North Clin Istanb
doi: 10.14744/nci.2018.82957

Original Article BIOCHEMISTRY

UNCORRECTED PROOF

The hepatoprotective effect of *Aloe vera* on ischemia-reperfusion injury in rats

Muserref Hilal Sehitoglu,[1] Ihsan Karaboga,[2] Asli Kiraz,[3] Hasan Ali Kiraz[4]

[1]Department of Medical Biochemistry, Canakkale Onsekiz Mart University Faculty of Medicine, Canakkale, Turkey

[2]Namik Kemal University School of Health, Tekirdag, Turkey

[3]Department of Medical Microbiology, Canakkale Onsekiz Mart University Faculty of Medicine, Canakkale, Turkey

[4]Department of Anesthesiology and Reanimation, Canakkale Onsekiz Mart University Faculty of Medicine, Canakkale, Turkey

ABSTRACT

OBJECTIVE: *Aloe vera* is known for its antioxidant properties. In this experimental study, we aimed to investigate the efficacy of *Aloe vera* in liver I/R injury in rats.

METHODS: Male Wistar Albino rats were divided into 3 groups: Sham group (n=7) had no medication or surgical procedures applied. I/R group (n=7) was the control group and had 45 minutes of abdominal aorta ischemia applied and rats were sacrificed 24 hours after reperfusion. I/R+AV group (n=7) was the treatment group given *Aloe vera* (30 mg/kg) every day with gastric gavage for a month before 45 minutes of abdominal aorta ischemia and rats were sacrificed 24 hours after reperfusion. Before sacrifice, all liver tissues were removed. Tissues were examined for histopathological investigation, iNOS immunoreactivity and tissue biochemistry, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities.

RESULTS: The SOD, CAT and GSH-Px levels of I/R+AV group were not significantly different from sham group ($p>0.05$) but were significantly higher when compared to I/R group. MDA levels of liver tissues were significantly lower ($p<0.05$) in I/R+AV group compared to I/R group. Disrupted hepatic cords, sinusoidal dilatation, haemorrhage, cytoplasmic vacuolisation of hepatocytes and intensive iNOS immunoreactivity were detected in I/R group. Decreased histopathological change score and iNOS immunoreactivity score were noticed in I/R+AV group compared with I/R group.

CONCLUSION: It was found that *Aloe vera* showed a hepatoprotective effect against I/R injury. Further research to determine the effective dose, administration method and effects of *Aloe vera* for liver transplantation is required.

Keywords: Aloe vera; hepatoprotective; ischemia reperfusion; rat.

Cite this article as: *Sehitoglu MH, Karaboga I, Kiraz A, Kiraz HA. The hepatoprotective effect of Aloe vera on ischemia-reperfusion injury in rats. North Clin Istanb*

Organ damage due to reperfusion after temporary ischemia is related to many clinical tableau. I/R injury occurs during a variety of surgical interventions like organ transplant and coronary bypass and due to diseases such as stroke, shock and cardiac infarctus. The destructive effects of I/R injury result in direct tissue damage due to formation of acute reactive oxygen radicals (ROS) following reoxygenation, a variety of events with harmful effects at the cellular level and linked inflammation, cell death and organ failure [1–3].

I/R injury in the liver causes a biphasic response in the form of acute and subacute phases. The acute phase is observed 3–6 hours after reperfusion and is characterized by free radical formation and activation of T lym-

Received: March 23, 2018 *Accepted:* October 02, 2018 *Online:* October 10, 2018

Correspondence: Dr. Muserref Hilal SEHITOGLU. Canakkale Onsekiz Mart Universitesi Tip Fakultesi, Dekanlik Binasi, 17100 Canakkale, Turkey.
Tel: +90 286 218 00 18 - 2103 e-mail: hill_al@hotmail.com

phocytes and Kupffer cells. The response in the subacute phase is intense neutrophil infiltration and is observed 18–24 hours after reperfusion [2]. When examined histopathologically, I/R injury causes cellular swelling, vacuolisation, destruction of endothelial cells and polymorphonuclear cell infiltration [4]. The nitric oxide (NO) synthase family forms a range of enzymes causing oxidative deanimation of L-arginine and NO formation. Induced NO-synthase (iNOS) is one of these with increased levels during inflammatory events contributing largely to NO synthesis. In tissues iNOS testing is possible with immunohistochemical staining [5].

In organisms, ROS are natural products occurring due to oxygen metabolism with small molecular structure which have destructive effects on cells when they are produced above the anti-oxidant capacity of the body. Reperfusion of the ischemic liver involves exposure to high levels of ROS and I/R injury is one of the best examples of this situation. When the balance between ROS and anti-oxidants is disrupted, oxidative stress occurs. The leading enzymes with protective effects against ROS are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). When the levels of these types of protective enzymes and molecules reduce, ROS increases forming a critical situation. Malondialdehyde (MDA) is a toxic metabolite formed by lipid peroxidation developing after ischemia and reperfusion, and is the most important marker of lipid peroxidation [6–8].

Preventing I/R injury to the liver carries great importance for healing after liver transplants and treatment of severe liver disease. This is because patients with I/R injury are more liable to mortal complications due to the development of a variety of pathophysiological processes causing Kupffer cell activation, and increases in ROS, inflammatory mediators and cytokines [9, 10]. There are a variety of studies proving that anti-oxidants are beneficial for I/R injury [3, 6, 11–13].

Aloe vera is a semi-tropical plant from the Liliaceae family with a broad range of application within traditional medications [14]. It has been shown that *Aloe vera* has a strong antioxidant and antimicrobial properties thanks to the phenolic compounds it contains. *Aloe vera* gel has almost two hundred bioactive ingredients such as minerals, vitamins, proteins, lipids, amino acids and polysaccharides [15, 16]. *Aloe vera* has proven to be an effective treatment agent in many diseases ranging from asthma, ulcers to wound healing and cancer [17]. We have shown that *Aloe vera* is effective against different tissue ischemia reperfusion injuries in various studies that we have done previously [18, 19]. Although *Aloe vera* has some healing effects on liver damage, no study has been done to determine whether it can prevent liver from ischemia reperfusion damage especially in organ transplantation. This study researched whether *Aloe vera*, with known anti-oxidant and anti-inflammatory effects, has a protective effect on I/R injury induced in rats.

MATERIALS AND METHODS

After receiving permission from Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee, decision no: 2014/13–1, a total of 21, 8–12 week old male Wistar Albino rats weighing 250–300 grams were obtained. During the study rats were housed in special cages with appropriate feeding conditions. The rats were kept in controlled temperature (23–25 °C) and lighting (12 hours light, 12 hours dark) conditions, with ad libitum access to food and water. Rats were randomly divided into 3 groups of 7 individuals:

Sham group (n=7): Underwent laparotomy and observation only (No I/R and no treatment) – to neutralise the surgery and the anesthesia effects.

I/R group (n=7): Control group. Underwent laparotomy and liver I/R performed but AV did not given.

I/R+AV group (n=7): Treatment group. Underwent laparotomy and liver I/R performed also AV was given.

Aloe vera was obtained from Herbalife Turkish distributor (Herbalife Inc, İstanbul, Turkey). It is used by many people as food supplements.

Dosage: In this study we decided the dose of *Aloe vera* as 30 mg/kg body weight on the basis of studies that used 10–120 mg/kg/day doses to reveal the therapeutic effects of AV [18–24]. Since we have previously demonstrated the efficacy of *Aloe vera* at a dose of 30 mg/kg, a single dose trial was deemed appropriate in this study.

Experimental procedure

Anaesthesia for all groups used xylazine (Bayer, İstanbul, Turkey) 5 mg/kg and ketamine hydrochloride (Parke Davis, İstanbul, Turkey) 50 mg/kg, with spontaneous respiration at room temperature. The rats were placed in supine position on the operating table under sterile conditions and skin and subdermal midline incision was opened. Intestines were pushed to the right and the abdominal aorta was reached from the midline. In groups

with ischemia induced, the abdominal aorta and vena cava inferior were carefully dissected and separated from each other. The abdominal aorta was clamped from the lower section of the renal artery turning immediately above the bifurcation. After 45 minutes clamping, the clamps were removed and reperfusion ensured. The abdomen was closed appropriately. Rats in all groups were sacrificed 24 hours later by administration of high dose anaesthetic, Ketamine (50 mg/kg). Immediately before sacrifice, the liver tissues were fully removed. Half of the tissue was stored in formaldehyde. Histopathological investigation, iNOS immunoreactivity and malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) tissue biochemistry tests of tissues exposed to ischemia were performed as stated below.

Biochemical investigations of rat liver tissues

After macroscopic analysis, rat tissues were kept at -80°C. Tissues for biochemical analysis were homogenized in the appropriate buffer for each method separately and supernatants were removed. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) levels of rat liver tissues were measured in duplicate with highly sensitive ELISA spectrophotometry. The protein concentrations were indicated by the Bradford method using Bradford reagent (Sigma Aldrich, Bradford reagent-B6916-1KT, USA). All the data was defined as the mean±standard deviation (SD) results based on per mg of protein.

Tissue SOD activities were measured with SOD assay kit (Biovision-K335-100; Milpitas, CA95035, USA) using highly sensitive ELISA spectrophotometry. The IC50 (50% inhibition activity of SOD) values was determined by this colorimetric method under 450 nm. The results were expressed as U/mL SOD per milligram protein (U/mL.mg protein).

CAT activity of rat liver tissues was determined with commercial colorimetric kit, S-341 Cell Biolabs' OxiselectTM Catalase Activity Assay Kit. The results, measured using highly sensitive ELISA spectrophotometry, were expressed as U/mL CAT per milligram protein (U/mL.mg protein).

Liver tissue GPx activities were measured with commercial Glutathione Peroxidase Activity Colorimetric Assay Kit (Biovision-K762-100; Milpitas, CA95035, USA) using highly sensitive ELISA spectrophotometry. The results were expressed as U/mL GPx per milligram protein (mU/mL.mg protein).

Lipid peroxidation was determined by the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a colorimetric (532 nm) product, proportional to the MDA present. Biovision-K739-100; Milpitas, CA95035, USA, Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit was used to determine MDA levels. The results were expressed as nmole MDA per milligram tissues (nmole/mg tissue).

Statistical analysis

Results were subjected to one-way analysis of variance (ANOVA) using SPSS 21.0 software (SPSS Inc., USA). Differences among the groups were obtained using Tukey's test option. Statistical significance was accepted as $p<0.05$. All data was expressed as mean±standard deviation (SD) in each group.

Histopathological examination

For histopathological examination, liver tissues were fixed in 4% formaldehyde for 48 hours. After fixation, tissues were embedded in paraffin and cut into 5 µm sections by microtome (Slee, 5061). Paraffin sections were stained with haematoxylin and eosin (H&E). H&E stained sections were examined under light microscope (Olympus CX41) using different magnifications by two histopathologists blind to the grouping of the animals to detect liver injury. Histopatholocal changes of sinusoidal dilatation, haemorrhage and vacuolisation were assessed in the liver tissue. Tissue damage was graded and scored as follows, 0; normal histological appearance, 1; mild, 2; moderate, and 3; severe.

Immunohistochemical examination

For examination of iNOS immunoreactivity in liver tissue, paraffin-embedded liver tissue was cut into 5 µm and deparaffinised in xylene then rehydrated in graded series of ethanol. Sections were washed in phospate buffer saline (PBS) and boiled in citrate buffer using an microwave oven at 90–100 °C for ten minutes. Endogenous peroxidase activity was blocked with H_2O_2 and sections were transferred to normal goat serum to block non-specific binding. Then sections were incubated with primary antibody (Anti-iNOS, ab15323) in humidified chamber at room temperature for 1 hour. After incubation of anti-iNOS, sections were incubated with biotinylated secondary antibody and streptavidin

Table 1. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities and levels of malondialdehyde (MDA) of rat liver tissues

Groups	SOD (U/ml.mg protein) Mean±SD	CAT (U/ml.mg protein) Mean±SD	GPx (mU/ml.mg protein) Mean±SD	MDA (nmol/mg tissue) Mean±SD
Liver				
Sham group	24.03±5.11	32.36±5.41	36.10±3.59	4.99±0.29
I/R	0.57±0.42*	10.40±3.35*	16.43±3.26*	18.30±1.46*
I/R+AV	17.97±1.99$^{\beta}$	29.60±2.28$^{\beta}$	31.14±4.65$^{\beta}$	7.70±2.30$^{\beta}$

SD: Standard deviation; I/R: Ischemia reperfusion; AV: Aloe vera. Means by the letter * and $^{\beta}$ are significantly different to the One-way ANOVA-Tukey's test in each coloumn ($p<0.05$).

peroxidase (Ultra Vision Detection System-HRP kit, Thermo Scientific/Lab Vision) at room temperature for 10 minutes, respectively. Chromogen 3-amino-9-ethylcarbazole (AEC Substrate System, Thermo Scientific/Lab Vision) was used and the sections were counterstained with haematoxylin. Anti-iNOS immunoreactivity was examined and scored semi-quantitatively from 0 to 3 as follows, 0; none, 1; weak, 2; moderate, and 3; intense for each liver tissue.

Statistical analysis

Data was analysed using SPSS 16.0 statistical software package for Windows (SPSS Inc, Chicago, USA). The results were reported as the mean±standard deviation. Groups were compared using the nonparametric Kruskal-Wallis test. The Mann-Whitney U test was used for binary comparisons. Spearman correlation test was used for histological evaluations of the relationship between the variables. A p value of less than 0.05 was considered significant.

RESULTS

The mean and standard deviation values of SOD, CAT, GPx, and MDA in all groups are shown in Table 1 and Figure 1. SOD, CAT and GPX levels of I/R group (0.57±0.42, 10.40±3.35, and 16.43±3.26 U/ml.mg protein, respectively) were found to be lower compared to the other groups and this was statistically significant ($p<0.05$). SOD, CAT and GPX levels of I/R+ AV group (17.97±1.99, 29.60±2.28 and 31.14±4.65 U/ml.mg protein, respectively) were not significantly different from Sham group ($p>0.05$), but were significantly higher when compared to I/R group. In the same way, MDA levels of liver tissues were significantly lower ($p<0.05$) in I/R+ AV group (7.70±2.30 nmol/mg tissue) compared to I/R group (18.30±1.46 nmol/mg tissue).

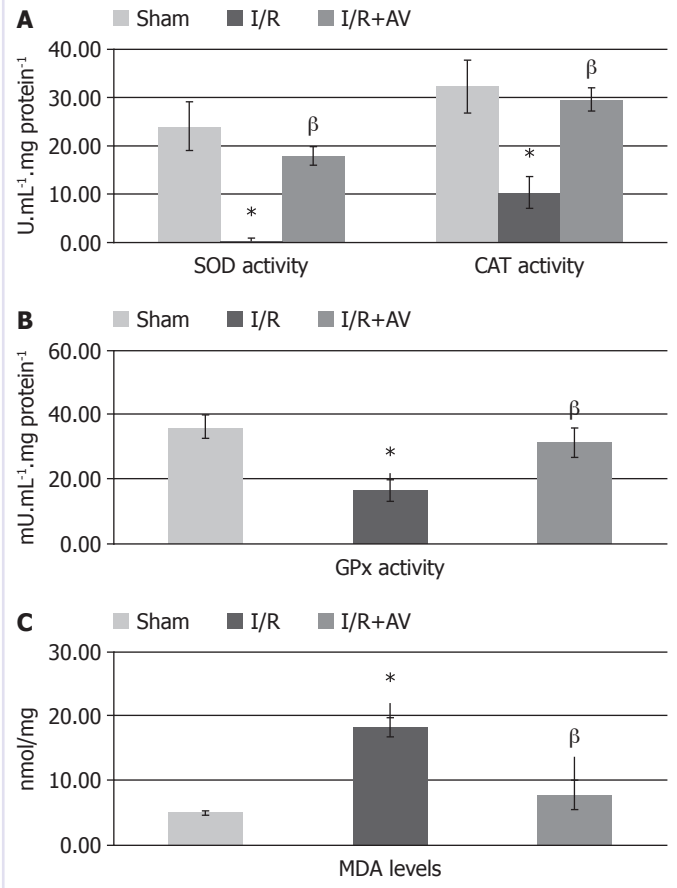

Figure 1. The effects of Aloe vera teraphy on superoxide dismutase (SOD) and catalase (CAT) **(A)**, glutathione peroxidase (GPX) **(B)** activities and levels of malondialdehyde (MDA) **(C)** of rat liver tissues. Aloe vera teraphy groups are significantly different compared to the ischemia groups ($p<0.05$).

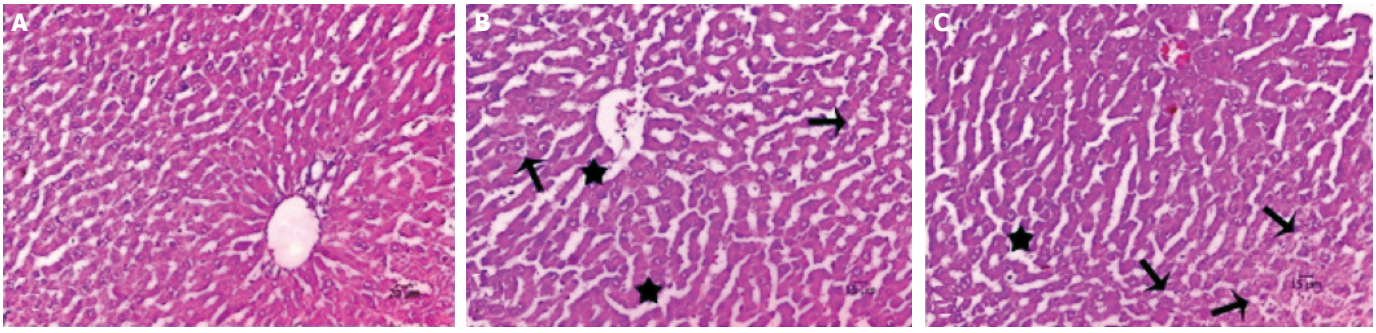

FIGURE 2. Hematoxylin & eosin (H&E) staining of liver tissue. **(A)** Sham group showing normal histological apperance, **(B)** I/R group showing disrupted hepatic cord and vacuolisation, **(C)** I/R+AV showing minimal sinusoidal dilation and vacuolisation (H&E, 200X, arrow; cytoplasmic vacuolisation, star; disrupted hepatic cords, arrow head; hemorrhage).

TABLE 2. Comparison of histopathological changes and iNOS immunreactivity scores of groups

Group	Sinusoiadal dilatation Mean±SD	Hemorrhage Mean±SD	Cytoplasmic vacuolisation Mean±SD	iNOS immunreactivity Mean±SD
Sham group	0	0	0	0.7±0.4
I/R	2.7±0.4[a]	2.4±0.5[a]	2.3±0.4[a]	2.8±0.4[a]
I/R + AV	1.5±0.5[a,b]	1.2±0.6[a,b]	1.5±0.5[a,b]	1.2±0.4[a,b]

iNOS: Induced NO-synthase; SD: Standard deviation; I/R: Ischemia reperfusion; AV: Aloe Vera; [a]Compared with Control group ($p<0.05$); [b]Compared with I/R group ($p<0.05$).

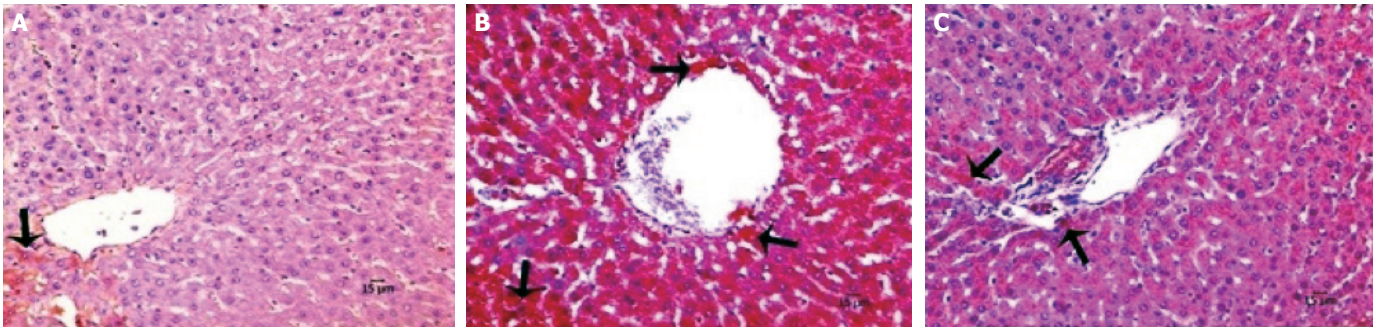

FIGURE 3. Immunohistochemical staining of liver tissue with Anti-iNOS. **(A)** Sham, **(B)** I/R, **(C)** I/R+AV. AV-treated groups showing decreased iNOS immunreactivity. (Hematoxylin counterstain, 200X, arrow; iNOS positive cells).

As revealed in Figure 2, normal liver architecture was observed in Sham group. Disrupted hepatic cords, sinusoidal dilatation, haemorrhage, cytoplasmic vacuolisation of hepatocytes and intensive iNOS immunreactivity was detected in I/R group. Decreased histopathological change score and iNOS immunreactivity score was noticed in I/R+ AV group (Table 2, $p<0.05$). Immunohistochemical findings of the groups are presented in Figure 3. iNOS immunoreactivity was clearly decreased in I/R+ AV group compared with I/R group ($p<0.05$).

DISCUSSION

Liver transplantation is a commonly used treatment

method for congenital and acquired disorders of the liver [2]. I/R injury in liver transplantation is closely related to non-functional or dysfunctional graft development and may result in graft rejection. ROS and reactive nitrogen radicals (RNS) play an important role during I/R injury by intracellular calcium overload and many cellular factors. In this situation, consumption of endogenous anti-oxidants due to release of ROS and RNS causes apoptotic and necrotic cell death to occur. Among endogenous intracellular anti-oxidants catalase, superoxide dismutase, glutathione peroxidase and allopurinol are the most known. iNOS is released by endothelial cells, hepatocytes and Kuppfer cells and is an enzyme showing activity independent of calcium. iNOS has protective and toxic effects with the type of effect linked to the stimulus type, iNOS expression levels and duration [6, 25].

Many extracellular anti-oxidant agents have been shown to have beneficial effects for treatment of liver I/R injury. Examples of these include α-tokoferol, ascorbate, coenzyme Q, pentoxifilin, lipoic acid, quercetin, cyanidine, green tea extract, N-acetylcystein, catalase derivatives, allopurinol and aminoguanidine. Many clinical and experimental studies on these types of anti-oxidants have produced different results which may be linked to the type of subjects used in the study, the administration method, duration and dose of the anti-oxidant agent used, duration of ischemia and many other factors [6].

Currently *Aloe vera* is used widely in food and drinks, pharmaceutical material and cosmetics. *Aloe* species are used generally in the world due to antitumor, anti-inflammatory, anti-oxidant and laxative effects [14, 26]. An experimental study on rats showed *Aloe vera* had neuroprotective effect on sciatic nerve I/R injury [27]. To date, though many studies have used *Aloe vera*, there is no study investigating the protective effect on I/R injury in liver.

In a study of the effects of aloe vera gel against oxidative stress-induced liver damage, it was determined that aloe vera reduced the formation of lipid peroxidation [28]. Also when the effects of aloe vera were examined on nephrotoxicity in rabbits, it was determined increased total antioxidant status, reduced total oxidant status, and increased serum catalase levels due to increased doses of *Aloe vera* [29]. *Aloe vera* has great effects to the antioxidant enzyme levels of streptozotocin-induced diabetes in rats. When superoxide dismutase, glutathione peroxidase, catalase and glutathione s -tranferase enzyme levels of rats given *Aloe vera* were compared to rats given glibenclamide used as reference antidiabetic drug, the results were not significantly different. *Aloe vera* was effective on enzyme levels up to at least glibenclamide and the antioxidant enzyme levels increased compared to diabetic rats [30]. In this study, it was found the catalase, superoxide dismutase and glutathione peroxidase enzyme levels in rats administered *Aloe vera* were significantly greater than in rats in I/R group. When levels of MDA, a marker of lipid peroxidation are examined, the MDA levels in the *Aloe vera* group were significantly lower than in I/R group. Our results suggest that *Aloe vera*'s antioxidant levels are beneficial for I/R injury of the liver.

It was seen that the iNOS expression levels are decreased in the *Aloe vera* treated rats in the liver injury induced by carbon tetrachloride [31]. Also in our previous study we have shown by histopathological examination that the increasing level of nNOS with the damage of cerebral ischemia was decreased by *Aloe vera* effect. nNOS levels of I/R+AV group (36.71±5.15) was found to be lower compared to the I/R group (62.71±11.91) [19]. Histopathological investigation revealed that the negative effects linked to I/R injury in the *Aloe vera* group were improved by a certain amount by *Aloe vera*. iNOS immunoreactivity was significantly lower in the *Aloe vera* group compared to I/R group, supporting the hepatoprotective effect of *Aloe vera* on I/R injury.

When it is considered that oxidative stress plays a role in countless diseases, determining a direct and in vivo effect on oxidative damage is very important. Currently oxidative stress is shown by new biological markers, and of these, allantoin measured in urine is promising [32]. In our study, newly-discovered oxidative stress markers were not examined and this is a limiting factor. There is a need to develop new agents with fewer side effects to replace these types of medications. We believe that more studies will be beneficial to patients and clinicians in determining the administration dose, method and possible effects of herbal material with proven anti-oxidant activity like *Aloe vera*.

Conflict of Interest: There is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Montalvo-Jave EE, Escalante-Tattersfield T, Ortega-Salgado JA, Piña E, Geller DA. Factors in the pathophysiology of the liver ischemia-reperfusion injury. J Surg Res 2008;147:153–9.
2. Fan C, Zwacka RM, Engelhardt JF. Therapeutic approaches for is-

chemia/reperfusion injury in the liver. J Mol Med (Berl) 1999;77:577–92.
3. Fouad AA, El-Rehany MA, Maghraby HK. The hepatoprotective effect of carnosine against ischemia/reperfusion liver injury in rats. Eur J Pharmacol 2007;572:61–8.
4. Kupiec-Weglinski JW, Busuttil RW. Ischemia and reperfusion injury in liver transplantation. Transplant Proc 2005;37:1653–6.
5. Kapral M, Wawszczyk J, Sośnicki S, Węglarz L. Down-regulation of inducible nitric oxide synthase expression by inositol hexaphosphate in human colon cancer cells. Acta Pol Pharm 2015;72:705–11.
6. Glantzounis GK, Salacinski HJ, Yang W, Davidson BR, Seifalian AM. The contemporary role of antioxidant therapy in attenuating liver ischemia-reperfusion injury: a review. Liver Transpl 2005;11:1031–47.
7. Halladin NL. Oxidative and inflammatory biomarkers of ischemia and reperfusion injuries. Dan Med J 2015;62:B5054.
8. Cichoż-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 2014;20:8082–91.
9. Abcouwer SF, Lin CM, Shanmugam S, Muthusamy A, Barber AJ, Antonetti DA. Minocycline prevents retinal inflammation and vascular permeability following ischemia-reperfusion injury. J Neuroinflammation 2013;10:149.
10. Zhai Y, Shen XD, O'Connell R, Gao F, Lassman C, Busuttil RW, et al. Cutting edge: TLR4 activation mediates liver ischemia/reperfusion inflammatory response via IFN regulatory factor 3-dependent MyD88-independent pathway. J Immunol 2004;173:7115–9.
11. Giakoustidis D, Papageorgiou G, Iliadis S, Giakoustidis A, Kostopoulou E, Kontos N, et al. The protective effect of alpha-tocopherol and GdCl3 against hepatic ischemia/reperfusion injury. Surg Today 2006;36:450–6.
12. Zhang WH, Li JY, Zhou Y. Melatonin abates liver ischemia/reperfusion injury by improving the balance between nitric oxide and endothelin. Hepatobiliary Pancreat Dis Int 2006;5:574–9.
13. Váli L, Stefanovits-Bányai E, Szentmihályi K, Fébel H, Sárdi E, Lugasi A, et al. Liver-protecting effects of table beet (Beta vulgaris var. rubra) during ischemia-reperfusion. Nutrition 2007;23:172–8.
14. Gamboa-Gómez CI, Rocha-Guzmán NE, Gallegos-Infante JA, Moreno-Jiménez MR, Vázquez-Cabral BD, González-Laredo RF. Plants with potential use on obesity and its complications. EXCLI J 2015;14:809–31.
15. Abdul Qadir M, Shahzadi SK, Bashir A, Munir A, Shahzad S. Evaluation of Phenolic Compounds and Antioxidant and Antimicrobial Activities of Some Common Herbs. Int J Anal Chem 2017;2017:3475738.
16. Moghaddasi MS. Aloe vera chemicals and usages. Advances in Environmental Biology 2010:464–9.
17. Olusegun A. One hundred medicinal uses of Aloe vera. Good Health Inc: Lagos; 2000.
18. Ikeno Y, Hubbard GB, Lee S, Yu BP, Herlihy JT. The influence of long-term Aloe vera ingestion on age-related disease in male Fischer 344 rats. Phytother Res 2002;16:712–8.
19. Guven M, Yuksel Y, Sehitoglu MH, Akman T, Aras AB, Cosar M. Aloe vera ameliorates cerebral ischemia/reperfusion injury in rats. Arch Clin Exp Surg 2016;5:85–93.
20. Wang Y, Cao L, Du G. Protective effects of Aloe vera extract on mitochondria of neuronal cells and rat brain. Zhongguo Zhong Yao Za Zhi 2010;35:364–8.
21. Mirshafiey A, Aghily B, Namaki S, Razavi A, Ghazavi A, Ekhtiari P, et al. Therapeutic approach by Aloe vera in experimental model of multiple sclerosis. Immunopharmacol Immunotoxicol 2010;32:410–5.
22. Parihar MS, Chaudhary M, Shetty R, Hemnani T. Susceptibility of hippocampus and cerebral cortex to oxidative damage in streptozotocin treated mice: prevention by extracts of Withania somnifera and Aloe vera. J Clin Neurosci 2004;11:397–402.
23. Lu ZQ, Deng YJ, Lu JX. Effect of aloe polysaccharide on caspase-3 expression following cerebral ischemia and reperfusion injury in rats. Mol Med Rep 2012;6:371–4.
24. Anilakumar KR, Sudarshanakrishna KR, Chandramohan G, Ilaiyaraja N, Khanum F, Bawa AS. Effect of Aloe vera gel extract on antioxidant enzymes and azoxymethane-induced oxidative stress in rats. Indian J Exp Biol 2010;48:837–42.
25. Kiraz HA, Poyraz F, Kip G, Erdem Ö, Alkan M, Arslan M, et al. The effect of levosimendan on myocardial ischemia-reperfusion injury in streptozotocin-induced diabetic rats. Libyan J Med 2015;10:29269.
26. Eshun K, He Q. Aloe vera: a valuable ingredient for the food, pharmaceutical and cosmetic industries-a review. Crit Rev Food Sci Nutr 2004;44:91–6.
27. Guven M, Gölge UH, Aslan E, Sehitoglu MH, Aras AB, Akman T, et al. The effect of aloe vera on ischemia-Reperfusion injury of sciatic nerve in rats. Biomed Pharmacother 2016;79:201–7.
28. Nahar T, Uddin B, Hossain S, Sikder AM, Ahmed S. Aloe vera gel protects liver from oxidative stress-induced damage in experimental rat model. J Complement Integr Med 2013;10.
29. Iftkhar A, Hasan IJ, Sarfraz M, Jafri L, Ashraf MA. Nephroprotective Effect of the Leaves of Aloe barbadensis (Aloe Vera) against Toxicity Induced by Diclofenac Sodium in Albino Rabbits. West Indian Med J 2015;64:462–7.
30. Rajasekaran S, Sivagnanam K, Subramanian S. Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. Pharmacol Rep 2005;57:90–6.
31. Kim SH, Cheon HJ, Yun N, Oh ST, Shin E, Shim KS, et al. Protective effect of a mixture of Aloe vera and Silybum marianum against carbon tetrachloride-induced acute hepatotoxicity and liver fibrosis. J Pharmacol Sci 2009;109:119–27.
32. Czerska M, Mikołajewska K, Zieliński M, Gromadzińska J, Wąsowicz W. Today's oxidative stress markers. Med Pr 2015;66:393–405.