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# Protective effect of *Asphodelus aestivus* Brot. on bladder injury due to ischemia-reperfusion: Biochemical and histopathological Study

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Severe oxidative stress caused by reperfusion after ischemia in the bladder causes serious functional and structural damage. New molecules with antioxidant effects are needed to protect against this damage. In our study, we aimed to investigate the effect of *Asphodelus aestivus* on the oxidant-antioxidant system in bladder ischemia/reperfusion (I/R) injury induced in rats biochemically and histopathologically. In our study, 24 three-month-old male Wistar-Albino rats weighing 250-300 g were randomly divided into 3 groups of 8 rats each: sham group (I/R+serum physiological, 1mL) (group 1), I/R group (group 2), I/R+*A. aestivus* group (50 mg/kg/day) (treatment group, group 3). At the end of the experiment, all rats were sacrificed and their bladder tissues were taken and divided into 2 parts for biochemical and histopathological examination. Protein amount, malondialdehyde (MDA) levels and superoxide dismutase (SOD) and catalase (CAT) enzyme activity levels were measured spectrophotometrically, and histopathological examination was performed under a light microscope. MDA levels were significantly higher and CAT activity was significantly lower in the I/R group compared to the sham and treatment groups ($P$<0.05). In histopathological examination, it was determined that there was a significant regression and/or decrease in the findings caused by I/R injury in the group treated with *A. aestivus*. *A. aestivus* has a protective effect in reducing oxidative stress in bladder I/R injury.

**Keywords**: Antioxidant, Catalase, Malondialdehyde, Oxidative stress, Superoxide dismutase

Lower urinary tract symptoms (LUTS) are common in both men and women, especially as they age[1]. Bladder I/R is a cause of LUTS that contributes to age-related structural and functional changes of the bladder[2]. Bladder ischemia and subsequent reperfusion are observed in age-related disorders such as urinary retention, atherosclerosis, vasospasm, embolism, and thrombosis[3]. Ischemia is a pathophysiological condition that occurs when blood flow in tissues or organs is reduced[4]. Although all tissues can withstand short-term ischemia, cell damage and/or death occur after a critical period of ischemia, depending on the cell type and organ[5]. Restoring blood flow through the reperfusion process in structures that have not received blood for a long time causes organ damage by causing accumulation of reactive species such as hydroxyl radical ($OH^{\bullet}$), superoxide anion radical ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydrogen peroxide ($H_2O_2$) and nitric oxide (NO), oxidative stress and an increase in inflammation[6].

In a situation such as I/R, oxidative stress occurs when the balance between ROS and antioxidants is disrupted in favour of oxidants due to an increase in ROS or a decrease in antioxidants[7]. Peroxidation is one of the most basic and typical results of oxidative stress. MDA, which can be produced as a secondary product during lipid peroxidation, is an indicator of fatty acid oxidation resulting from the decomposition of arachidonic acid and larger polyunsaturated fatty acids (PUFA) by enzymatic or non-enzymatic means[5,8]. Studies have observed an increase in MDA levels due to bladder I/R damage[9,10].

Effective defence mechanisms that prevent the formation of ROS and the damage they cause, and protect tissues from oxidative damage, are known as "antioxidants"[11]. SOD and CAT are among the endogenous antioxidant enzymes that play a role in preventing the damage caused by ROS[7]. SOD catalyses the dismutation of $O_2^{\bullet-}$ producing $H_2O_2$ and the $O_2$ molecule[12]. CAT breaks down two molecules of $H_2O_2$ into two molecules of water ($H_2O$) and one molecule of $O_2$[13].

Since endogenous antioxidant levels are not sufficient to combat increased ROS to prevent reperfusion injury, the importance of researching exogenous antioxidants is increasing[14]. *A. aestivus*, which grows in the mountains of Eastern and Southeastern Anatolia and Central Anatolia in Turkey, in the pastures of the Eastern Mediterranean region and the Aegean region and is popularly known as "ciris" grass, is a plant species belonging to the *Asphodelaceae* family[15,16]. It can be consumed as a vegetable in Turkish cuisine[15]. It is stated that *A. aestivus* has antimicrobial and antioxidant

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properties in addition to its white blood cell (WBC) enhancing properties[17].

There are studies in the literature investigating the effects of various antioxidant substances against bladder I/R damage[10,18]. In addition, there are also studies analysing the biochemical content of *A. aestivus*[19,20]. However, we did not come across a study in the literature investigating the effect of *A. aestivus* in protecting against bladder I/R damage. In our current study, we aimed to investigate the effects of *A. aestivus* on MDA concentration, SOD and CAT enzyme activity levels in bladder tissue during I/R damage and whether it has a histopathological protective effect.

## Materials and Methods

The study was conducted at Kahramanmaraş Sütçü İmam University Laboratory of Experimental Animals.

### Extract preparation

In this study, extracts prepared with KCl (potassium chloride) of *A. aestivus* leaves collected from the Eastern Mediterranean region of Turkey in March 2019 were used. The plant material's identity was verified by Professor Ali Kaygısız at the Faculty of Agriculture.

### Design of experimental groups and surgical procedure

In our study, all rats were kept at a room temperature of 22±2 ºC with a 12-hour light and 12-hour dark period and fed with standard rat food and water until the time of the experiment. All rats were given 50 mg/kg Ketamine (Ketalar vial, Eczacıbaşı Turkey) intramuscularly as an anesthetic. After anesthesia, median abdominal laparotomy was performed. Then, the bladder was reached and the abdominal aorta was closed with a clamp, the laparotomy incision was closed and a 30-minute ischemia was created. After the ischemia period, the laparotomy incision was reopened, the clamp was removed, and reperfusion was achieved for 30 minutes. Afterwards, the animals of all three groups were sacrificed and their bladder tissues were taken.

Sham group (group 1, n=8): 1 mL physiological saline (0.9% NaCl) was given to the rats once by gavage 24 hours before anesthesia and surgical procedure. Following reperfusion, physiological saline was given again (single dose). I/R group (group 2, n=8): After the anesthesia and surgical procedure applied to the rats, ischemia and reperfusion were applied to the bladder. I/R+*A. aestivus* group (group 3, n=8): 50 mg/kg/day *A. aestivus* extract was given to the rats via gavage once, 24 hours before anesthesia and surgical procedure. After reperfusion, 50 mg/kg/day (single dose) *A. aestivus* extract was given.

At the end of the experiment, the removed bladder tissue was divided into two equal parts, one part was kept for biochemical analysis (at -80 °C) and the other part was kept for histopathological examination (in 10% buffered neutral formaldehyde) until the time of the experiment.

Biochemical evaluations were made in Kahramanmaraş Sütçü İmam University Medical Biochemistry Department, and histopathological evaluations were made in Kahramanmaraş Sütçü İmam University Health Practice and Research Hospital Pathology Department.

### Biochemical analysis

Bladder tissues were homogenised at a ratio of 1/9 (weight/volume) in cold 1.15% KCI on ice with a homogeniser (Ultra Turraks) for 3 minutes at 16,000rpm. Afterwards, the homogenates were centrifuged at 14000rpm for 30 minutes at +4 ºC, and protein and MDA levels and SOD and CAT enzyme activities were measured in the resulting supernatants using spectrophotometric methods (Shimadzu UV-1800).

Protein quantification, MDA level determination, SOD and CAT enzyme activity determination in bladder tissue were performed as described by Lowry *et al.*[21], Ohkawa *et al.*[22], Fridovich[23], Beutler[24], respectively. The data obtained as a result of MDA (nmol/mL), SOD (U/mL) and CAT (U/mL) analysis were given as nmol/mg, U/mg and U/mg, respectively, in proportion to the amount of tissue protein (mg/mL).

### Histopathological evaluation

For histopathological examination, tissues were fixed in 10% buffered neutral formaldehyde solution for 24 hours. All samples were routinely monitored on a tissue tracking device and paraffin blocks were prepared. Serial sections of 5 µm were prepared for each tissue sample from these paraffin blocks with a microtome device and stained with Hematoxylin-Eosin (H&E) dye. Evaluation of the study was performed blindly by the same pathologist. The prepared preparations were subjected to histopathological examination with a light microscope (Olympus BX41). The groups were

evaluated in terms of epithelial desquamation, inflammatory effect, congestion and muscular hypertrophy.

**Statistical analysis**

The data were evaluated using the SPSS (Statistical Package for Social Sciences) 20 program. In the evaluation of our biochemical data, the Non Parametric Kruskal-Wallis test was used for comparison analyses between three groups, and the Mann-Whitney U test was used for comparison analyses between two groups. The results are given as mean±standard deviation (mean±sd.). For both tests, a value of $P<0.05$ was considered statistically significant.

## Results and Discussion

**Biochemical findings**

In the I/R group compared to the sham and treatment groups; MDA levels are statistically significantly higher ($P=0.028$ and $P=0.010$, respectively), while CAT activity is statistically significantly lower ($P=0.006$ and $P=0.003$, respectively). There is no statistically significant difference in MDA levels ($P=0.626$) and CAT activity ($P=0.685$) between the sham and treatment groups. There was no statistically significant result between the groups in terms of SOD activity [between I/R and sham groups ($P=0.273$), between I/R and treatment groups ($P=0.153$) and between sham and treatment groups ($P=0.570$)] (Table 1).

**Histopathological findings**

In the sham group; It was determined that there was a decrease in congestion (Fig. 1A). In the I/R group; Congestion, desquamation of the epithelium, muscular hypertrophy and inflammation (edema) were observed (**Fig.** 1B). In the I/R+*A. aestivus* group, a decrease in congestion, muscular hypertrophy, inflammation and epithelial desquamation was observed (Fig. 1C).

Bladder ischemia occurs in both men and women as a result of decreased blood flow[25,26]. Muscles with persistent overactive contractions, such as the detrusor muscle, trigger oxidative stress by producing excessive free radicals[25]. Chronic ischemic changes due to repeated I/R and atherosclerosis during the micturition cycle can produce ROS, leading to oxidative stress, bladder denervation, and expression of tissue-damaging molecules in the bladder wall[1,27]. This condition may contribute to the development of bladder dysfunction and LUTS by impairing the contraction and relaxation functions of the bladder, causing bladder hyperactivity to progress towards decreased bladder activity[1,18,27]. In a study conducted in rabbits, it was shown that moderate ischemia caused bladder hyperactivity and severe ischemia caused a decrease in bladder activity[28]. Saito *et al.*[29] showed that ischemia did not cause a significant decrease in contractile responses, but reperfusion caused additional damage to rat bladder smooth muscles.

During I/R injury, ROS release increases and free radicals easily react with unsaturated bonds of cholesterol and fatty acids in the cell membrane, causing lipid peroxidation[8,30]. Sezginer *et al.*[31] showed that MDA levels increase in severe partial bladder outlet obstruction. Increases in lipid peroxidation can lead to nerve and smooth muscle membrane damage[32]. In an *in vitro* study by Radu *et al.*[33], significant increases in lipid peroxidation in rabbit bladder smooth muscle were found to be consistent with a significant decrease in bladder contractility following I/R. Lin *et al.*[34] showed in their study on rabbit bladder that the increase in tissue MDA levels reached the highest level, especially 30 min after reperfusion, and MDA content returned to normal levels 2 hours later.

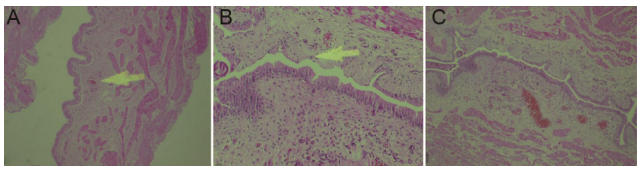


Fig. 1 — Muscular hypertrophy formation in the area under the surface epithelium (A); Epithelial desquamation at the lumen opening (B); Congestion (formation of small vascular structures), inflammation image (C).

Table 1 — MDA, SOD and CAT levels of the groups

| | Sham (Group 1, n=8) | I/R (Group 2, n=8) | I/R+*A. aestivus* (Group 3, n=8) |
|---|---|---|---|
| MDA (nmol/mg) | 0.45±0.11 | 0.71±0.16* | 0.44±0.08** |
| SOD (U/mg) | 3.13±0.91 | 2.34±0.83 | 3.76±1.87 |
| CAT (U/mg) | 0.054±0.009 | 0.012±0.001* | 0.056±0.018** |

[**P*-Compared with the sham group, ***P*-Compared with the I/R group (Mann-Whitney U test). MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase]

Benign prostatic hyperplasia is a common disease in middle-aged and elderly men and can cause acute urinary retention at certain periods of patients' lives. Acute urinary retention can also cause an increase in MDA levels in the bladder tissue[35]. In these patients, a decrease in blood flow (ischemic change) is observed due to the increase in intra-bladder pressure during micturition or the increased pressure of the bladder wall during the urine filling phase, followed by an increase in blood flow and oxygen tension after micturition (reperfusion phase)[1]. In the study of Averbeck *et al.*[7], where the relationship between preoperative parameters and oxidative stress markers in the bladder wall of men who underwent open prostatectomy was evaluated, in men with severe bladder outlet obstruction compared to grade III-IV bladder outlet obstruction (bladder outlet obstruction degree V-VI according to Schaefer[36] nomogram); Compared to patients with mild LUTS, MDA concentration was found to be statistically significantly higher in patients with severe LUTS[7].

The effectiveness of some exogenous antioxidant substances in defence against lipid peroxidation caused by oxidative stress in bladder I/R injury[6,37] and their effects on changes in SOD and CAT levels[9,18] have been examined in various studies. Tissue MDA levels decreased in bladder I/R injury with oxytocin treatment in the study by Erkanli Şentürk *et al*.[6], and with riboflavin and n-acetylcysteine treatment in the study by Ertaş[18]. In the study of Tinay *et al*.[10], with quercetin treatment, which is known for its antioxidant and protective effects, increased MDA levels in the bladder tissue in rat I/R injury decreased and decreased SOD activities increased.

*A. aestivus* is a plant that grows in calcareous soils in agricultural lands, roadsides and pastures in the Mediterranean basin[38]. It is traditionally used among the public in the treatment of many diseases[39]. There are various studies analysing the biochemical content of *A. aestivus*. In the study of Karataş *et al*.[17], the result of analysis by High Performance Liquid Chromatography (HPLC), it has been shown that *A. aestivus* is a very good source of vitamins C and B3, and also contains sufficient amounts of GSH, vitamins B1, B6 and B9. According to the study conducted by Güzel *et al.*[19], *A. aestivus* extracts are a natural source of antioxidants with a moderate free radical inhibitory effect. The biochemical content of *A. aestivus* may also vary as it is obtained from different regions. In the study of Unal *et al*.[38], *A. aestivus* was collected from the eastern and western parts of Tunceli. Vitamin A and vitamin C concentrations in Western Tunceli *A. aestivus* were found to be higher than in eastern samples[38]. In the study of Peksel *et al.*[40], leaves of *A. aestivus,* while the metal chelating and radical scavenging activities of the water extract are higher than the ethanolic extract; the β-carotene bleaching effect, total antioxidant activity and antifungal properties of the ethanol extract were found to be higher than the water extract[41]. In our current study, as a result of our biochemical analysis, MDA levels decreased statistically significantly in the *A. aestivus* treated group compared to the I/R group, while CAT enzyme activity increased statistically significantly. Although there was no significant difference between the groups in terms of SOD activity, the values of the I/R+*A. aestivus* group were above the sham group. Failure to obtain a significant result in SOD activity may be due to many factors. Many factors that affect the effectiveness of *A. aestivus* on the antioxidant system, such as the geographical region where *A. aestivus* was obtained, the parts of *A. aestivus* such as leaves, stems and similar parts used, and the extraction method may also have affected the SOD activity. In addition, the dose of *A. aestivus* may have been insufficient to further increase SOD activity against oxidants that increase in bladder I/R.

Acute ischemia causes an acute inflammatory response characterised by activation of endothelin and neutrophils through an increase in the permeability and expression of different adhesion molecules, and activated neutrophils induce tissue damage. Although reperfusion is necessary for the survival of ischemic tissue, it itself leads to cell death through apoptosis[5,6]. Apoptosis, although an important process in maintaining structural integrity, is an active cell destruction process in bladder tissues after I/R injury. Antioxidants block or delay this process[10]. In the study of Tinay *et al.*[10], quercetin treatment protected bladder tissue contractility against acute I/R injury by reducing oxidative stress and apoptosis induced by I/R. In the study of Erkanli Şentürk *et al.*[6], excessive damage to the urothelium, dilatation in intercellular connections, and inflammatory cell infiltration were observed in the bladder I/R injury group. A decrease in the severity of bladder injury was observed in the group administered oxytocin for I/R injury[6]. In Ertaş's[18] study, improvement of I/R-induced edema and oxidant damage with riboflavin and n-

acetylcysteine treatment resulted in improvement of the thinning of the bladder wall. In our current study, a decrease in congestion was observed in the sham group. While congestion, desquamation of the epithelium, muscular hypertrophy and inflammation (edema) were observed in the I/R group; in the I/R+ *A. aestivus* group, a significant regression and/or decrease in the findings caused by I/R injury was detected.

## Conclusion

In our current study, MDA levels were statistically significantly higher in the I/R group compared to the sham and treatment groups; and CAT activity was statistically significantly lower. Histopathologically, it was determined that *A. aestivus* treatment caused a significant regression in the findings caused by I/R damage. Although no statistically significant result was obtained regarding SOD activity in the treatment of bladder I/R injury with *A. aestivus*, our findings suggest that *A. aestivus* has an antioxidant effect and a histopathological protective effect on bladder tissue in I/R injury.

We recommend that further studies be conducted on the usability of *A. aestivus* in treatment by comparing the antioxidant properties of extracts prepared from different regions of *A. aestivus* obtained from different geographical regions and with different extraction methods.

## Ethics statement

The ethics committee approval for this study was obtained from the Kahramanmaraş Sütçü İmam University Faculty of Medicine Experimental Animal Ethics Committee (Date: 10.10.2018, Session No: 2018/10, Decision No: 04).

In all animal procedures used, care was taken to strictly comply with the "The European Convention on Animal Care and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals".

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## Conflict of interests

The authors declare no conflict of interests.

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