antibody [PCNA] (Thermo cat number 11-9910-71) (Alenzi et al., 2010) and a constituent of the DNA replication process, also related with growth regulation. PCNA expression was used for the immunohistochemistry using commercial monoclonal primary antibodies. Formalin was used for fixation of the obtained liver tissue, and the samples were sealed in paraffin and 5–6-μm sections were cut using a microtome (Leica RM2255, Germany), and then air-dried at 36–37 °C. The endogenous peroxidase was blocked by 0.3% H_2O_2 for 15 min in a sodium citrate buffer (pH 6) in a microwave oven after antigen retrieval. Samples were left for 12 h at 4 °C in the primary antibody (1:100) and a horseradish peroxidase (HRP) UltraVision detection kit (Thermo cat number TL-125-QHD; Thermo Fisher Scientific Inc., Waltham, MA, USA) was used as a secondary antibody. Next, 3,3'-diaminobenzidine (DAB) was utilized as a chromogen substrate for the HRP. A unique isotype was used as a control for the nonspecific

background staining and the normal structure of liver sections was obtained with hematoxilin from the related groups. Next, 5 randomly selected PCNA-stained cells were used to detect the proliferative index. Pictures of the tissues were acquired using a light microscope (Olympus BX51) (Nygard et al., 2015).

2.6. TUNEL (apoptosis) Assay

The terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) method was used with an In Situ Cell Death Detection Kit Fluorescein Kit (Roche-11684795910) to demonstrate the apoptosis of the liver tissue. According to the procedure, 5–6-µm-thick liver tissues were obtained, deparaffinized, and rehydrated, respectively. Next, they were washed with phosphate buffered saline (PBS). The samples were immersed in 0.01 M of sodium citrate buffer in a microwave oven, at 350 W for 5 min, after washing for antigen retrieval. Next, they were allowed to cool for 20 min at 20 °C and washed 2 times with PBS for 5 min, and then left to incubate for 60 min at 37 °C in a damp dark place, in a TUNEL reaction mixture. The tissues were then counterstained with 4,6-diamidino-2′-phenylindole after the washing process. All of the tissues were examined with an Olympus BX51 fluorescent microscope at 450–500 nm after they had been covered with a glycerol solution. TUNEL positive cells in the liver tissue were counted to predict the apoptotic index in 20 randomly selected areas. The calculation was done according to the method of Bayatli et al. 2013.

2.7. Evaluation of the DNA structure with the comet assay

Low melting point agar (LMPA) (0.5%) was prepared at 37 °C. Cells of the liver suspension were dissolved in 100 µL of LMPA. Subsequently, a normal melting point agar (NMPA) solution was prepared at about 1% and then the slides were precoated in it. These slides were placed on ice cubes for about 5 min while awaiting agar solidification and then plunged into a cold lysis solution and left it in the refrigerator. The slides were electrophoresed for 20 min at 25 V and 300 mA after being left in this solution for 20 min without applying any current. After completion of the electrophoresis step, the slides were removed from the electrophoresis tank and the neutralization solution was used 3 times for washing. Ethidium bromide was used for staining the slides at 20 µg/mL, which were then read using a fluorescence microscope for at least 10 min (Pandır, 2018).

2.8. Data analysis

SPSS v. 11.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to analyze the variations among the samples. The analysis of variance (ANOVA) and Tukey tests were used to calculate significance. $P < 0.05$ was accepted as statistically significant. Data were expressed as the mean ± standard deviation.

3. Results

3.1. Evaluation of the biochemical results

Biochemical parameters, such as the ALP, AST, ALT, albumin, triglyceride, and total protein, were investigated to assess the hepatic function. When the SS, VE, SS + VE groups were compared to the control group, there were no considerable changes in the biochemical parameters in the blood tissues after 6 h. The LPS group had changed significantly when compared to the control group for the biochemical parameters examined. The LPS + VE and SS groups had significantly lower ALP, ALT, and AST levels, but had higher levels of albumin, triglycerides, and total protein than the LPS group ($P < 0.05$), but they differed from the parameters of the control animals (Table 1).

3.2. Changes in antioxidant capacity of liver tissue

Changes in the liver antioxidant capacity as a result of LPS and/or VE + SS are shown in Figures 1 and 2. The TEAC

Table 1. Liver function tests and lipid profiles of rats treated with LPS and/or VE + SS.

Parameters	Groups							
	Control	SS	VE	SS + VE	LPS	LPS + SS	LPS + VE	LPS + SS + VE
AST (U/L)	35.45 ± 1.03^a	35.24 ± 1,45^a	35.21 ± 2,47^a	35.21 ± 2.78^a	48.12 ± 8.64^b	42.12 ± 3.74^c	39.23 ± 4.75^c	37.58 ± 5.26^d
ALT (U/L)	9.02 ± 1.33^a	9.22 ± 0.05^a	9.14 ± 0.07^a	10.24 ± 1.78^a	25.52 ± 0.12^b	19.14 ± 1.24^c	20.21 ± 2.74^c	18.25 ± 2.85^c
ALP (U/L)	15.02 ± 2.25^a	17.02 ± 1.23^a	17.96 ± 1.75^a	18.25 ± 0.28^a	38.02 ± 3.12^b	22.23 ± 1.52^c	23.54 ± 2.05^c	21.02 ± 1.54^c
Total protein (g/dL)	15.23 ± 2.65^a	17.25 ± 1.75^a	18.25 ± 2.54^a	16.23 ± 2.85^a	5.52 ± 0.04^b	10.45 ± 0.03^c	11.85 ± 0.03^c	14.45 ± 0.26^c
Albumin (g/dL)	10.26 ± 0.03^a	9.78 ± 1.58^a	10.48 ± 1.89^a	9.23 ± 1.23^a	2.14 ± 0.08^b	4.26 ± 0.01^c	5.26 ± 1.22^c	7.45 ± 0.03^c
Triglycerides (mg/dL)	40.25 ± 8.23^a	39.23 ± 8.25^a	38.05 ± 4.02^a	39.52 ± 6.56^a	12.23 ± 5.45^b	18.25 ± 2.45^c	19.56 ± 5.12^c	24.45 ± 1.48^c

Superscripted letters indicate significant differences in the rat liver tissue of the control and different treatment groups. Significance was accepted as $P < 0.05$.

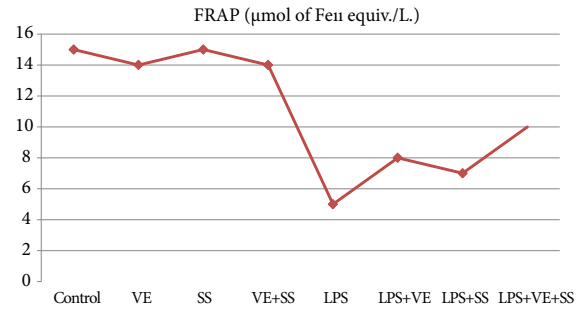


Figure 1. FRAP values (µmol of Fe^{II} equiv/gram tissue) in the liver of rats treated with LPS (10 mg/kg bw), VE (200 mg/kg bw), and SS (0.35 mg/kg bw). Significance was accepted as $P < 0.05$.

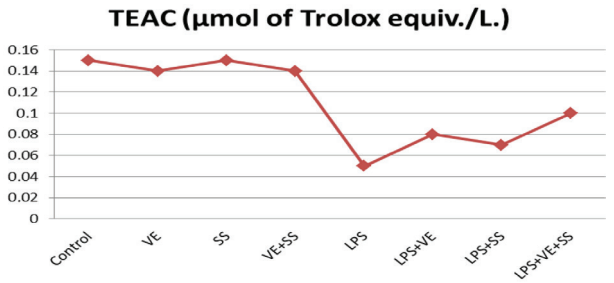


Figure 2. TEAC values (µmol of Trolox equiv/gram tissue) in the liver of rats treated with LPS (10 mg/kg bw), VE (200 mg/kg bw), and SS (0.35 mg/kg bw). Significance was accepted as $P < 0.05$.

and FRAP values of the cells treated with VE, SS, and VE + SS were statistically similar to those of the nontreated cells. LPS caused a considerable decrease in the FRAP and TEAC values of the liver tissue only in the LPS group. In the VE + LPS, SS + LPS, and SS + LPS + VE groups, a considerable increase in FRAP and TEAC values was observed. When compared with the VE + LPS, SS + LPS, and SS + LPS + VE groups, there was a significant ($P < 0.05$) decrease in the FRAP and TEAC values in LPS group ($P < 0.05$) (Figure 2). The antioxidant capacity of the liver tissues increased significantly in the VE + LPS, SS + LPS, and SS + LPS + VE groups when compared to the LPS group.

3.3. Histopathology and immunohistochemistry

Light microscopic examination of the livers revealed that the livers in the control, VE, SS, and VE + SS groups were normal in structure. Regulation of the hepatocytes was seen as usual, the cytoplasm seemed regular, and there were visible sinusoids in many areas (Figures 3a and 3b). However, the livers of the animals in the LPS group exhibited histopathological changes; the livers of the animals in the LPS group exhibited dilation of the sinusoids, leukocyte infiltration, necrosis, vacuolar degeneration, vascular congestion, and binucleated hepatocytes hemorrhage (Figures 4a–4d). In the VE + LPS group, the animals exhibited vacuolar degeneration, necrosis, dilation of the sinusoids, vascular congestion, and hemorrhage (Figures 5a and 5b). In the SS + LPS group, the animals exhibited dilation of the sinusoids, necrosis, vacuolar degeneration, vascular congestion, and hemorrhage (Figures 6a and 6b). In the LPS + VE + SS group, the animals only exhibited hemorrhage and necrosis (Figures 7a and 7b). All of the histopathological changes are listed and shown in Table 2. The PCNA expression was weaker in the control group than in the treatment groups (Figures 8a and 8b), but the LPS group had a strong PCNA expression (Figures 9a and 9b). The PCNA expression was moderate in the VE + LPS and SS + LPS groups, but it was weak in the SS + LPS + VE group (Figures 9c– 9f).

3.4. TUNEL assay

To detect apoptotic cells in the liver tissues, TUNEL staining was applied (Figure 10). The apoptotic index counts are shown in Table 3. The number of apoptotic cells in the treatment groups was statistically increased when compared to the control group. Coadministration of VE and SS with LPS reduced the increased apoptotic cells compared to when LPS was administered alone. There was no change in the number of apoptotic cells in the VE, SS, and SS + VE groups when compared to the control (Table 3, Figure 10).

3.5. Evaluation of the genetic substances

Changes in the genetic substances were detected in the liver tissue with some parameters. The results also demonstrated that the DNA tail%, tail length, and moment did not alter in groups that were not administered LPS (Table 4) (Figures 11a–11c).

LPS, applied to the liver tissue at 10 mg/kg bw for 6 h, induced a considerable increase in the fragments of DNA that were viewed on the agarose gel (Figures 11d and 11e, Table 4). The results of the comet assay indicated a 104.02 ± 9.25-µm increase in the average tail length after the administration of LPS when compared to the control group (Table 4). At the end of 6 h, there was an important decrease in the DNA tail% and tail length in the LPS + VE and/or SS groups when compared to the LPS group (Figures 11f–11i, Table 4).

4. Discussion

Sepsis is a clinical condition that can lead to multiple organ damage and death, characterized by hemodynamic and metabolic changes (Hotchkiss and Karl, 2003). In response to severe infection, the immune system and neuroendocrine systems have an important effect on protection against sepsis (Klijn et al., 2008). Microvascular dysfunction is considered to be an important factor for

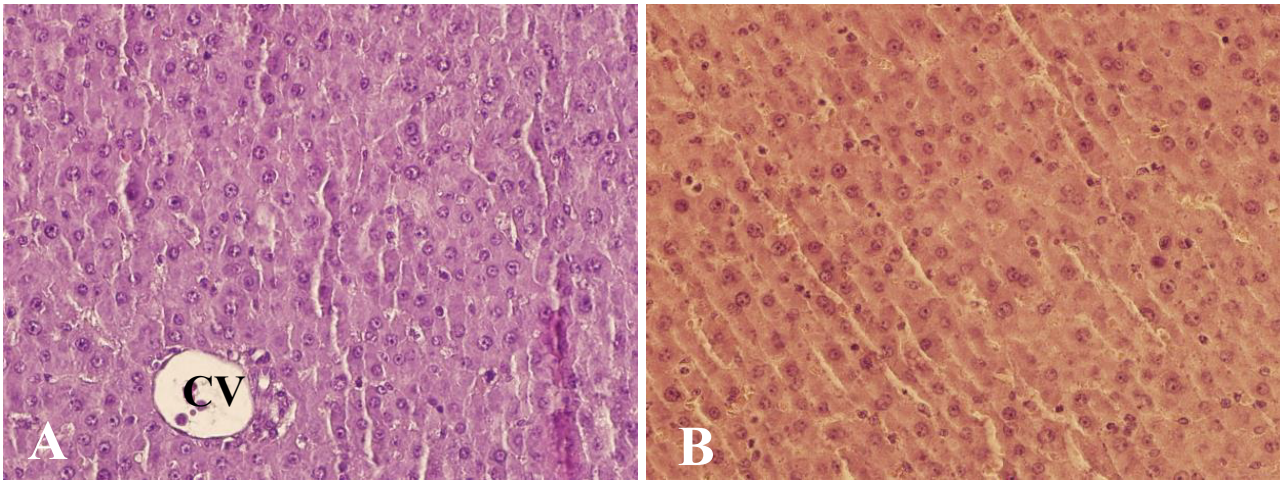


Figure 3. Liver section of the control rats, CV: central vein at 200×. Histological structures (A, B) of the control, VE, SS, and VE + SS-treated rats were similar to the control group.

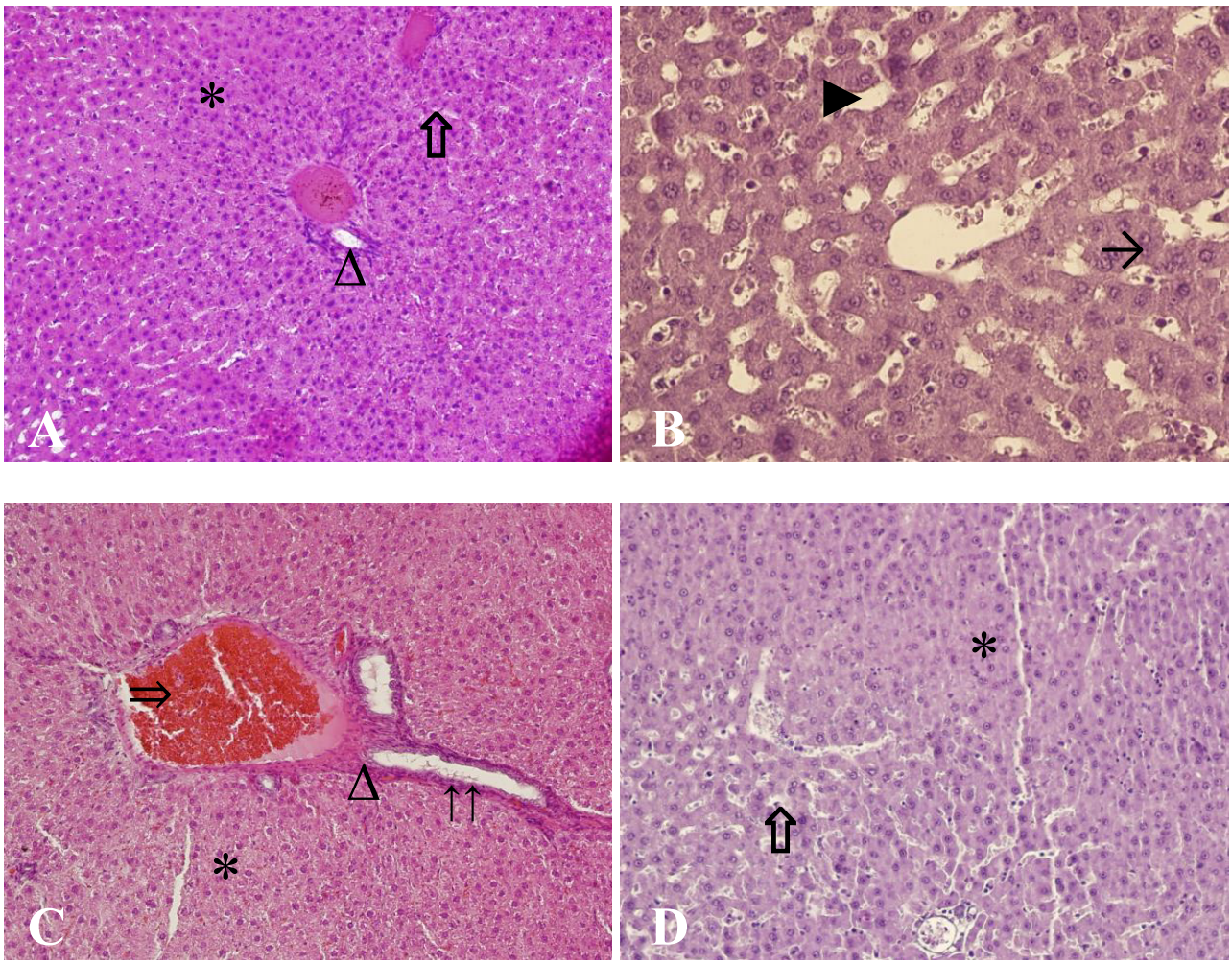


Figure 4. Liver sections of the LPS-treated rats. (A) Showing Δ: leukocyte infiltration, *: necrosis, Δ: dilation of the sinusoids at 200×; (B) ►: vacuolar degeneration, →: binucleated hepatocytes at 400×; (C) ⇒: hemorrhage, Δ: leukocyte infiltration, *: necrosis, ↑↑: vascular congestion; and (D) ⇑: dilation of the sinusoids and *: necrosis at 200×.

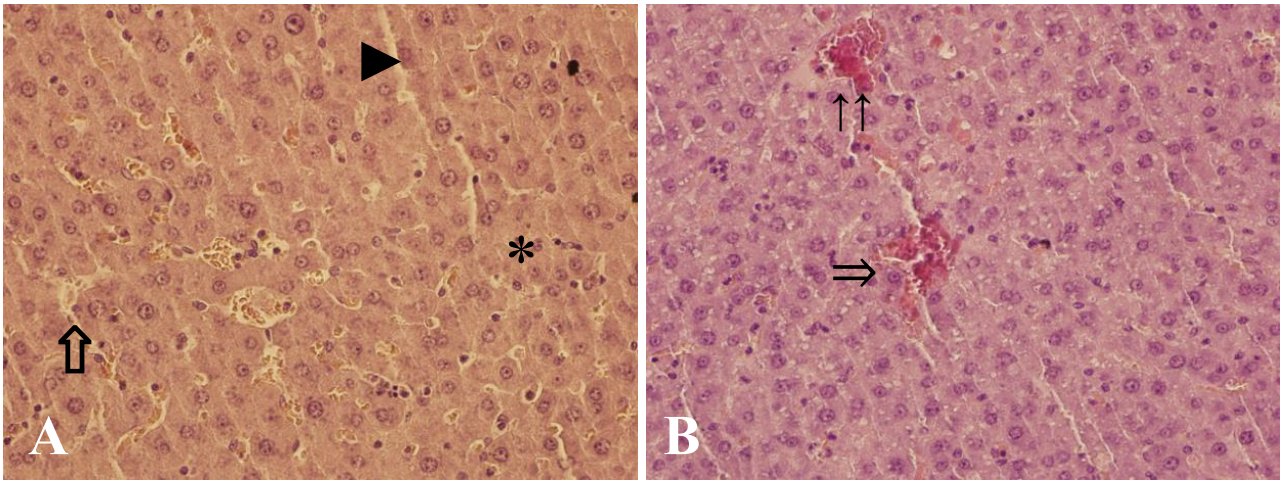


Figure 5. Liver sections of LPS + VE-treated rats. (A) Showing ⇑: dilation of the sinusoids, *: necrosis, and ▶: vacuolar degeneration; and (B) ↑↑: vascular congestion and ⇒: hemorrhage at 200×.

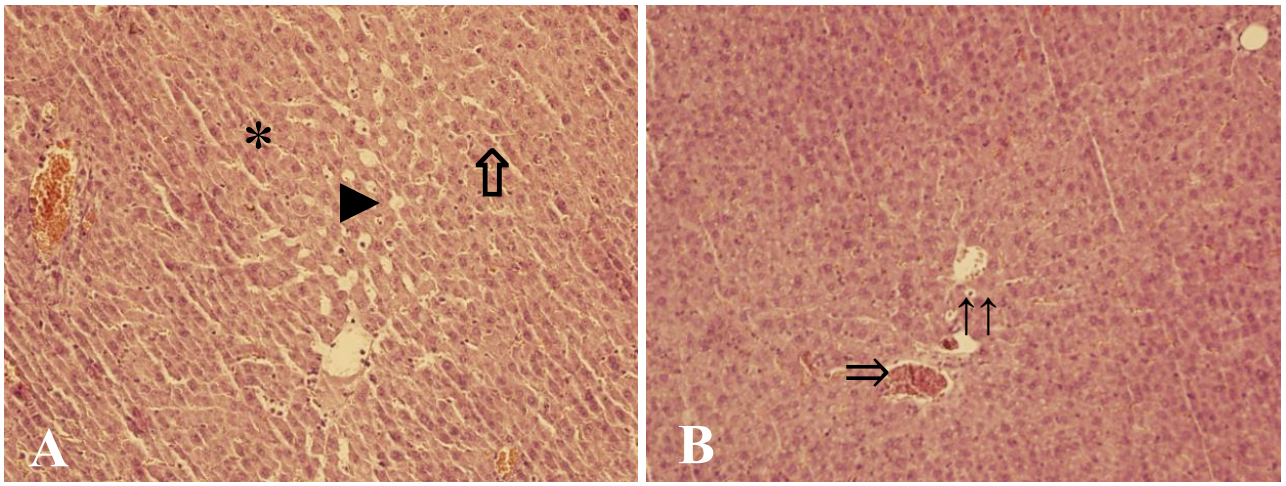


Figure 6. Liver sections of the LPS + SS-treated rats. Showing (A) ⇑: dilation of the sinusoids, *: necrosis, and ▶: vacuolar degeneration; and (B) ↑↑: vascular congestion and ⇒: hemorrhage at 200×.

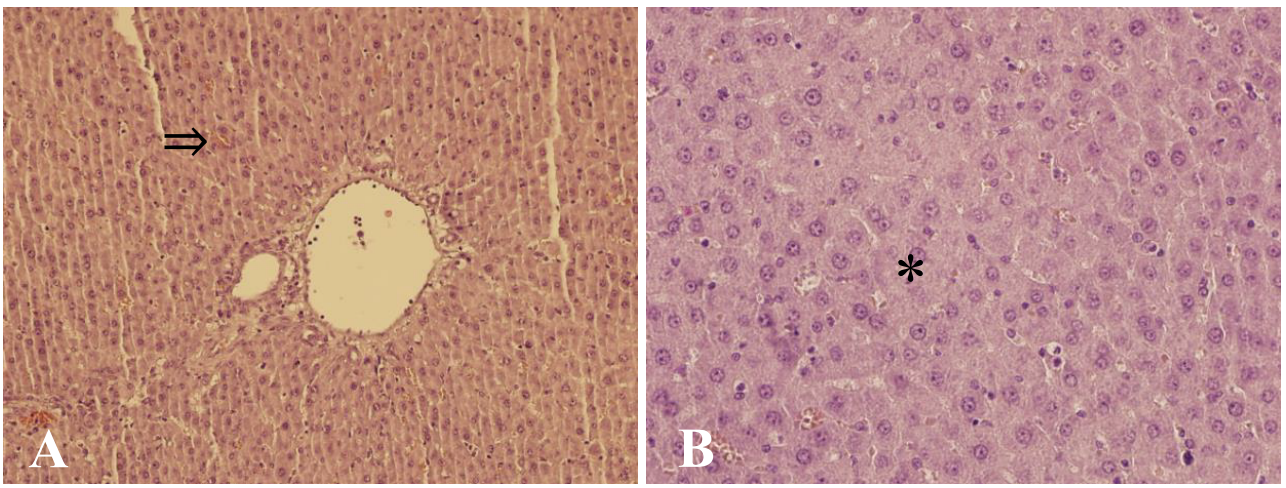


Figure 7. Liver sections of the LPS + VE + SS-treated rats. Showing (A) ⇒: hemorrhage and (B) *: necrosis at 200×.

Table 2. Grading of the histopathological changes in the liver sections.

Groups	Necrosis	Hemorrhage	Vacuolar degeneration	Vascular congestion	Binucleated hepatocytes	Leukocyte infiltration	Dilation of sinusoids
Control	-	-	-	-	-	-	-
VE	-	-	-	-	-	-	-
SS	-	-	-	-	-	-	-
SS + VE	-	-	-	-	-	-	-
LPS	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
LPS + VE	+ +	+ +	+ +	+ +	-	-	+ +
LPS + SS	+ +	+ +	+ +	+ +	-	-	+ +
LPS + SS + VE	+	+	-	-	-	-	-

The features were scored as follows: none (-), mild (+), moderate (+ +) and severe (+ + +).

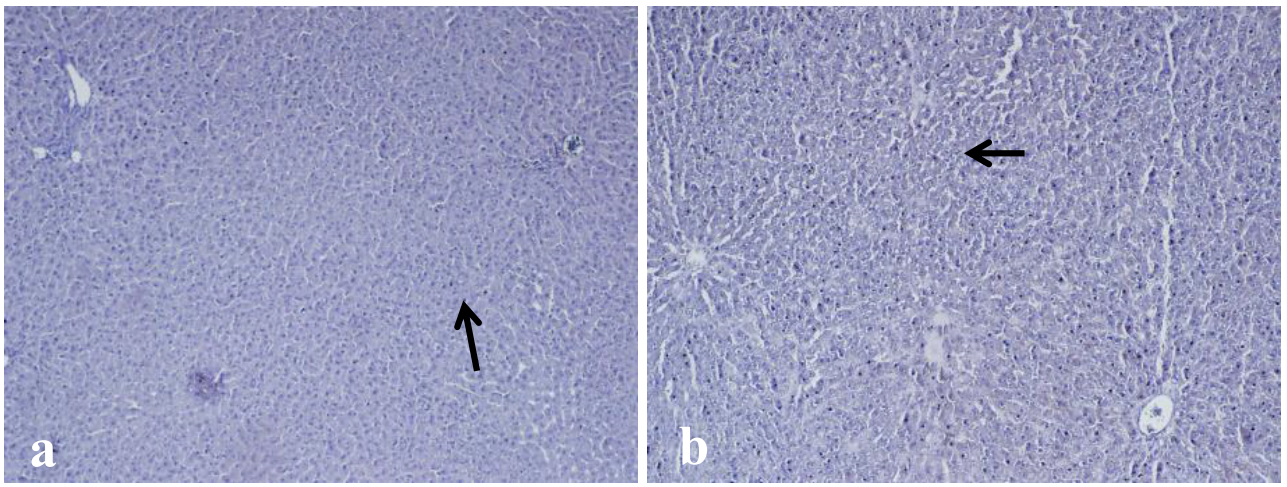


Figure 8. Control, VE-, SS-, and VE + SS-treated rats, showing PCNA protein expression with immunohistochemical analysis in male rat liver. Single arrow PCNA expression in the group shows multiple apoptotic cells at 200×.

sepsis-related circulatory failure and organ dysfunction (Cauwels, 2007). In this study, LPS was administered at 10 mg/kg bw, i.p. Pathological changes, the antioxidant capacity, and DNA structure were observed, and liver function tests were performed, and the lipid profile was evaluated in the rat liver after 6 h. None of the rats used herein died as a result of the experiments.

The liver is a vital organ that synthesizes and metabolizes many substances necessary for organisms (Jeschke, 2009). It is very sensitive to chemical substances and pesticides, because they are taken into the body to the main sites of biotransformation and enter the body through the liver. Furthermore, the concentration of enzymes that metabolize these substances in the liver is high in the cytochrome P-450 monooxygenase system. These substances cause the etiology and pathology of liver disorders. AST, ALT, and ALP, from serum enzymes, are essential for the evaluation of liver damage. This study showed significantly higher ALP, AST, ALT, total cholesterol levels and a significant reduction of triglyceride levels in LPS-induced liver tissue injury. All of these parameter changes, induced by LPS exposure, were at least partially normalized when SS and VE were given together with LPS.

Sepsis causes organ dysfunctions and cell hypoxia, apoptosis, and death. Apoptosis has a major effect on the rate of sepsis of the parenchymal tissue. Apoptosis has been found in the liver, lungs, intestinal system, and kidneys (Hotchkiss et al., 1999). Immunohistochemical findings of the PCNA showed that carcinogenesis resulted in DNA damage. Its expression may be beneficial as an indicator of cell multiplication. Nevertheless, when compared to the other multiplication indicators, they showed advanced results because of their features (Scott et al., 1991). Herein, the PCNA expression was analyzed in both the treatment

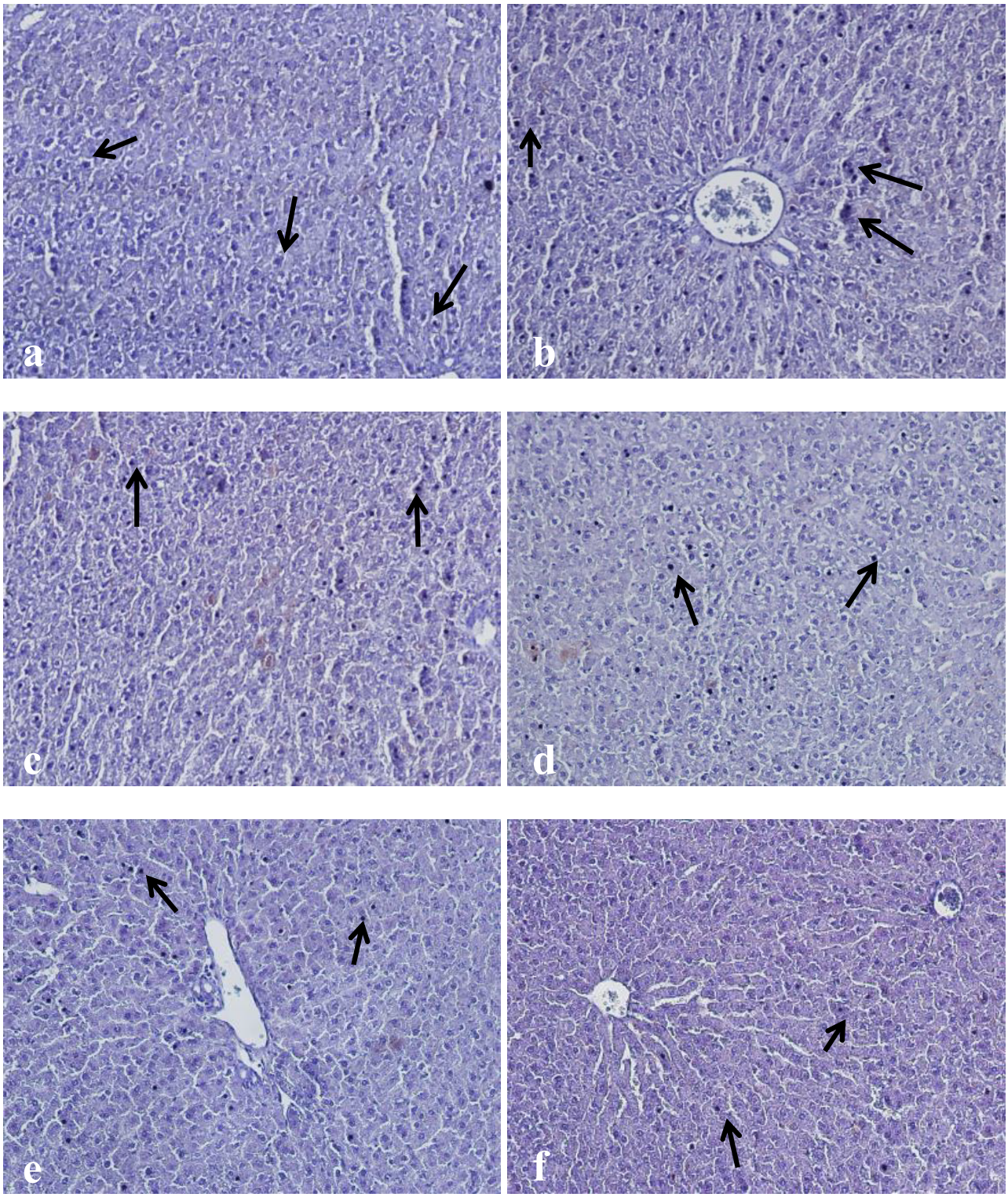


Figure 9. LPS group with VE and SS in male rat liver, showing PCNA protein expression with immunohistochemical analysis. (**a, b**) LPS, (**c**) LPS + VE, (**d**) LPS + SS, and (**e, f**) LPS + SS + VE groups. Single arrow PCNA expression in the LPS, LPS and/or VE + SS groups show multiple apoptotic cells at 200×.

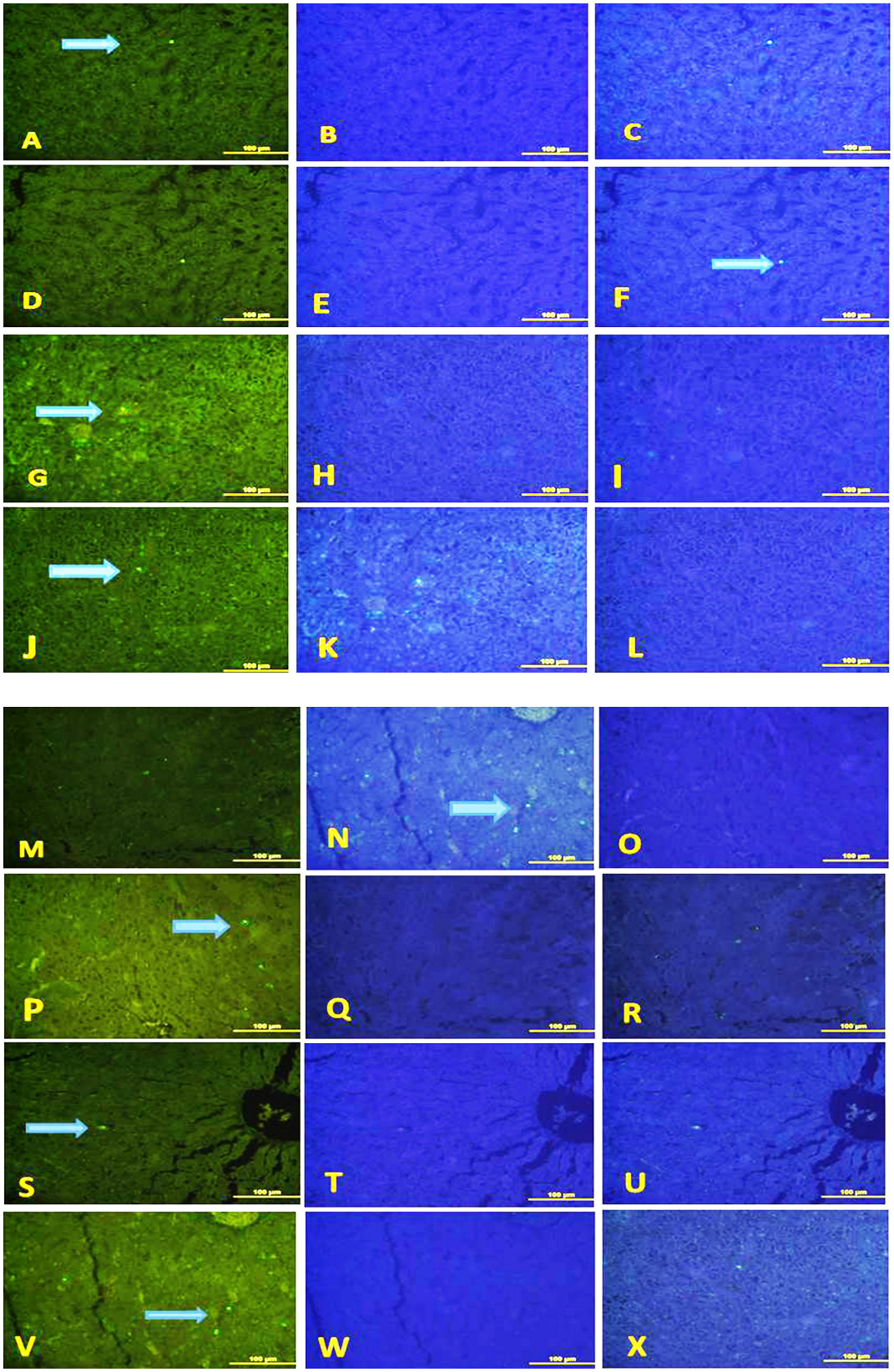


Figure 10. Histological features of the apoptotic areas in the liver of the rats. A, B, C → Control; D, E, F → SS; G, H, I →VE; J, K, L → SS + VE; M, N, O → LPS; P, Q, R → LPS + SS; S, T, U, → LPS + VE; and V, W, X → LPS + SS + VE.

Table 3. Results of the TUNEL assay in the rat liver of the control and treatment groups.

Groups	Control	SS	VE	SS + VE	LPS	LPS + SS	LPS + VE	LPS + SS + VE	P
Tunel positive cell count N = 35 area	0.53 ± 0.25 [a]	0.54 ± 0.74 [a]	0.60 ± 0.81 [a]	0.52 ± 0.60 [a]	1.91 ±1.46 [b]	1.00 ± 1.26 [c]	0.99 ± 0.74 [c]	0.88 ± 1.01 [d]	<0.05

Superscripted letters indicate significant differences in the rat liver tissue of the control and different treatment groups. Significance was accepted as $P < 0.05$.

Table 4. Estimated mean values of the DNA tail%, tail length, and tail moment of the comet assay by image analysis after treatment of the liver cells with different chemicals.

	Tail% DNA Mean ± SD	Tail length Mean ± SD	Tail moment Mean ± SD
Control	45.81 ± 5.25[a]	29.78 ± 3.65[a]	13.64 ± 1.74[a]
VE	52.24 ± 8.45[a]	26.05 ± 8.12[a]	13.61 ± 3.56[a]
SS	57.05 ± 9.45[a]	28.64 ± 6.25[a]	16.34 ± 3.45[a]
VE + SS	55.39 ± 6.82[a]	37.82 ± 5.63[a]	20.95 ± 7.25[a]
LPS	99.17 ± 6.45[b]	104.02 ± 9.25[b]	102.16 ± 10.23[b]
LPS + VE	91.26 ± 9.45[c]	77.23 ± 9.45[c]	74.44 ± 20.15[c]
LPS + SS	96.4 ± 11.23[c]	81.91 ± 9.54[c]	78.96 ± 10.21[c]
LPS + VE + SS	75.63 ± 8.62[d]	54.18 ± 9.26[d]	40.98 ± 7.41[d]

Superscripted letters indicate significant differences in the rat liver tissue of the control and different treatment groups. Significance was accepted as $P < 0.05$.

and control groups. The results indicated that the expression of PCNA in the LPS-treated groups was stronger than in the nontreated groups. The SS + LPS, VE + LPS, and SS + LPS + VE groups showed a lower expression of PCNA than the LPS and control groups.

VE and SS regulate toxicity by the antioxidant mechanism, which constitutes a significant portion of the nonenzymatic antioxidant defense system (Karabulut-Bulan et al., 2008). SS and VE deficiency affects chemical metabolism and toxicity. Both protect biological membranes from oxidative degeneration. VE and SS are protective elements against some diseases (Rana and Verma, 1997; Ellis et al., 1984; Tong and Wang, 1998). It has also been reported that VE and SS are associated with the prevention of oxidative damage in tissues (Ganther, 1978). In a study by Pandır et al. (2017), it was observed that chemicals induced oxidative stress in the liver, brain, and kidneys, and VE and SS provided protective effects against oxidative stress and complex effects in the antioxidant system of rats. In their study, Singh et al. (2006) concluded that VE and SS had a synergistic effect on humoral immune response. Fischer et al. (2001) reported that VE and SS deficiency significantly reduced the expression level of genes important for antioxidant defense, cell cycle, and apoptosis inhibition. Our work detected that LPS induced oxidative stress in the liver cells of rats via the formation of free radicals and changes in the cellular antioxidant defense system. These changes were determined through the FRAP and TEAC of the liver cells. The antioxidant capacity of cells related to antioxidant defense mechanisms were changed by LPS. Alterations of the FRAP and TEAC values may have exemplified a feature of the cellular response to increased ROS levels caused by LPS toxicity in this study. Changes in the antioxidant capacity of the liver cells increased significantly with SS and VE in treatment of the LPS-administered group.

Messarah et al. (2012) reported that in rats, arsenic caused weak pathological changes, such as cellular debris and cytoplasmic vacuolization in the central vein in the liver, and when SS was administered together with VE, the histological structure of the liver was impaired. Our light microscopic analyses showed that animals exposed to LPS exhibited leukocyte infiltration, dilation of the sinusoids, necrosis, binucleated hepatocytes hemorrhage, vacuolar degeneration, and vascular congestion in the liver of the animals in the LPS group. However, in the VE and SS + LPS groups, the animals exhibited dilation of the sinusoids, necrosis, vacuolar degeneration, vascular congestion, and hemorrhage. These changes were less severe in the LPS + VE and/or SS groups. Thus, SS and VE could ameliorate the liver damage induced by LPS exposure.

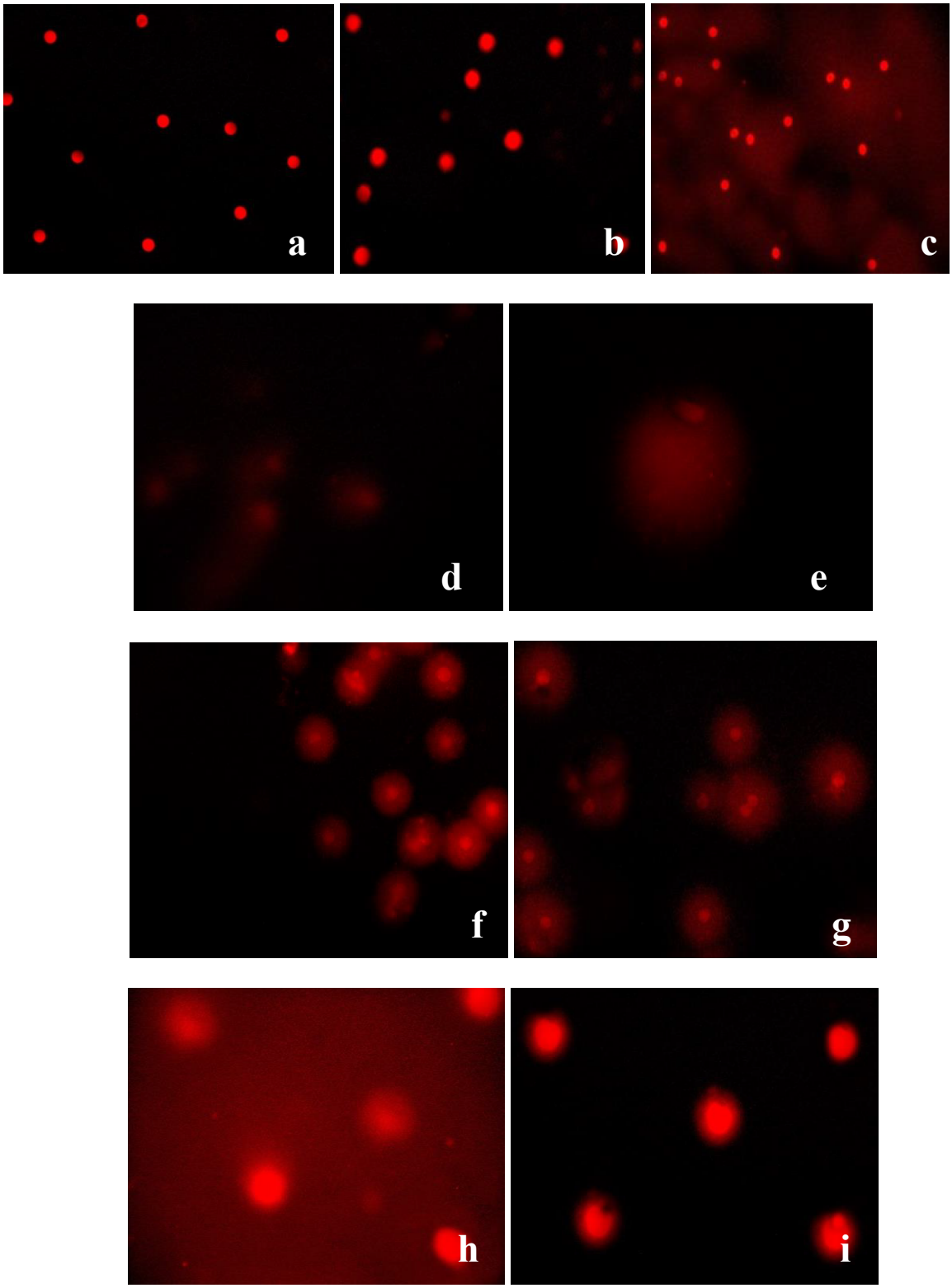


Figure 11. DNA damage in rat liver exposed to LPS + VE and/or SS. (**a**) Control group, (**b**) VE, (**c**) SS, (**d, e**) LPS, (**f**) LPS + VE , (**g**) LPS + SS, and (**h, i**) LPS + VE + SS groups.

DNA, a stable molecule, can also undergo spontaneous chemical oxidative damage like lipids, carbohydrates, and proteins. It has been suggested that in every cell of the human body, DNA is exposed to oxidative damage 10^3 times a day (Halliwell and Gutteridge, 1999). The fast, sensitive fluorescent microscopic (Come assay) method used to detect single-cell damage is increasingly being used to measure DNA damage in experimental studies (Green et al., 1996; Baltacı et al., 1998). After treatment with LPS, assessment of the comet assay studies of the DNA of the liver cells was performed using the DNA tail% and tail lengths in this study. The DNA was not significantly affected in the VE, SS, and VE + SS groups when compared with the control group. The DNA tail% and tail lengths were changed with LPS when compared with the control group ($P < 0.05$). These outcomes suggested that LPS + SS + VE had a protective role against changes as the result of LPS in the DNA structure.

Apoptosis is an extremely organized procedure used to remove undesired or damaged cells in organisms (Cilenk et al., 2016). In the study of Li et al. (2017), TUNEL assay results also revealed that an abundance of hepatocytes went through apoptosis over time, which was consistent with the histopathology outcomes. Zhou et al. (2017) investigated the role of GRP78 in the pathogenesis of nonalcoholic steatohepatitis (NASH) in rats. H&E, TUNEL, and immunohistochemistry staining were applied to detect histological changes, apoptosis, and macrophage infiltration in the NASH liver tissue, respectively. In this study, macrophage infiltration and hepatocytic apoptosis was considerably elevated in the livers of the rats from the NASH group when compared with the control group. Herein, the number of apoptotic cells in the LPS, LPS + SS, and LPS + SS + VE groups was statistically significant when compared to the control group. It was also found that the number of apoptotic cells in the LPS, LPS + SS, and LPS + SS + VE groups was statistically meaningful when compared to the control group. Coadministration of VE and/or SS with LPS reduced the increased the number of apoptotic cells compared to when LPS was administered alone. No change in the number of apoptotic cells was observed in the SS, VE, and VE + SS groups when compared to the control. It was concluded that LPS may have increased apoptosis by inducing oxidative stress.

In a previous study, it was argued that LPS showed harmful effects on animals to the point of cell damage (Doğanyiğit et al., 2013). Recent studies have shown that SS and VE have important roles in many tissues, as well as their effects on cell membrane resistance. These effects are due to the widespread availability of SS and VE receptors in many tissues. SS and VE play a particularly immunosuppressive role in the immunoregulatory system. The influence of SS and VE on cytokine release from monocytes and lymphocytes in the immune system is thought to play a key role. SS and/or VE treatment with LPS reversed histopathologic changes through antioxidant capacity, against the DNA damage that occurred in the liver tissue when compared to treatment with LPS. Biochemical evaluations and ultrastructural alterations in this study demonstrated that LPS affects liver cells in rats, even if it was administered at a dose less than 10 mg/kg bw. While VE and SS inhibited the formation of the study parameters, it also decreased LPS hepatotoxicity, but more detailed work needs to be done in this regard.

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