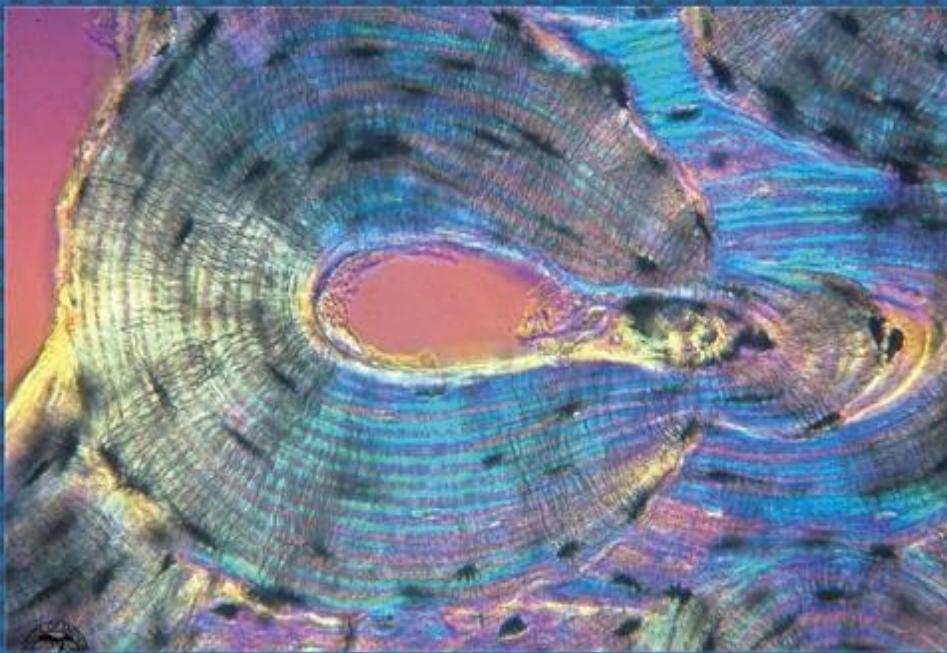




EGYPTIAN ACADEMIC JOURNAL OF  
**BIOLOGICAL SCIENCES**  
HISTOLOGY & HISTOCHEMISTRY

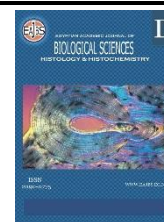
D



ISSN  
2090-0775

[WWW.EAJBS.EG.NET](http://WWW.EAJBS.EG.NET)

Vol. 14 No. 2 (2022)



**Evaluation of the Protective Role of Intravesical Chondroitin Sulfate Versus Intravesical Dimethyl Sulfoxide on The Glycosaminoglycan's, Uroplakin and Restoration of Barrier Function of the Urinary Bladder in A Rat model of Ketamine Induced Interstitial Cystitis. (Histological and Immunohistochemical Study)**

**Walaa Adel Abd el Moez Ahmed**

Department of Anatomy and Embryology. Faculty of Medicine, Ain-Shams University. Abbassia, Postal code: 11591 Cairo, Egypt.

Email\*: [Walaa\\_adel@med.asu.edu.eg](mailto:Walaa_adel@med.asu.edu.eg)

**ARTICLE INFO**

Article History

Received:16/9/2022

Accepted:20/11/2022

Available:26/11/2022

**Keywords:**

Cystitis,  
ketamine;  
DMSO;  
Chondroitin  
sulphate.

**ABSTRACT**

Ketamine recently used as a treatment of resistant depression and bipolar disorder. It has been noted that chronic use of ketamine may induce cystitis and urological toxicity. The purpose of the present study is to examine the protective role of intravesical chondroitin sulphate versus DMSO against ketamine effect on the urinary bladder. Twenty- eight adult albino rats divided into 4 groups seven rats per each: Group (I) received 1ml saline by intraperitoneal injection. Group (II) received 30mg/kg/d ketamine by intraperitoneal injection. Group (III) received 30 mg/kg/d ketamine + intravesical 1ml DMSO 50%. Group (IV) received 30 mg/kg/d ketamine + intravesical 1 mL of 2% chondroitin sulfate. Group (II) showed areas of urothelial cell degeneration and surface ulcerations. Mast cells appeared in the lamina propria and all layers of the urothelium. Marked distortion of the superficial umbrella cells, wide cracks appeared separating them from each other. There was significant increase in IL8 and UPIII expression in group II in comparison to group I with (P value<0.5). Group III showed restoration of normal shape of the superficial cells in some areas; with persistence of surface cracking. Furthermore, there was significant decrease in IL8 and UPIII expression in group III in comparison to group II with (P value<0.5). The best results observed in group IV. Dimethyl sulfoxide (DMSO) induced moderate improvement on the bladder urothelium and decreased inflammation. Chondroitin sulfate rendered a remarkable improvement via its strong anti-inflammatory effect.

**INTRODUCTION**

Interstitial cystitis (IC) is a chronic illness of the urinary bladder resulting from multiple etiological causes (Ueda *et al.*, 2021). Several etiological mechanisms are believed to contribute to the pathophysiology of interstitial cystitis (IC), However, disruption of the glycosaminoglycan (GAG) layer is considered to be the most important histological factor leading to its clinical manifestation (Towner *et al.*, 2021). Proteoglycans, such as chondroitin sulphate and hyaluronic acid, make up the GAG layer (Digesu *et al.*,2020). In addition, GAG layer is essential to the protective and barrier functions of the urinary bladder (Wyndaele *et al.*, 2019). Instability of the GAG layer increases the permeability of the urothelial layer, leading to subepithelial inflammatory processes and lower urinary tract symptoms, such as bladder pain and urinary frequency. (Wang *et al.*, 2021).

Ketamine has been used to treat pain, depression, and mood changes for many years (Xie *et al.*, 2021). In recent years, ketamine cystitis (KC) has emerged as a unique ailment due to its adaptability. It has been identified as a chronic ketamine-associated ulcerative cystitis (Ou *et al.*, 2018). In addition to these side effects, ketamine cystitis patients also have diminished bladder volume, limited bladder compliance, and hyperactive bladder. Several pathological techniques clarify the adverse impacts of ketamine and its metabolites, including norketamine and hydroxynorketamine, on urothelial cells (Kutscher and Greene, 2022). The urothelium functions as a barrier by acting as the bladder's initial line of defense and protecting the bladder stroma (Wan *et al.*, 2018). Toxic metabolites of ketamine immediately rupture the urothelium by disrupting the GAG layer, allowing urine irritant particles to rapidly enter the bladder via the weakening bladder wall, which ultimately induces an inflammatory reaction. It has been postulated that the dense GAG layer is the primary component of the urinary bladder barrier. The use of standard intravesical medicines including GAG family components, such as heparin sulphate and chondroitin sulphate, can enhance urothelial layer recovery by replenishing the GAG layer. Oral and intravesical treatments are currently available for IC (Lin *et al.*, 2022).

Dimethyl sulfoxide (DMSO) is a solvent used in industry. Its medicinal qualities, including its anti-inflammatory, immunological, and analgesic effects, receive considerable study. The FDA approved Rimso-50® (50% DMSO solution) in 1978, and the American Urological Association advises DMSO intravesical instillation as a second-line therapy. Due to a lack of clinical trials, the European Association of Urology guidelines do not support its usage. Recent research indicates that the efficacy of DMSO in the treatment of interstitial cystitis has not yet been

established (Homma *et al.*, 2020). Recently, chondroitin sulphate has been used in cases of interstitial cystitis to restore the protective barrier lost due to epithelial dysfunction. In addition, chondroitin sulphate has potential advantages over heparin, pentosane polysulfate, and hyaluronic acid when it comes to simple barrier restoration. It has no impact on the coagulation cascade or the various receptor systems that heparinoids and hyaluronic acid affect. Moreover, it is less expensive and more stable than the agents listed earlier (Goddard and Janssen, 2018).

## MATERIALS AND METHODS

### Experimental Animals:

28 mature female albino rats weighing between 150 and 200 g were raised in the animal house of the medical research centre (MRC), school of medicine, Ain Shams University. Rats were kept in stainless steel cages and given a week to acclimate to experimental settings before any intervention. Rats that were afflicted with an illness, had been used in a previous experiment, or had difficulty walking were removed from the investigation. The study was approved by A Committee on Animal Research Ethics (CARE) –College of Medicine-Ain Shams University, with ethical number: FWA 000017585. Rats were exposed to a 12-hour light/dark cycle and had unrestricted access to food and water, in addition to optimal environmental conditions and ventilation.

### Experimental Design & Procedures:

#### Drugs Used in The Experiment:

**1-Ketamax (Ketamine Hcl®):** Ketamine vials 10ml for iv injection. The drug was purchased from Panpharma online marketing.

**2-DMSO (Rimso-50®):** (50% DMSO solution); it was purchased from sigma company.

#### 3-Chondroitin-sulfate 2%:

Chondroitin sulfate® powder 2% was purchased from sigma company.

#### Rats Catheterization:

Female rats were sedated with 10% ketamine (60 mg/kg, i.m.) and 2% xylazine (5 mg/kg, i.m.) for 45 minutes to facilitate bladder catheterization and to minimize discomfort and pain. Rats were catheterized using a 24gauge catheter. The rats were positioned in the dorsal recumbent position while under anesthesia. In addition, micturition was stimulated by massaging the lower abdominal region. The external urethral opening was located and xylocaine gel was used to lubricate it. The catheter was gently placed into the external urethral orifice by 3 mm, parallel to the distal urethral axis and spinal cord from cranial to caudal. The catheter was inserted 7 mm farther into the urinary bladder, bringing the total insertion.

#### **Experimental groups:**

Twenty-eight rats were divided into 4 groups/ 7rats each:

**Group (I): (Control group):** Rats received 1ml normal saline (0.9 NaCl) by intraperitoneal injection every day for 4 weeks' duration.

**Group (II): (ketamine-treated group):** received 30mg/kg/d ketamine by intraperitoneal injection every day for 4 weeks' duration.

**Group (III): (ketamine+ DMSO):** received 30mg/kg/d ketamine by intraperitoneal injection every day for 4 weeks' duration+ intravesical 1ml DMSO 50% applied every other day for a total 3 sessions per week through 24gauge catheter inserted transurethral for 4weeks duration.

**Group (IV): (ketamine+ chondroitin sulfate- treated groups):** Received 30mg/kg/d ketamine by intraperitoneal injection every day for 4 weeks' duration +intravesical 2% sodium chondroitin sulfate dissolved in 1ml normal saline applied every other day for a total 3 sessions per week through 24gauge catheter inserted transurethral for 4weeks' duration. At the end of experimental period, the rats were anesthetized by ip injection of 7mg/kg sodium thiopental then sacrificed and the bladders were dissected and possessed

for histopathological and immunohistochemical assessments.

#### **Light Microscopic Study:**

The urinary bladder was dissected and fixed in 10% neutral formalin for 10 days before being processed into paraffin blocks for light microscopic examination. Blocks of paraffin were sliced to a thickness of 5-7 m and stained with Hematoxylin and Eosin (H&E), Masson's Trichrome stain, and PAS stain. Using an Olympus light microscope coupled with an automatic photo micrographic camera system, stained sections were inspected. Kodak ASA400 film was used to capture the color prints. Preparation of Semithin Sections Following a 4-hour phosphate buffer wash of the urine bladder samples, the specimens were processed for preparation of semithin sections. Using a glass knife, one-micron-thick portions were sliced into semithin slices. They were stained with toluidine blue at a concentration of 1%.

#### **Immunohistochemical Staining Procedure:**

Using uroplakin-III (UP-III) polyclonal antibody, the degree of epithelial integrity and epithelial damage was evaluated. Interleukin8 measures the intensity of an inflammation. For the demonstration of (UP-III) and interleukin 8 immunohistochemistry, paraffin slices were utilized. Rinsed in phosphate-buffered saline (PBS), sections were treated with rabbit antiserum produced against pure soluble uroplakin isolated from rat urine bladder. The sample was then treated with a biotinylated anti-rabbit antibody from donkey. They were stained with Avidin Biotin Peroxidase, and Hematoxylin and Eosin were used as counter stain. Normal (UP-III) expression was characterized as an even distribution across the urothelium, primarily on the epithelial cell surface. Weak expression and distribution throughout the urothelium were classified as an abnormal expression. Alternatively, the expression of IL8 was recognized by brownish cytoplasmic

staining of inflammatory cells in the lamina propria or by cytoplasmic staining of urothelial cells.

#### **Scanning Electron Microscopy:**

Glutaraldehyde -fixed specimens were prepared for scanning electron microscopy analysis. The specimens were dehydrated in ascending concentrations of ethyl alcohol, a 1:1 mixture of absolute alcohol and acetone, and then acetone by itself. The specimens were dried using liquid carbon dioxide BALTK CPD30 at the critical point. The specimens were affixed to aluminum studs and gold-coated using BALTK SCD500. The samples were analyzed using a Philips, XL30 scanning electron microscope.

#### **Quantitative Image Analysis:**

In the Histology and cell biology Department of Ain Shams University's Faculty of Medicine, the image analyzer was utilized to take measurements (Image J programme). The percentage of immunostaining was determined in five fields from five non-overlapping serial sections of five animals from each group. First, the image analyzer was automatically calibrated to convert the measurement units (pixels) generated by the application into actual micrometer units. Prior to quantification, the positively immunostained regions for interleukin 8 and UPIII in each field were masked with a blue binary color.

### **RESULTS**

#### **Histopathological Results:**

Light microscopic examination of H&E and semithin sections-stained sections of urinary bladder of group(I) showed the urothelium appeared composed of multiple layers. The superficial cells called umbrella cells appeared covered with the thickened plaques on their luminal surface (Figs.1A,2A). Moreover, the PAS-stained sections revealed strong positive reaction appeared as continuous layer on the luminal edge of the superficial cells (Fig.3A). Besides, the scanning electron microscopy showed the superficial umbrella cells(S) with well-

defined borders and tight cell junctions appeared on the lateral borders of the cells (Fig.7A).

In ketamine-treated group (II) some areas of the urothelium appeared with surface ulceration and with degenerated cells either with dark shrunken pyknotic nuclei or with karyolytic nuclei. The lamina propria also showed inflammatory cell infiltrate (Fig.1B). Semithin sections revealed the mast cells invading the different layers of the bladder urothelium (Fig.2B). PAS-stained sections revealed the GAG layer which appeared focal and discontinuous on the luminal edge of the urothelium (Fig.3B). Scanning electron microscopy revealed distortion of the superficial umbrella cells, wide cracks appeared separating the superficial cells away from each other (Fig.7B).

On other hand, there was moderate improvement in DMSO-treated group (III); there was marked improvement in the condition of urothelium and its nuclei and most of thickened plaques on the luminal surface of the urothelium were apparently restored (Fig.1C). There was slight decrease in the number of mast cells in DMSO-treated group (Fig.2C). The GAG layer on the luminal edge of the urothelium appeared faint and focal (Fig.3C). In addition, scanning electron microscopy revealed restoration of normal shape of the superficial cells in some areas; with persistence of the surface cracking (Fig.7C). The best results were observed in chondroitin sulfate treated group (IV); The urothelium appeared with normal thickness, normal shape of most of its nuclei and restoration of the thickened plaques on its luminal surface (Fig.1D). In Semithin sections there was marked decrease in the number of mast cells (Fig.2D). Besides, the GAG layer appeared as continuous layer on the luminal edge of the urothelium in PAS-stained sections (Fig.3D). Finally, scanning electron microscopy revealed restoration of the superficial umbrella cells and the tight cell junctions on their

lateral borders on most areas of urinary bladder surface (Fig.7D).

**Immunohistochemical Results:**

-The stained sections of UPIII of the control group (I) showed strong positive immune reaction to UPIII; the reaction appeared as strong brownish cytoplasmic staining of the urothelial cells (Figs.4A,5A). In contrast, in ketamine-treated group(II) there was negative immune reaction to UPIII (Figs.4B,5B). Moreover, there was moderate immune reaction to UPIII in DEMSO- treated group (III) appeared as faint brownish cytoplasmic reaction of the basal urothelial cells (Fig.4C,5C). Furthermore, there was Strong positive immune reaction to UPIII expressed in chondroitin sulfate treated group III (Fig.4D,5D).

-The immunohistochemical stained sections of the control group revealed weak immune reaction to IL8; the reaction appeared as faint brownish

staining of the lamina propria (Fig.6A). In addition, there was strong positive immune reaction to IL8 appeared in the lamina propria (Fig.6B). Moderate immune reaction to IL8 was observed in DMSO treated group (III) (Fig.6C) and very weak reaction to IL8 in the lamina propria of chondroitin sulfate treated group (IV) (Fig.6D).

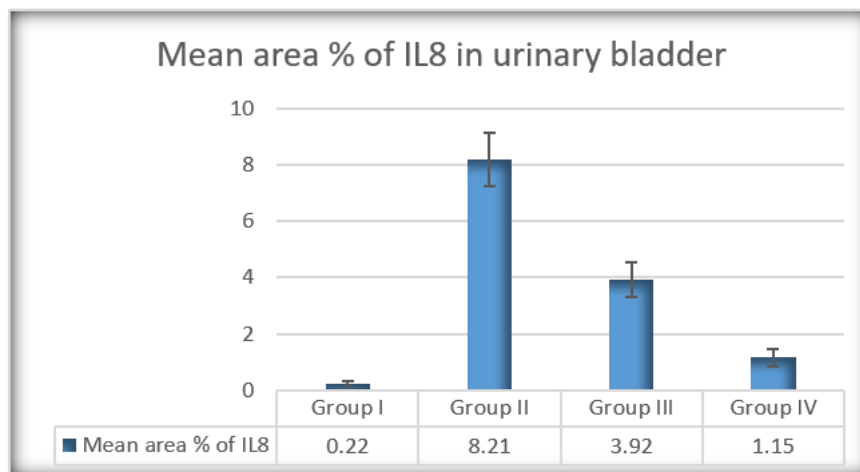
**Histomorphometric Results:**

**Mean Area Percentage to IL8:**

Group I demonstrated a significant decrease compared to other groups (p<0.05). The percentage of stained area in Group II increased significantly compared to the other groups (p<0.05). Group III demonstrated a significant decrease relative to group II, but a significant increase relative to groups I and IV (p<0.05). Group IV demonstrated a significant decrease relative to groups II and III, but a significant increase relative to group I (p<0.05).

**Table 1:** Mean area % of IL8 in the lamina propria of urinary bladder

Groups	Group I	Group II	Group III	Group IV
Mean area % of IL8	0.22	8.21	3.92	1.15
SD	0.08	0.95	0.62	0.32



Group I showed significant decrease in the area percentage stained with IL8 as compared to that of other groups(p<0.05). Group II showed significant increase as compared to that of other groups(p<0.05). Group III showed significant decrease as compared to group II, but it showed significant increase as compared to groups I, IV(p<0.05). Group IV showed significant decrease as compared to group II & III, but it showed significant increase as compared to groups I(p<0.05).

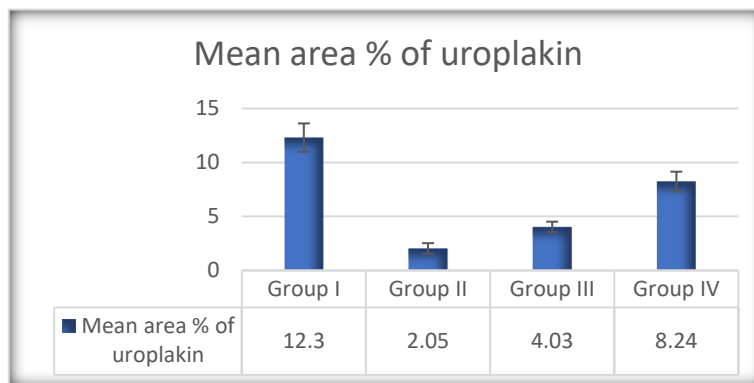
**Mean Area Percentage to UPIII:**

The percentage of area stained with UPIII was significantly higher in Group I ( $p<0.05$ ) compared to the other groups. Group II demonstrated a significant decrease relative to the other groups ( $p<0.05$ ). Group III demonstrated

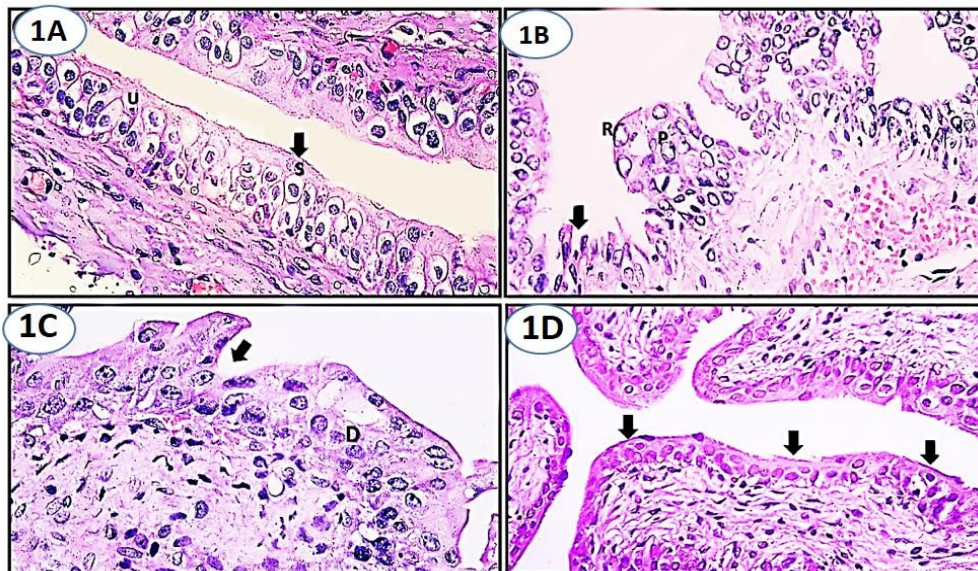
a significant increase relative to group II ( $p<0.05$ ), but a significant decrease relative to groups I and IV ( $p<0.05$ ). Group IV demonstrated a significant increase relative to groups II and III, but a significant decrease relative to group I ( $p<0.05$ ).

**Table 2:** Mean area % of Uroplakin in the urothelium of urinary bladder.

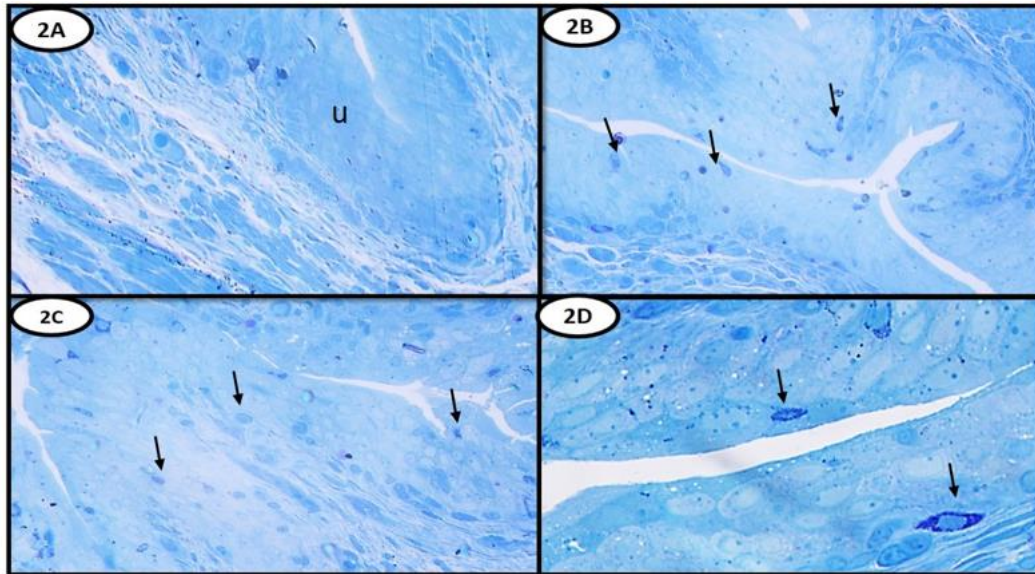
group	Group I	Group II	Group III	Group IV
Mean area % of uroplakin	12.3	2.05	4.03	8.24
SD	1.32	0.48	0.49	0.91



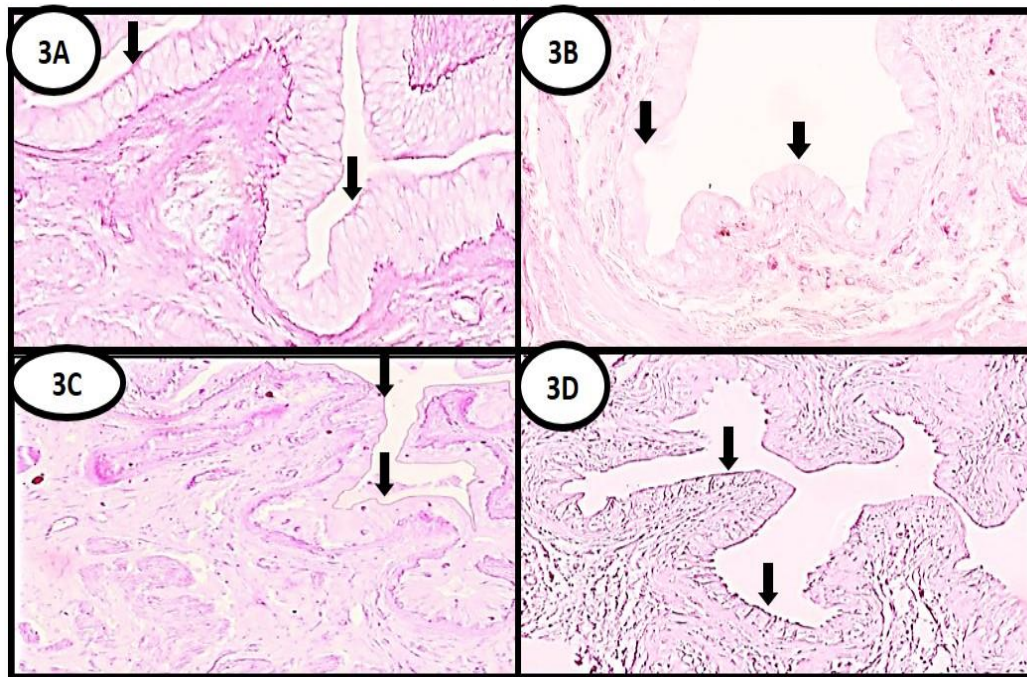
Group I showed significant increase in the area percentage stained with UPIII as compared to that of other groups ( $p<0.05$ ). Group II showed significant decrease as compared to that of other groups ( $p<0.05$ ). Group III showed significant increase as compared to group II, but it showed significant decrease as compared to groups I, IV ( $p<0.05$ ). Group IV showed significant increase as compared to group II & III, but it showed significant decrease as compared to groups I ( $p<0.05$ ).



**Fig.1:** (A) showing the different layers of the bladder urothelium(u) consisting of multiple layers with the superficial umbrella cells(S) and thickened plaques on their luminal surface(black arrows).(B): the urothelium showed surface ulceration(black arrow) and most of its nuclei appeared degenerated either with rarified nuclei(R) Or dark pyknotic nuclei.(C) the urothelium appeared with persistent surface ulceration(black arrow) and with degenerated nuclei (D).(D) the urothelium appeared of normal thickness and normal shape of most of its nuclei with the thickened plaques appeared on most of its luminal surface (black arrows) (H&EX400).

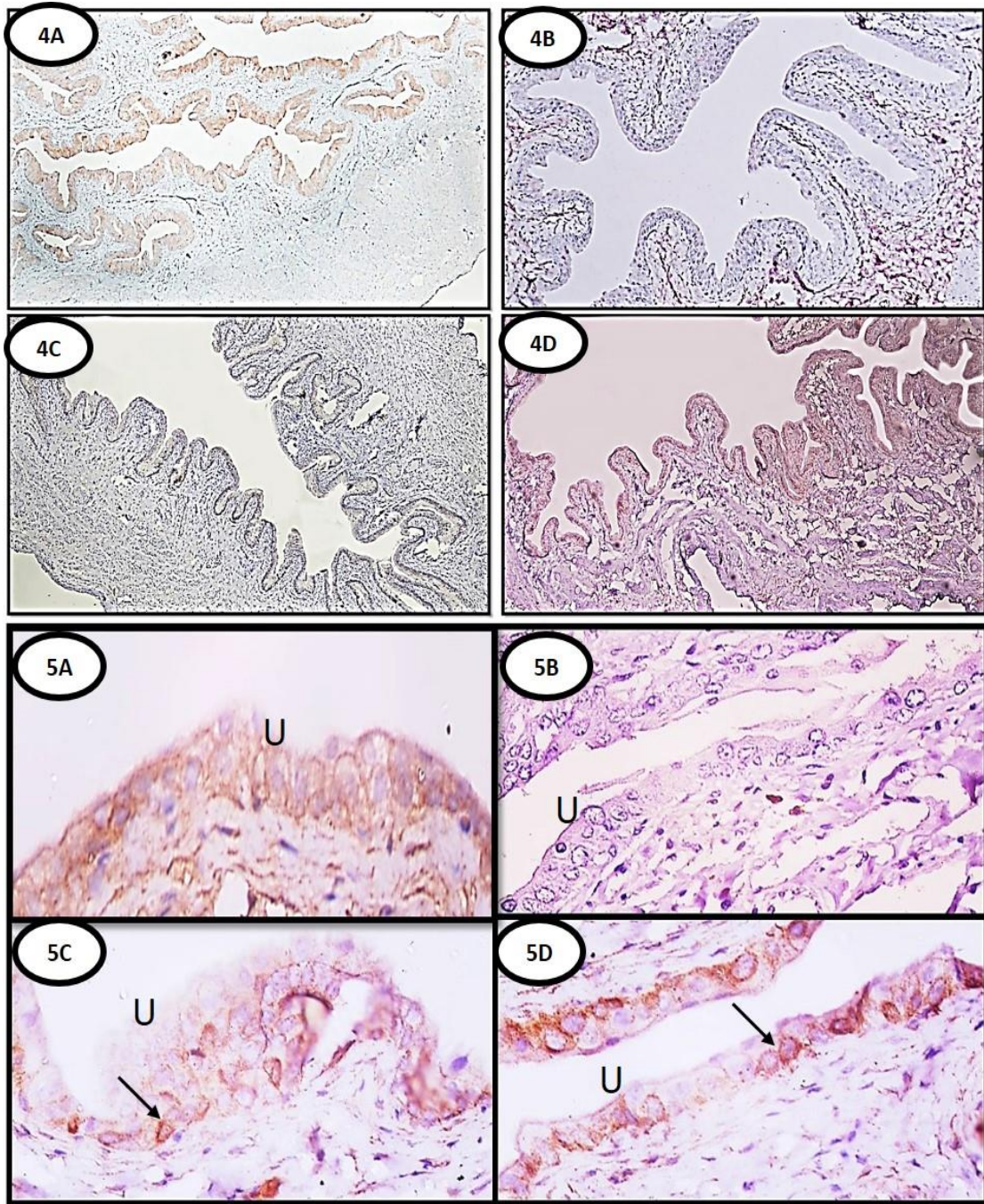


**Fig.2:** (A) showing layers of bladder urothelium(U). (B): showing the mast cells appeared in the lamina propria and invading the different layers of the urothelium (black arrows). (2C): showing few number of mast cells appeared in the urothelium (black arrows). (D): showing few number of mast cells in the urothelium(U) (black arrows) (Toluidine blux400).

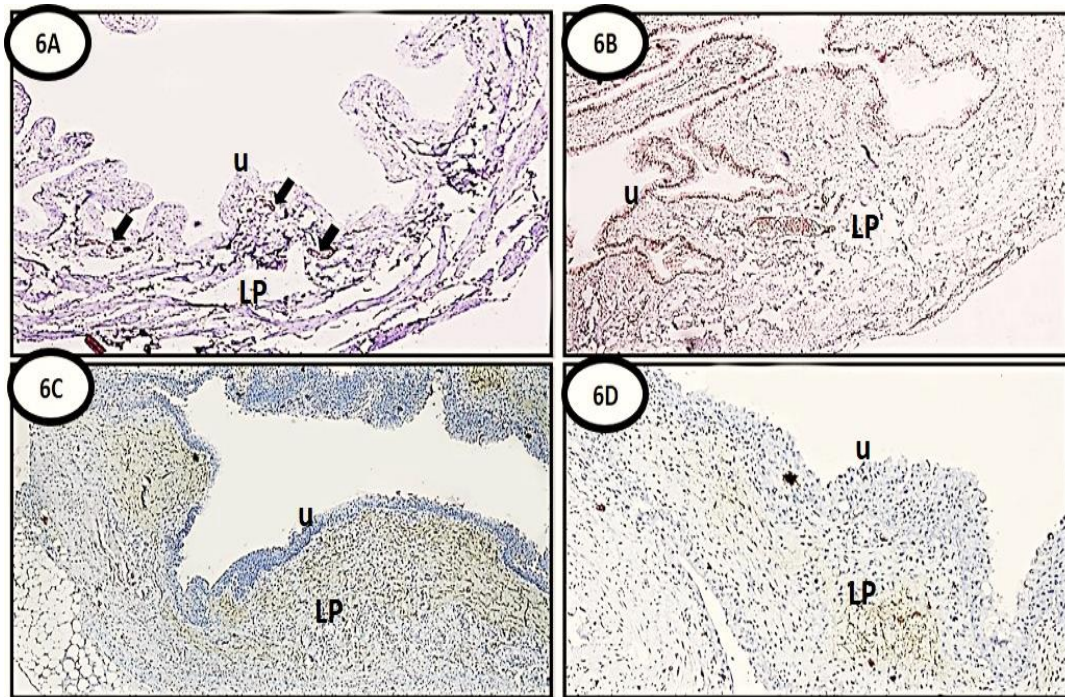


**Fig.3:** (A) showing strong positive PAS reaction appeared as continues layer on the luminal surface of the urothelium (black arrows). (B): showing very weak focal reaction appeared as discontinues layer on the luminal surface of some areas of the urothelium (black arrows). (3C): showing faint reaction on the luminal surface of the urothelium (black arrows). (3D): showing moderate reaction appeared on the luminal surface of some areas of the urothelium (black arrows) (PASX400)

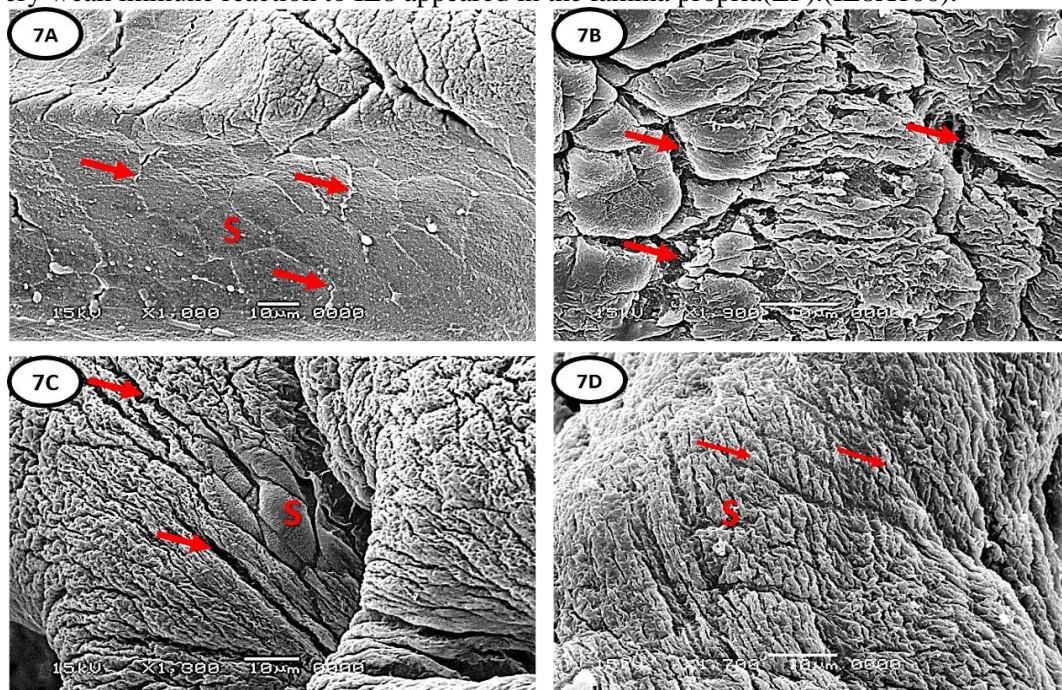




**Figs.(4A,5A):** showing strong positive immune reaction to UPIII appeared in all urothelial cell layers(u). With higher magnification; the reaction appeared as brownish staining of the cytoplasm of all urothelial cell layer(U) (black arrows). **(Figs. 4B,5B):** showing negative immune reaction to UPIII in the cytoplasm of the urothelium (black arrows). **(Figs.4C,5C):** showing moderate immune reaction to UPIII appeared in the cytoplasm of basal urothelial cells (black arrows). **(Fig.4D,5D):** showing strong positive immune reaction to UPIII, the reaction appeared concentrated more in the basal urothelial cells (black arrows).



**Fig.6:** (A) showing very weak and focal immune reaction to IL8 appeared in the lamina propria(LP) (black arrows). (B): showing strong positive immune reaction to IL8; the reaction appeared affecting both the urothelium(u) and the lamina propria(LP). (C): showing moderate immune reaction to IL8 the reaction was confined on the lamina propria(LP). (D): showing very weak immune reaction to IL8 appeared in the lamina propria(LP).(IL8X100).



**Fig.7:** (A) showing the superficial umbrella cells(S) with well-defined borders and tight cell junctions appeared on the lateral borders of the cells (red arrows). (B) showing marked distortion of the superficial umbrella cells, wide cracks appeared separating the superficial cells away from each other. (C) showing restoration of hexagonal shape of the superficial cells in some areas; with persistence of the surface cracking. (D) showing restoration of the superficial umbrella cells and the tight cell junctions on their lateral borders on most areas of urinary bladder surface. (SEM, X1000,1900,1300,1700).

## DISCUSSION

Interstitial cystitis is a chronic urological illness that has developed globally; numerous investigations have been conducted to identify potential etiological elements and explain the pathogenesis of this disorder. In addition, there is a growing concern over the potential therapeutic substances that may be applied and ensure positive outcomes and symptom relief for patients. Recently, many therapeutic compounds that could be delivered locally and systemically have been found; however, their efficacy in easing symptoms and enhancing the patient's lifestyle has not been demonstrated. This issue still requires multiple studies and is the subject of current research. In the realms of anesthesia and pain management, ketamine is a unique medication.

Recent usage of ketamine as an antidepressant is a worldwide cause for worry because of its related pathology, ketamine cystitis. In this study, a rat model of ketamine-induced interstitial cystitis was developed, and the potential protective role of various intravesical medicines was explored. The findings demonstrated that long-term ketamine usage generated significant histological and histomorphometric alterations. There was significant disturbance of the usual architecture of the urinary bladder wall. In addition, the majority of urothelium appeared ulcerated and deteriorated. In addition, PAS-stained sections revealed a significant rupture of the thickened plaques and the GAG layer on the luminal surface.

Due to the presence of thickened plaques and the GAG layer on the luminal surface of umbrella cells (Montalbetti *et al.*, 2019) (Wu *et al.*, 2022), during the filling phase of the urinary bladder, urinary solutes never come into contact with the urothelium. Any disruption of the GAG layer and the thickened plaques may increase the umbrella cells' permeability to urea,

calcium, and potassium, among other urinary solutes (Towner *et al.*, 2021). This may exert a toxic impact on the urothelium of the bladder, resulting in significant cell disruption and disruption of the suburothelial blood vessels (Dalghi *et al.*, 2020).

On the other hand, the disruption of the suburothelial blood arteries results in the extravasation of inflammatory cells, such as mast cells, into the lamina propria and the various layers of the urothelium, as demonstrated in Semithin sections. In addition, there was a high positive immunological response to IL8, an essential inflammatory cytokine that affects both the urothelium and the lamina propria, indicating that the bladder wall was severely inflamed.

In prior research works, it has been demonstrated that the existence of inflammatory cells is an essential sign of the presence of an inflammation and that a decrease in their number represents the efficacy of various treatment methods (Almeida Pinto, 2019) (Almeida de Oliveira *et al.*, 2022) (Jiang *et al.*, 2022). Mast cells play an important role in the pathophysiology of IC and are responsible for the release of various nociceptive mediators, neuropeptide Y, and Substance-P. Consequently, IC patients exhibit an increase in positive sympathetic nerves (Akiyama *et al.*, 2018) (Malik *et al.*, 2018) (Wen *et al.*, 2022). Previous experimental models have demonstrated a connection between an increase in the number of nerve fibers in the bladder and mast cells. According to studies (Almeida Pinto, 2019) (Almeida de Oliveira *et al.*, 2022) (D'Amico *et al.*, 2021), the activation of mast cells and sympathetic nerve fibers may exacerbate the symptoms of inflammatory disorders such as inflammatory bowel disease and irritable bowel syndrome. In other investigations, elevated levels of IL8 have been recorded and linked with the severity of interstitial cystitis symptoms

as well as the degree of mast cell infiltration into the urothelium of the bladder. This demonstrates that IL-8 may operate as a critical chemokine for mast cells and that mast cells may also stimulate the urothelium to secrete IL-8 (Jiang *et al.*, 2022) (Li *et al.*, 2020).

Uroplakin is a protein made by the urothelial cells on the surface of mammals. Transmembrane proteins belonging to the uroplakin protein family are essential for the mechanical stabilization and barrier function of the urothelial surface. Uroplakin III is the most important of this family's UPI, II, and III proteins (Jackson *et al.*, 2020) (Zwaans *et al.*, 2021). Currently, the replacement of urothelial GAG layer by intravesical installation of chondroitin sulfate, hyaluronic acid and pentosan poly sulfate attracts a great attention. There are several theories about their clinical uses and instillation regimens (Daniels *et al.*, 2018) (Rooney *et al.*, 2020). In the present study, the outcomes of intravesical chondroitin sulfate versus DMSO on the histological changes induced by ketamine were clarified and analyzed.

In the current investigation, there was a moderate improvement in thickness of the urothelium in DMSO-treated group. However, some of the nuclei remained profoundly stained, while others remained rarified. In addition, there was minor reduction in the extravasation of inflammatory cells from injured blood vessels. Besides, there was a continuous rise in the expression of IL8 in the lamina propria, but not in the urothelium. All prior data indicate that DMSO has a mild anti-inflammatory effect. On other hand, there was a mild reactivity to uroplakin III in the cytoplasm of the urothelial cells, indicating its minor significance in restoring the normal structure of the uroplakin protein, the normal barrier function of the urothelium, and thickened plaques.

Compared to the DMSO-treated group, the chondroitin sulphate-treated group rendered the best results. The

urothelium looked to have a normal thickness and form for the majority of its nuclei. The extravasated mast cells decreased significantly. In addition, there was moderate immune response to IL8 in the lamina propria. All prior findings point to the potent anti-inflammatory effect of chondroitin sulphate and its potential role in reducing the severity of interstitial cystitis symptoms.

In addition, there was a robust expression of UPIII in the cytoplasm of the urothelium and a spectacular recovery of the thickened plaques on the majority of the luminal surface of the urothelial cells. According to the presented data, chondroitin sulphate plays a vital role in the protection of the GAG layer and UPIII, as well as in the improvement of the barrier function of the bladder urothelium, by virtue of its potent anti-inflammatory effect and its ability to replenish the luminal GAG layer of the bladder urothelium.

**Conclusion:** Long-term use of ketamine generated profound alterations in the urinary bladder wall; DMSO rendered moderate protective potential; and there was a moderate improvement in the inflammatory condition, and structure of uroplakin III in the urothelium. Chondroitin sulphate, on the other hand, provided the optimal protective capacity against the histological changes generated by ketamine on the bladder wall due to its potent anti-inflammatory impact and its ability to restore the normal structure of the urothelium's GAG layer.

## REFERENCES

- Akiyama Y., Maeda D., Morikawa T., et al., (2018): Digital quantitative analysis of mast cell infiltration in interstitial cystitis. *Neurourology and Urodynamics*, 37(2), pp.650-657. DOI: 10.1002/nau.23365, indexed in PubMed: 29065222.
- Almeida Pinto R., (2019): Bladder Pain Syndrome/Interstitial Cystitis. In *Postmenopausal Diseases*

- and Disorders* (pp. 121-132). Springer, Cham. DOI: 10.1111/jcmm.17132, indexed in PubMed: 34953040.
- Almeida de Oliveira L.S., de Moura Bandeira S.R., Gomes Gonçalves R.L., et al., (2022): The Isopropyl Gallate Counteracts Cyclophosphamide -Induced Hemorrhagic Cystitis in Mice. *Biology*, 11(5), p.728. DOI: 10.3390/biology11050728, indexed in PubMed: 35625456.
- Dalghi M.G., Montalbetti N., Carattino M.D. et al., (2020): The urothelium: life in a liquid environment. *Physiological reviews*, 100(4), pp.1621-1705. DOI: 10.1152/physrev.00041.2019, indexed in PubMed: 32191559.
- D'Amico R., Trovato Salinaro A., Cordaro M., et al., (2021): Hidrox® and Chronic Cystitis: Biochemical Evaluation of Inflammation, Oxidative Stress, and Pain. *Antioxidants*, 10(7), p.1046. DOI: 10.3390/antiox10071046, indexed in PubMed: 34209690.
- Daniels A.M., Schulte A.R. and Herndon C.M., (2018): Interstitial cystitis: an update on the disease process and treatment. *Journal of Pain & Palliative Care Pharmacotherapy*, 32(1), pp.49-58. DOI: 10.1080/15360288.2018.1476433, indexed in PubMed: 30212267.
- Digesu G.A., Tailor V., Bhide A.A. et al., (2020): The role of bladder instillation in the treatment of bladder pain syndrome: Is intravesical treatment an effective option for patients with bladder pain as well as LUTS? *International urogynecology journal*, 31(7), pp:1387-1392. DOI: 10.1007/s00192-020-04303-7, indexed in PubMed: 32358624.
- Goddard J.C. and Janssen D.A., (2018): Intravesical hyaluronic acid and chondroitin sulfate for recurrent urinary tract infections: systematic review and meta-analysis. *International urogynecology journal*, 29(7), pp.933-942. DOI: 10.1007/s00192-017-3508-z, indexed in PubMed: 29181550.
- Homma Y., Akiyama Y., Tomoe H., et al., (2020): Clinical guidelines for interstitial cystitis/bladder pain syndrome. *International Journal of Urology*, 27(7), pp:578-589. DOI: 10.1111/iju.14234, indexed in PubMed: 32291805.
- Jackson A.R., Ching C.B., McHugh K.M. et al., (2020): Roles for urothelium in normal and aberrant urinary tract development. *Nature Reviews Urology*, 17(8), pp:459-468. DOI: 10.1038/s41585-020-0348-2, indexed in PubMed: 32647226.
- Jiang Y.H., Jhang J.F., Lee Y.K. et al., (2022): Low-Energy Shock Wave Plus Intravesical Instillation of Botulinum Toxin A for Interstitial Cystitis /Bladder Pain Syndrome: Pathophysiology and Preliminary Result of a Novel Minimally Invasive Treatment. *Biomedicines*, 10(2), p.396. DOI: 10.3390/biomedicines10020396, indexed in PubMed: 35203604.
- Jiang Y.H., Jhang J.F. and Kuo H.C., (2022): Can We Use Urinary Cytokine/Chemokine Analysis in Discriminating Ulcer-Type Interstitial Cystitis/Bladder Pain Syndrome? *Diagnostics*, 12(5), p.1093. DOI: 10.3390/diagnostics

- 12051093, indexed in PubMed: 35626252.
- Kutscher E. and Greene R.E., (2022): Ketamine Cystitis: An Under recognized Cause of Dysuria. *Journal of General Internal Medicine*, 37(5), pp.1286-1289. DOI: 10.1007/s11606-021-07297-0, indexed in PubMed: 34981355.
- Li, W., Yang, F., Zhan, H., Liu, B., Cai, J., Luo, Y. and Zhou, X., (2020): Houuttuynia cordata Extract Ameliorates Bladder Damage and Improves Bladder Symptoms via Anti-Inflammatory Effect in Rats with Interstitial Cystitis. *Evidence -Based Complementary and Alternative Medicine*, 2020. DOI: 10.1155/2020/9026901, indexed in PubMed: 33133219.
- Lin J.W., Lin Y.C., Liu J.M., (2022): Nor ketamine, the Main Metabolite of Ketamine, Induces Mitochondria-Dependent and ER Stress-Triggered Apoptotic Death in Urothelial Cells via a Ca<sup>2+</sup>-Regulated ERK1/2-Activating Pathway. *International journal of molecular sciences*, 23(9), p.4666. DOI: 10.3390/ijms23094666, indexed in PubMed: 35563057.
- Malik S.T., Birch B.R., Voegeli D., et al., (2018): Distribution of mast cell subtypes in interstitial cystitis: implications for novel diagnostic and therapeutic strategies? *Journal of Clinical Pathology*, 71(9), pp.840-844. DOI: 10.1136/jclinpath-2017-204881, indexed in PubMed: 29764932.
- Montalbetti N., Rooney J.G., Rued A.C. et al., (2019): Molecular determinants of afferent sensitization in a rat model of cystitis with urothelial barrier dysfunction. *Journal of Neurophysiology*, 122(3), pp:1136-1146. DOI: 10.1152/jn.00306.2019, indexed in PubMed: 31314637.
- Ou Y.L., Liu C.Y., Cha T.L., et al., (2018): Complete reversal of the clinical symptoms and image morphology of ketamine cystitis after intravesical hyaluronic acid instillation: A case report. *Medicine*, 97(28). DOI: 10.1097/MD.00000000000011500, indexed in PubMed: 29995815.
- Rooney P., Ryan C., McDermott B.J., et al., (2020): Effect of Glycosaminoglycan Replacement on Markers of Interstitial Cystitis in Vitro. *Frontiers in pharmacology*, 11, p.575043. DOI: 10.3389/fphar.2020.575043, indexed in PubMed: 33390947.
- Towner, R.A., Smith, N., Saunders, D. et al., (2021): MRI as a Tool to Assess Interstitial Cystitis Associated Bladder and Brain Pathologies. *Diagnostics*, 11(12), p.2298. DOI: 10.3390/diagnostics11122298, indexed in PubMed:34943535.
- Towner R.A., Greenwood-Van Meerveld B., Mohammadi E., (2021): Super GAG biopolymers for treatment of excessive bladder permeability. *Pharmacology Research & Perspectives*, 9(1), p. e00709. DOI: 10.1002/prp2.709, indexed in PubMed: 33540486.
- Ueda T., Hanno P.M., Saito R., et al., (2021): Current understanding and future perspectives of interstitial cystitis/bladder pain syndrome. *International Neurourology Journal*, 25(2): p.99. DOI: 10.5213/inj.2142084.042, indexed in PubMed:34218637.
- Wang S., Jin S., Shu Q. et al., (2021): Strategies to get drugs across

- bladder penetrating barriers for improving bladder cancer therapy. *Pharmaceutics*, 13(2) : p.166. DOI: 10.3390/pharmaceutics13020166, indexed in PubMed: 33513793.
- Wan Q., Xiong G., Liu G., et al., (2018): Urothelium with barrier function differentiated from human urine-derived stem cells for potential use in urinary tract reconstruction. *Stem cell research & therapy*, 9(1), pp:1-13. DOI: 10.1186/s13287-018-1035-6, indexed in PubMed: 30409188.
- Wen C., Xie L. and Hu C., (2022): Roles of mesenchymal stem cells and exosomes in interstitial cystitis/bladder pain syndrome. *Journal of Cellular and Molecular Medicine*, 26(3), pp.624-635.
- Wu S.Y., Jiang Y.H., Jhang J.F., et al., (2022): Inflammation and Barrier Function Deficits in the Bladder Urothelium of Patients with Chronic Spinal Cord Injury and Recurrent Urinary Tract Infections. *Biomedicines*, 10(2), p.220. DOI: 10.3390/biomedicines10020220, indexed in PubMed: 35203430.
- Wyndaele J.J.J., Riedl C., Taneja R., et al., (2019): GAG replenishment therapy for bladder pain syndrome /interstitial cystitis. *Neurourology and urodynamic*, 38(2), pp:535-544. DOI: 10.1002/nau.23900, indexed in PubMed: 30592544.
- Xie X., Liang J., Huang R., et al., (2021): Molecular pathways underlying tissue injuries in the bladder with ketamine cystitis. *The FASEB Journal*, 35(7), p: e21703. DOI: 10.1096/fj.202100437, indexed in PubMed: 34105799.
- Zwaans B.M., Carabulea A.L., Bartolone S.N., et al., (2021): Voiding defects in acute radiation cystitis driven by urothelial barrier defect through loss of E-cadherin, ZO-1 and Uroplakin III. *Scientific reports*, 11(1), pp.1-10. DOI: 10.1038/s41598-021-98303-2, indexed in PubMed: 34588475