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# The effects of alpha-lipoic acid (ALA) on the urinary bladder injury in rats exposed to chronic stress: A histochemical study

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ABSTRACT

Objective: In the present study, we aimed to investigate the morphological and biochemical effects of alpha-lipoic acid (ALA) on bladder injury caused by water avoidance stress (WAS) and to show its effect on the number of degranulated mast cells, which increase after stress.

Materials and Methods: Wistar albino rats were subjected to WAS and the animals in the treatment group were injected ALA. After the urinary bladder tissues were subjected to routine tissue processing, hematoxylin-eosin staining and periodic acid-Schiff reaction were applied to observe general morphology and acidic toluidine blue method to investigate mast cells. Biochemical assessments of malondialdehyde (MDA) and glutathione (GSH) were also obtained. Transmission electron microscope was used for the ultrastructural, and scanning electron microscope for the topographical analyses.

Results: The experiments showed that chronic stress caused injury in the bladder, increased degranulated and total number of mast cells and decreased GSH and increased MDA levels. ALA treatment after WAS ameliorated bladder injury in most areas, decreased degranulated and total mast cell number and increased GSH and decreased MDA levels.

Conclusion: It was concluded that ALA can be a useful agent in the treatment of interstitial cystitis.

Keywords: Alpha-lipoic acid (ALA), Interstitial cystitis, Mast cell, Stress, Urinary bladder

## 1. INTRODUCTION

Interstitial cystitis (IC) is a symptom syndrome complex characterized by persistent pain and urinary frequency and/or urgency [1, 2]. Bacterial and viral infections, hormonal and neuropsychologic disorders, allergy and immune disorders related to collagen diseases may cause IC, however, exact reasons are not well understood [3, 4].

Physical and psychological stress affect negatively urinary bladder epithelium [5]. One of the mechanisms related to stress is mast cell activation, which is a characteristic of IC [6]. Studies have reported that mast cells were significantly increased and associated with bladder pain and inflammation in patients with IC and in animal models [7]. Stress causes release of corticotropin-releasing hormone, which is a potent activator of mast cell degranulation. The inflammation effect of stress shows itself in the urinary bladder as damage. Being exposed to various stress conditions causes urothelial damage and increase in the number of mast cells [8, 9]. Mast cells in the urinary bladder and intestines are localized very close to the neurons including neuropeptides and neurotransmitters. In the presence of urothelial damage and factors causing bladder inflammation, nerves release various neuropeptides when stimulated. These neuropeptides cause inflammation. In the pathophysiology of IC, mechanisms such as release of neuropeptides from mast cells play a role [10, 11].

Mast cells are located in the connective tissue in humans and rats [12]. The etiology of IC is not well understood; however, mast cells were reported to play a role in inflammation and pain. Mast cells act as modulators of nociceptive neurons via release of histamine, serotonin, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6 [13].

Electron microscopic studies showed that mast cell activation was present and they were localized around neuronal

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projections in IC [11]. The characteristic of mast cells is that they contain dense granules in their cytoplasm. Granules show metachromasia when stained with toluidine blue. Mast cells are primarily localized around the blood vessels, nerves, gland ducts, and epithelial serous and synovial membranes surrounded by loose connective tissue. Mucosal mast cells are found in the urinary bladder and gastrointestinal system, and connective tissue mast cells in the lung and skin [14]. Being exposed to various stress conditions causes urothelial damage, leading to increase in the number of mast cells. Increased mast cell activation causes release of inflammatory mediators such as histamine, proteases, prostaglandins, leukotrienes, and cytokines. Mast cells play a role in inflammatory diseases and allergic reactions [15].

Alpha-lipoic acid (ALA) or thioctic acid is a natural organic sulphur compound and a ketoacid dehydrogenase mitochondrial complex that can be produced by plants, animals and humans [16]. ALA and its metabolites function as antioxidants [10, 17]. It was reported that ALA was used as a therapeutic agent in diseases such as diabetic neuropathy and stroke [18]. It was also reported that it played a neuroprotective role in Parkinson's disease [19]. ALA and dihydrolipoic acid (DHLA) was shown to prevent oxidative stress by repressing reactive oxygen species [20]. ALA and DHLA play a role in regeneration of endogen and exogen antioxidants such as vitamin C and E, and glutathione (GSH), chelation of metal ions, and in repair of oxidized proteins [21, 22].

In the present study, we aimed to investigate the effects of ALA on bladder damage caused by chronic water avoidance stress (WAS), morphologically and biochemically, and to show the effect of WAS on mast cell number and morphology.

## 2. MATERIALS and METHODS

Female Wistar albino rats (250-300 g) were used in the present study. Animals were obtained from Marmara University, The Experimental Animal Implementation and Research Center. All experiments were approved by the Marmara University Local Ethical Committee for Experimental Animals (approval number 03.2009.mar). The animals were housed with free access to water and food in a 12-h light/dark cycle and humidity controlled room (21±2°C and 65-70% humidity). For WAS, a platform having a size of 8x8 cm was placed in a water container with a diameter of 90 cm and a depth of 50 cm. The platform was placed 1 cm above the water and the animals were placed on that platform.

Experimental groups were as follows:

1. Control group (n=10): Tissues were obtained from this group of animals without any procedure.

ALA group (n=10): This group of animals were given 25 mg/kg ALA (i.p.) for 5 days, once a day, on the same hours with the other groups.

2. WAS group (n=10): This group of animals were exposed to WAS for 5 days on the same hours with the other groups for the same duration (2 h).

3. WAS+ALA group (n=10): This group of animals were exposed to WAS and immediately, 25 mg/kg ALA (i.p.) was injected once a day.

4. Animals were decapitated after 5 days and bladder tissues were obtained and fixed in 10% formaldehyde for 24 h for light microscopic observation. For malondialdehyde (MDA) and GSH measurements, tissues were kept at – 20°C until biochemical evaluations were performed.

### *Light Microscopy*

After fixation, tissues were dehydrated in increasing series of ethanol and cleared in toluene. The tissues were incubated in 60°C incubator in liquid paraffin and then embedded. Five-micron-thick sections were obtained on a rotary microtome. Sections were stained with hematoxylin and eosin for demonstrating bladder morphology and with periodic acid Schiff (PAS) reaction for observing glycosaminoglycan (GAG) layer.

Serial sections were obtained and stained with toluidine blue to examine mast cell morphology and number. Granulated and degranulated mast cells were counted separately in randomly selected 10 areas under x40 objective of an Olympus BX51 photomicroscope (Olympus, Tokyo, Japan) with the aid of a metric ocular.

### *Transmission Electron Microscopy*

Bladdder tissues were fixed in 2.5% glutaraldehyde and then postfixed in 1% osmium tetroxide for 1 h. After dehydrating in increasing series of ethanol, tissues were cleared in propylene oxide and incubated in propylene oxide/epon (1:1) mixture overnight. On the next day, tissues were incubated in pure epon, embedded in epon, and then polymerized in 60°C incubator overnight. Semi-thin sections were obtained by an ultramicrotome (Leica Ultracut R) and stained with toluidine blue for proper orientation. Thin sections were obtained on grids coated with Coat-Quick 'G' pen. Sections were contrasted with uranyl acetate and lead citrate, and examined and photographed under an SIS Morada CCD camera (Olympus, Tokyo, Japan) attached JEOL-JEM-1200EX II transmission electron microscope (JEOL, Tokyo, Japan).

### *Scanning Electron Microscopy*

Bladdder tissues were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide for 1 h, dehydrated in increasing series of ethanol, incubated in amyl acetate, and then dried in critical point dryer (BIO-RAD E3000). Tissues were coated with gold in a gold coating instrument (BIO-RAD-SC502) and examined and photographed under a JEOL JSM-5200 scanning electron microscope (JEOL, Tokyo, Japan).

### *Biochemical Evaluations*

### *Malondialdehyde (MDA) Measurement*

Amount of MDA, an indicator of lipid peroxidation, was determined by Beuge method [23]. After decapitation, bladder tissues were washed in saline, dried and weighed. 10% bladder homogenate was prepared on ice in Ika Werk homogenizator with 150 mM KCl solution. Thiobarbituric acid (1 ml, 0.375%) was added onto the homogenate, left in boiled water bath, and the tubes were cooled at room temperature. After centrifugating (Hettich Universal) at 3000 cycles/min, top phase were taken and absorbance of the resulting color was determined in a spectrophotometer at 532 nm.

### *Glutathione (GSH) Measurement*

Glutathione measurement was done according to Ellman method [24]. 10% homogenates, which were prepared for MDA measurement, were used in GSH measurement. 0.4 ml 10% homogenate was mixed with 0.2 ml 20% thiobarbituric acid homogenate, and centrifuged (Hettich Universal) at 3000 cycles/min. GSH was examined at top phase, and the precipitate was thrown away. 1 ml 0.3 M $Na_2HPO_4$+0.05 ml Ellman solution was added and mixed, and incubated for 5 min. Absorbance of the resulting color was determined in a spectrophotometer at 412 nm.

### Statistical Analysis

Data were interpreted as mean±S.E.M. and evaluated with One-Way ANOVA and Tukey-Kramer multiple comparison tests. Significance level was determined as $p<0.05$.

## 3. RESULTS

### *General Morphology*

Mucosa was in normal appearance in control (Figure 1a) and ALA (Figure 1b) groups, and epithelium was intact. There was severe epithelial damage and it was observed that epithelial cells shed into the lumen in WAS group (Figure 1c). Although epithelial damage was evident in some areas in WAS+ALA group, the epithelium was generally intact (Figure 1d).

GAG layer was intact in control (Figure 1e) and ALA (Figure 1f) groups. Disorganized GAG layer and epithelial damage was observed in WAS group and leukocytes which migrated to this region were seen (Figure 1g). Although there was disorganization in GAG layer in some regions, this layer was generally intact in WAS+ALA group (Figure 1h).

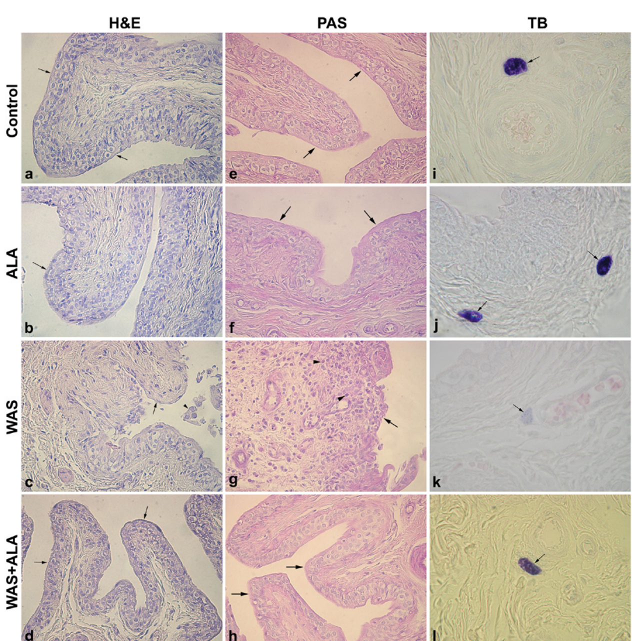


***Figure 1.*** *Normal appearance of epithelium (arrows) in control (a) and ALA (b) groups. (c) Shed epithelium (arrow) and residual cells (arrowhead) in the lumen in WAS group. (d) Normal appearance of epithelium in WAS+ALA group (arrows). Normal appearing GAG layer (arrows) in control (e) and ALA (f) groups. (g) Shed epithelium in the lumen and damaged GAG layer (arrow), and inflammatory cell infiltration (arrowhead) in WAS group. (h) Normal appearance of GAG layer (arrows) in WAS+ALA group. (i) Granulated mast cell (arrow) around a blood vessel in control group. (j) Granulated mast cells (arrows) in the mucosal layer in ALA group. (k) Regranulated mast cell (arrow) around a blood vessel in the mucosal layer in WAS group. (l) Granulated mast cell (arrow) around a blood vessel in WAS+ALA group. (a-d) Hematoxylin and eosin (H&E) staining. (e-h) Periodic acid-Schiff (PAS) reaction. (i-l) Toluidine blue staining. Original magnification: x400.*

### *Mast cell Morphology*

Although, some granulated mast cells were observed in mucosal regions of bladder sections in control and ALA groups, mast cells were generally granulated in these groups (Figure 1i, 1j). Besides granulated and degranulated mast cells in mucosa and near the smooth muscle in WAS group, regranulated mast cells were also observed (Figure 1k). Granulated mast cells and a few degranulated mast cells were seen in WAS+ALA group (Figure 1l).

Mast cells were generally granulated in semi-thin sections in mucosal regions of control (Figure 2a) and ALA (Figure 2b) groups. Granulated and degranulated mast cells were seen both in mucosa and near the smooth muscle in WAS group (Figure 2c). Mast cells were generally located near the blood vessels. Many mast cells were observed to migrate to the epithelium and degranulated in this region in WAS group. Besides granulated mast cells in mucosal region, a few degranulated cells were also observed in WAS+ALA group (Figure 2d).


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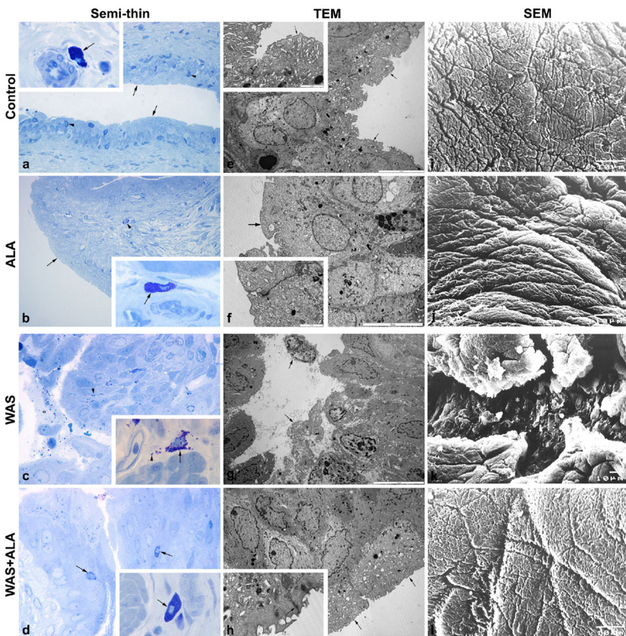


*Figure 2. (a) Normal epithelial layer (arrows) and granulated mast cells (arrowhead) in the epithelium in control group. Inset: Granulated mast cell (arrow). (b) Normal epithelial layer (arrow) and granulated mast cell (arrow) in ALA group. Inset: Granulated mast cell (arrow). (c) Epithelial cell remnants (asterisk) in the lumen and released mast cell granules (arrowhead) in WAS group. Inset: Released mast cell granules (arrowhead) from a degranulated mast cell (arrow) in the mucosa. (d) Granulated mast cells (arrows) in the mucosa in WAS+ALA group. Inset: Granulated mast cell (arrow). (e) Normal urothelium (arrows) in control group. Inset: Normal urothelium (arrow) and normal appearing tight junction (arrowhead). (f) Normal urothelium (arrow) and granulated mast cell (m) in the epithelium in ALA group. Inset: Normal appearing tight junction (arrow). (g) Epithelial cells shed into the lumen (arrows) in WAS group. (h) Normal urothelium (arrows) in WAS+ALA group. Inset: Normal appearing tight junction (arrow). Normal appearing urothelium in control (i) and ALA (j) groups. (k) Epithelial cells were shed into the lumen (asterisk) in WAS group. (l) Normal appearing urothelium in WAS+ALA group. (a-d) Toluidine blue stained semi-thin sections. Original magnification: x400, insets: x1000. (e-h) TEM imaging. Bars: 10 μm, insets: 2 μm. (i-l) SEM micrographs. Bars: 10 μm.*

### *Transmission Electron Microscopy*

In semi-thin sections of control (Figure 2e) and ALA (Figure 2f) groups, epithelium was normal and intact. Tight junctions were also intact and fusiform vesicles were normal in appearance in both goups. A few mast cells were present near the blood vessels. Severe epithelial degeneration was seen in WAS group (Figure 2g). Epithelial cells shed into lumen, intercellular junctions were opened, and there were vacuoles in the cells. The number of fusiform vesicles was decreased. Besides epithelial damage in some regions, intercellular openings, and degranulated mast cells in the epithelium, there was generally an amelioration of these findings in WAS+ALA group (Figure 2h). Tight junctions were intact in this group.

### *Scanning Electron Microscopy*

Urothelial surface was normal in control (Figure 2i) and ALA (Figure 2j) groups. There was severe epithelial shedding in WAS group (Figure 2k). Besides epithelial shedding in some regions, most regions appeared normal in WAS+ALA group (Figure 2l) compared to WAS group.

### *Mast Cell Number*

The number of granulated and degranulated mast cells in the mucosal layer of control and ALA groups was similar (Figure 3a). The number of granulated ($p<0.05$) and degranulated ($p<0.01$) mast cells was increased compared to control group. The number of granulated and degranulated mast cells was decreased in WAS+ALA group compared to WAS group ($p<0.001$).

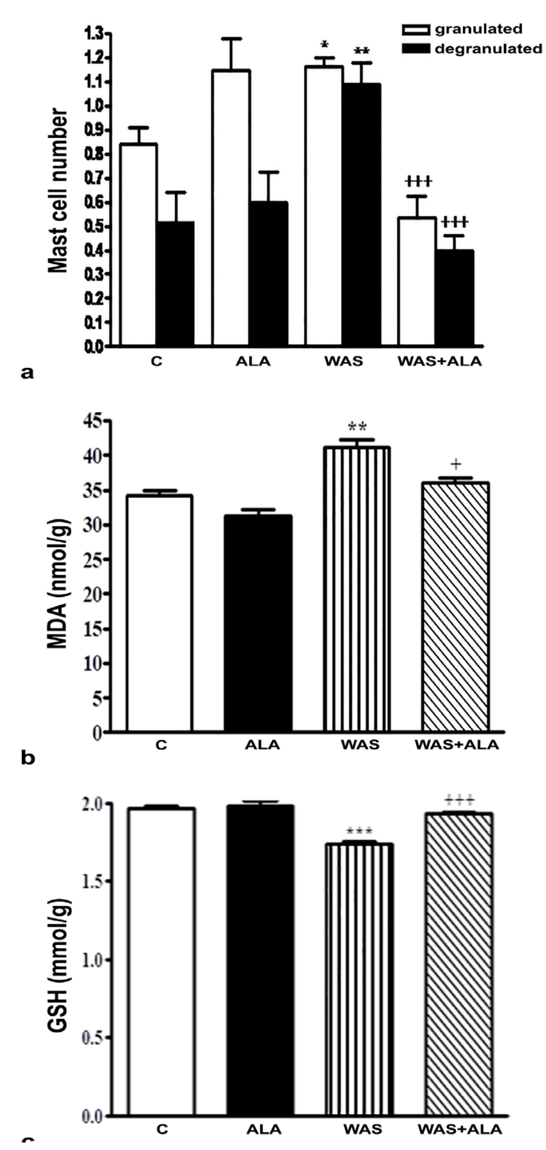


*Figure 3. Statistical analysis. (a) Mast cell number. *p<0.05, **p<0.01; compared to control group. +++p<0.001; compared to WAS group. (b) MDA analysis. **p<0.01; compared to control groups. +p<0.05; compared to WAS group. (c) GSH analysis. ***p<0.001; compared to control groups. +++p<0.001, compared to WAS group.*



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### *MDA Measurement*

In the present study, WAS caused lipid peroxidation in the bladder tissue and MDA levels, which is an indicator of lipid peroxidation, was increased compared to the control groups (Figure 3b, $p<0.01$). MDA levels in WAS+ALA group was decreased compared to the WAS group ($p<0.05$).

### *GSH Measurement*

Glutathione was decreased in WAS group compared to control groups (Figure 3c, $p<0.001$). GSH level was increased in WAS+ALA group compared to WAS group ($p<0.001$). Values of control and ALA groups were similar.

## 4. DISCUSSION

In the present study, ALA, a potent antioxidant, was used in rats exposed to WAS, and was observed to have ameliorating effects on bladder tissue. WAS is a model of physical and psychological stress and accepted as moderate stress [25-27].

WAS is a life stress causing psychological effects. It was reported that WAS applied for 10 days caused increased anxiety and pain in rats [28]. Studies have shown that repeated psychological stress in rodents induced bladder dysfunction [29]. In the studies using WAS model, bladder epithelium was observed to be injured and epithelial morphology was changed [26, 30]. It was also observed that WAS induced bladder overactivity in mice [31]. WAS mimics features of urinary frequency and bladder hyperalgesia seen in patients with IC [32]. In the present study, WAS was applied for 5 days in the rats. As a result, in line with the previous studies, bladder epithelium was injured. This injury demonstrated itself as opening of intercellular junctions, shedding of epithelial cells into the lumen, and decrease in the number of fusiform vesicles.

Injury in the GAG layer causes activation of the submucosal sensory neurons and pain [10]. Proinflammatory mediators are released due to triggering of substance P release. As a result, damage in the surface urothelial cells occur and epithelial cells are shed into the lumen. In the present study, GAG layer damage was demonstrated by PAS reaction, and in light and electron microscopic examinations, opening of intercellular junctions and epithelial cells in the lumen was observed in WAS group.

Clinical and laboratory studies proved that mast cells play a central role in the pathophysiology of IC [33]. Mast cell increase and activation, and prevention of this increase by medical treatment and deactivation of proinflammatory mediators was observed in patients with IC. Mast cells in smooth muscle and mucosal layers of urinary bladder in these patients were increased [34]. Mast cells are activated by a series of mechanisms in the bladder wall. Epithelial permeability increase due to potassium ion flow may cause mast cell activation. It was reported that there was an anatomical and functional relation between mast cells and neurons. Scanning electron microscopic studies showed that mast cells were located in the neighborhood of endothelial cells and neuronal projections [35, 36]. In human studies and animal models, a series of free neuropeptides and neurotransmitters were shown activating submucosal nerves and mast cells in the epithelium. Neuroinflammatory and neuroendocrine alterations in the bladder of patients with IC contribute to pain symptoms and frequent urination in these patients [33]. The inflammatory progression of IC was found to be related to the expression levels of inflammatory cytokines such as IL-6, IL-17, and IL-1β, and also related to mast cell activation [6, 37, 38]. In the present study, only mucosal mast cells were counted and mast cells in the smooth muscle layer were not included. Degranulated mast cells were increased in number in WAS group. Degranulated cells were observed in smooth muscle layer, although quantitative analysis was not performed. Antioxidant ALA treatment was observed to repress degranulation.

Alpha-lipoic acid might be useful clinically in the treatment of IC [39]. Urothelial expression of fractalkine and its receptor was reported to be increased in chronic cystitis model in mouse [40]. Fractalkine is a protein belonging to cytokine family [41]. Fractalkine receptors were increased in cytitis caused by cyclophosphamide in rat [40]. Because the cystitis caused by cyclophosphamide exposure corresponds to IC in humans, reducing fractalkine in the treatment of this clinical situation by ALA treatment was accepted as a potential target [40, 42]. Two hypotheses were suggested in the mechanism of effect of ALA. The first one is that ALA is a potential agent reducing fractalkine mRNA and its protein expression [43]. ALA also decreases fractalkine mediated inflammatory processes. The second hypothesis is that ALA has the capacity to inhibit TNF-α induced fractalkine expression.

In chronic cystitis models in rat, a prominent decrease in the antioxidant defense parameters was observed. Harmful effects of oxidative stress were reversed by powerful antioxidant effects of ALA in inflamed bladder [42]. It was also reported that ALA might prevent contractile function disorder in bladder tissue *in vitro* [44]. It was suggested that ALA might be used as a new strategy in treatment of IC [42]. In the present study, it was observed that bladder injury findings due to oxidative stress decreased by ALA treatment; therefore, similar to the above studies, we suggest it should be supported by clinical studies so that ALA can be used as a therapeutic agent.

Previous studies have shown that increased free oxygen radicals and lipid peroxidation played a role in the pathogenesis of many diseases [45]. In chronic WAS studies, increased MDA levels and decreased GSH levels were observed [46]. Similarly, we demonstrated significantly increased MDA levels in the bladder tissue of stress model in rat, which is a sign of lipid peroxidation; and in parallel with this finding, we observed significantly decreased GSH levels. ALA treatment decreased MDA levels and increased GSH levels. These findings indicate that ALA decreases oxidative stress in the bladder in IC model [47]. ALA was shown previously to be an antioxidant in the bladder tissue [48].

## 5. CONCLUSION

In conclusion, in the present study, it was shown that bladder mucosa injury and mast cell degranulation, related to oxidative stress, in WAS model in rat were reversed by ALA tratment.


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We suggest that these findings should be supported by clinical studies and ALA should be tried as a therapeutic agent in the treatment of IC.

### Compliance with Ethical Standards

**Ethical Approval:** All experiments were done according to the National Guidelines on Animal Experimentation and were approved by the Marmara University Local Ethical Committee for Experimental Animals (approval number 03.2009.mar).

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**Conflict of Interest:** The authors declare that there are no conflicts of interest.

**Author Contributions:** NY and EC: did the experiments, HZT: did the biochemical analysis and critical revision of the article, SS: analyzed the data and wrote the article. All authors approved the final version of the article.

## REFERENCES

[1] Akiyama Y, Hanno P. Phenotyping of interstitial cystitis/bladder pain syndrome. Int J Urol 2019; 26(Suppl 1):17-19. doi: 10.1111/iju.13969.

[2] Akiyama Y, Luo Y, Hanno PM, Maeda D, Homma Y. Interstitial cystitis/bladder pain syndrome: The evolving landscape, animal models and future perspectives. Int J Urol 2020; 27(6):491-503. doi: 10.1111/iju.14229.

[3] Koziol JA, Clark DC, Gittes RF, Tan EM. The natural history of interstitial cystitis: a survey of 374 patients. J Urol 1993; 149:465-9. doi: 10.1016/s0022-5347(17)36120-7.

[4] Sant GR. Interstitial cystitis. Curr Opin Obst Gynecol 1997; 9:332-6.

[5] Atuğ F, Canoruç N. İnterstisyel sistit: Kronik pelvik ağrı sendromu. Dicle Tıp Dergisi 2005; 32:204-10.

[6] Logadottir Y, Delbro D, Fall M, Gjertsson I, Jirholt P, Lindholm C, Peeker R. Cytokine expression in patients with bladder pain syndrome/interstitial cystitis ESSIC type 3C. J Urol 2014; 192:1564-8. doi: 10.1016/j.juro.2014.04.099.

[7] Kochiashvili G, Kochiashvili D. Urinary IL-33 and galectin-3 increase in patients with interstitial cystitis/bladder pain syndrome. Georgian Med News 2014; 232-233:12-5.

[8] Fall M, Johansson SL, Aldenborg F. Chronic interstitial cystitis: A heterogeneous syndrome. J Urol 1987; 137:35-8. doi: 10.1016/s0022-5347(17)43863-8.

[9] Kandere-Grzybowska K, Gheorghe D, Priller J, Esposito P, Huang M, Gerard N, Theoharides TC. Stress-induced dura vascular permeability does not develop in mast cell-deficient and neurokinin-1 receptor knockout mice. Brain Res 2003; 980:213-20. doi: 10.1016/s0006-8993(03)02975-5.

[10] Jasmin L, Janni G, Ohara PT, Rabkin SD. CNS induced neurogenic cystitis is associated with bladder mast cell degranulation in the rat. J Urol 2000; 164:852-5. doi: 10.1097/00005.392.200009010-00061.

[11] Letourneau R, Pang X, Sant GR, Theoharides TC. Intragranular activation of bladder mast cells and their association with nerve processes in interstitial cystitis. Br J Urol 1996; 77:41-54. doi: 10.1046/j.1464-410x.1996.08178.x.

[12] Ercan F, Çetinel Ş. Mast hücrelerinin enflamasyondaki rolü: insan ve deneysel hayvan modelleri üzerinde yapılan çalışmaların değerlendirilmesi. Marmara Medical Journal 2008; 21:179-86.

[13] Chatterjea D, Martinov T. Mast cells: versatile gatekeepers of pain. Mol Immunol 2015; 63:38-44. doi: 10.1016/j.molimm.2014.03.001.

[14] Parsons CL, Lilly JD, Stein P. Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis). J Urol 1991; 145:732-5. doi: 10.1016/s0022-5347(17)38437-9.

[15] Ercan F, Akıcı A, Ersoy Y, Hürdag C, Erin N. Inhibition of substance P activity prevents stress-induced bladder damage. Regul Pept 2006; 133:82-9. doi: 10.1016/j.regpep.2005.09.024.

[16] Salehi B, Berkay Yılmaz Y, Antika G, Boyunegmez Tumer T, Fawzi Mahomoodally M, Lobine D, Akram M, Riaz M, Capanoglu E, Sharopov F, Martins N, Cho WC, Sharifi-Rad J. Insights on the use of α – lipoic acid for therapeutic purposes. Biomolecules 2019; 9:356. doi: 10.3390/biom9080356.

[17] Senoglu M, Nacitarhan V, Kurutas EB, Senoglu N, Altun İ, Atli Y, Ozbag D. Intraperitoneal alpha-lipoic acid to prevent neural damage after crush injury to the rat sciatic nerve. J Brachial Plex Peripher Nerve Inj 2009; 4:22. doi: 10.1186/1749-7221-4-22.

[18] Ghibu S, Lauzier B, Delemasure S, Amoureux S, Sicard P, Vergely C, Muresan A, Mogosan C, Rochette L. Antioxidant properties of alpha-lipoic acid: effects on red blood membrane permeability and adaptation of isolated rat heart to reversible ischemia. Mol Cell Biochem 2009; 320:141-8. doi: 10.1007/s11010.008.9916-0.

[19] Zhang J, Wang M, Zhao Y, Zhang Y, Gao Y, Zhang X, Yang G. Alpha-lipoic acid improved motor function in MPTP-induced Parkinsonian mice by reducing neuroinflammation in the nigral and spinal cord. Neurosci Lett 2022; 781:136669. doi: 10.1016/j.neulet.2022.136669.

[20] Jiang Y, Gong D, Liu H, Yang C, Sun Z, Kong C. Ability of alpha-lipoic acid to reverse the diabetic cystopathy in a rat model. Acta Pharmacol Sin 2008; 29(6):713-9. doi: 10.1111/j.1745-7254.2008.00790.x.

[21] Biewenga GP, Haene GR, Bast A. The pharmacology of the antioxidant lipoic acid. Gen Pharmacol 1997; 29:315-31. doi: 10.1016/s0306-3623(96)00474-0.

[22] Packer L. α-Lipoic acid: metabolic antioxidant which regulates NF-κB signal transduction and protects against oxidative injury. Drug Metab 1998; 30:245-75. doi: 10.3109/036.025.39808996311.

[23] Beuge JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978; 52:302-11. doi: 10.1016/s0076-6879(78)52032-6.

[24] Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882-8.

[25] Cikler E, Ercan F, Çetinel S, Contuk G, Sener G. The protective effects of melatonin against water avoidance stress-induced mast cell degranulation in dermis. Acta Histochem 2005; 106:467-75. doi: 10.1016/j.acthis.2004.10.001.

[26] Cikler E, Ersoy Y, Çetinel Ş, Ercan F. The leukotriene D4 receptor antagonist, montelukast, inhibits mast cell degranulation in the dermis induced by water avoidance stress. Acta Histochem 2009; 111:112-8. doi: 10.1016/j.acthis.2008.04.006.

[27] Çetinel Ş, Ercan F, Conturk G, Sener G. Protective effect of melatonin on water avoidance stress induced degeneration of the bladder. J Urol 2005; 173:267-70. doi: 10.1097/01.ju.000.014.5891.35810.56.

[28] Robbins MT, DeBerry J, Ness TJ. Chronic psychological stress enhances nociceptive processing in the urinary bladder in high-anxiety rats. Physiol Behav 2008; 91:544-50. doi: 10.1016/j.physbeh.2007.04.009.

[29] West EG, Sellers DJ, Chess-Williams R, McDermott C. Bladder overactivity induced by psychological stress in female mice is associated with enhanced bladder contractility. Life Sci 2021; 265:118735. doi: 10.1016/j.lfs.2020.118735.

[30] Zeybek A, Ercan F, Cetinel S, Cikler E, Sağlam B, Sener G. Taurine ameliorates stress-induced degenerations of gastrointestinal tract and liver. Dig Dis Sci 2007; 51:1853-61. doi: 10.1007/s10620.006.9425-5.

[31] Mills KA, West EG, Sellers DJ, Chess-Williams R, McDermott C. Psychological stress induced bladder overactivity in female mice is associated with enhanced afferent nerve activity. Sci Rep 2021; 11:17508. doi: 10.1038/s41598.021.97053-5.

[32] Sanford MT, Yeh JC, Mao JJ, Guo Y, Wang Z, Zhang R, Holschneider DP, Rodriguez LV. Voluntary exercise improves voiding function and bladder hyperalgesia in an animal model of stress-induced visceral hypersensitivity: a multidisciplinary approach to the study of urologic chronic pelvic pain syndrome research network study. Neurourol Urodyn 2020; 39:603-12. doi:10.1002/nau.24270.

[33] Grannum RS, Duraisamy K, James ME, Theoharides TC. The mast cell in interstitial cystitis: Role in pathophysiology and pathogenesis. Urology 2007; 69(4 Suppl.):34-40. doi: 10.1016/j.urology.2006.08.1109.

[34] Kurtoğlu B, Yörükoğlu K, Sevinç M, Kırkalı Z. İnterstisyel sistitin patogenezi üzerine histokimyasal ve immunohistokimyasal bir çalışma. Turgut Özal Tıp Merkezi Dergisi 1998; 5:126-32.

[35] Theoharides TC, Kempuraj D, Sant GR. Mast cell involvement in interstitial cystitis: A review of human and experimental evidence. Urology 2001; 57(6 Suppl. 1): 47-55. doi: 10.1016/s0090-4295(01)01129-3.

[36] Theoharides TC. Mast cells and stress – a psychoneuroimmunological perspective. J Clin Psychopharmacol 2002; 22:103-6. doi: 10.1097/00004.714.200204000-00001.

[37] Grover S, Srivastava A, Lee R, Tewari AK, Te AE. Role of inflammation in bladder function and interstitial cystitis. Ther Adv Urol 2011; 3:19-33. doi: 10.1177/175.628.7211398255.

[38] Offiah I, Didangelos A, Dawes J, Cartwright R, Khullar V, Bradbury EJ, O'Sullivan S, Williams D, Chessell IP, Pallas K, Graham G, O'Reilly BA, McMahon SB. The expression of inflammatory mediators in bladder pain syndrome. Eur Urol 2016; 70:283-90. doi: 10.1016/j.eururo.2016.02.058.

[39] Pastuszka A, Slusarczyk K, Koszutski T, Kawalski H, Kudela G. Alpha-lipoic acid may be a clinically useful therapy in interstitial cystitis. Med Hypotheses 2007; 69:957-65. doi: 10.1016/j.mehy.2007.01.058.

[40] Yuridullah R, Corrow KA, Malley SE, Vizzard MA. Expression of fractalkine and fractalkine receptor in urinary bladder after cyclophosphamide (CYP)-induced cystitis. Auton Neurosci 2006; 126-127:380-9. doi: 10.1016/j.autneu.2006.02.030.

[41] El-Mansoury M, Boucher W, Sant GR, Theoharides TC. Increased urine histamine and methylhistamine in interstitial cystitis. J Urol 1994; 152:350-53. doi: 10.1016/s0022-5347(17)32737-4.

[42] Bilska A, Wlodek L. Lipoic acid – the drug of the future? Pharmacol Rep 2005; 57:570-7.

[43] Sung MJ, Kim W, Ahn SY, Cho CH, Koh GY, Moon SO. Protective effect of alpha lipoic acid in lipopolysaccharide induced endothelial fractalkine expression. Circ Res 2005; 97:880-90. doi: 10.1161/01.RES.000.018.6522.89544.4D.

[44] Levin RM, Danek M, Whitbeck C, Haugaard N. Effect of ethanol on the response of the rat urinary bladder to in vitro ischemia: protective effect of alpha-lipoic acid. Mol Cell Biochem 2005; 271:133-41. doi: 10.1007/s11010.005.5870-2.

[45] Altan N, Dinçel AS, Koca C. Diabetes mellitus ve oksidatif stress. Turk J Biochem 2006; 31:51-6.

[46] Paris M, Kidd PhD. Glutathione: systemic protectant against oxidative and free radical damage. Alt Med Rev 1997; 2:155-76.

[47] Busse E, Zimmer G, Schopohl B. Influence of alpha-lipoic acid on intracellular glutathione in vitro and in vivo. Arzneimittelforschung 1992; 42:829-31.

[48] Ekiz A, Özdemir-Kumral ZN, Erşahin M, Tuğtepe H, Velioğlu Öğünç A, Akakın D, Kıran D, Özsavcı D, Biber N, Hakan T, Çağlayan Yeğen B, Şener G, Toklu HZ. Functional and structural changes of the urinary bladder following spinal cord injury; treatment with alpha lipoic acid. Neurourol Urodyn 2017; 36:1061-8. doi: 10.1002/nau.23083.


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