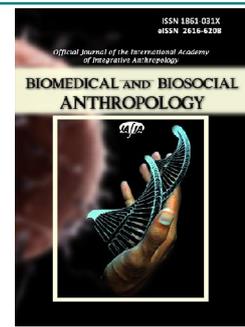




## BIOMEDICAL AND BIOSOCIAL ANTHROPOLOGY

Official Journal of the International Academy  
of Integrative Anthropology

journal homepage: <http://bba-journal.com>



## Development of a method for serial intravesical administration of drugs in experiment

**Kostiuk O. H., Hodovan N. L., Taran I. V., Mashevska O. V., Grebeniuk D. I.**

National Pirogov Memorial Medical University, Vinnytsya, Ukraine

### ARTICLE INFO

Received: 06 August, 2020

Accepted: 07 September, 2020

UDC: 616-092.9+612.62-  
089+591.462.2+59.089

### CORRESPONDING AUTHOR

e-mail: [scienceandroid@gmail.com](mailto:scienceandroid@gmail.com)  
Taran I. V.

*The technical difficulties of serial intravesical administration of drugs are a significant problem in preclinical studies of the effectiveness of local chemotherapy for superficial bladder cancer. The aim of the study was to develop and evaluate the effectiveness of the method of serial intravesical administration of drugs in the experiment. The study included 49 Wistar rats of both sexes. Five proposed methods of intravesical administration of drugs were investigated: retrograde catheterization of the urinary bladder under anesthesia, method of transcutaneous puncture of the urinary bladder in rats, intraoperative puncture of the bladder, method of subcutaneous fixation of the bladder for subsequent puncture in the experiment, method of serial intravesical administration of drugs in female rats using special catheter. The obtained data were processed using a package of statistical programs SPSS 20.0 for Windows. The disadvantage of the first technique is its trauma - each insertion of the catheter is accompanied by trauma of the urethral mucosa and associated pain. The manipulations were failed because of edema of the urethral mucosa. The manipulation should be performed under general anesthesia. The second technique also requires general anesthesia. The aggressive cytostatic action of Doxorubicin prevented the closure of the puncture hole and caused the development of complications. Sealing the bladder with a collagen plate when performing the third technique prevents the release of the drug into the abdominal cavity, however, the adhesion of the tissues of the surrounding organs to the plate promotes the formation of an adhesive conglomerate and makes further manipulations impossible. The withdrawal of the bladder from the abdominal cavity during the implementation of the fourth technique, theoretically, should have helped to avoid the complications observed during the third technique. However, insufficiently reliable fixation of the urinary and adhesions in the area of the implanted ring make this technique such that it does not correspond to the tasks set. The fifth method was the only one proposed that met all the assigned tasks. This technique made it possible to perform a series of 5 intravesical injections of Doxorubicin without general anesthesia and without the development of postoperative complications from the postoperative wound and abdominal cavity. The proposed method of serial intravesical administration of drugs in female rats using a special catheter makes it technically easy to perform serial intravesical administration of drugs without the use of general anesthesia and without the development of complications from the postoperative wound and abdominal cavity.*

**Keywords:** non-muscle invasive bladder cancer; intravesical, experimental, rats.

### Introduction

Due to the trend of constant increase in overall morbidity and mortality, bladder cancer remains a pressing problem in modern medicine in general and oncology in particular.

Bladder cancer is the fourth most common cancer in the United States and Europe. In terms of mortality, this pathology ranks seventh and eighth in the structure of

mortality from oncopathology in the United States [14] and Europe, respectively [5]. Tumors occur 3-4 times more often in men than in women. In one of 26 men will develop bladder cancer during their lifetime [14]. The overall five-year survival rate in Europe is about 68 % [3].

The situation in Ukraine is not better. Thus, almost 5,000

new cases of the disease and more than 2,000 deaths from this pathology are registered annually [2].

Due to the constant improvement of diagnostic algorithms, more and more newly diagnosed cases (according to some data up to 75 %) are superficial bladder cancer, when the tumor affects only the mucous membrane or submucosal layer [1].

The standard of treatment for patients with superficial bladder cancer is transurethral resection of the tumor [17]. Intravesical chemotherapy and immunotherapy are used as adjuvant therapy after transurethral resection to prevent the development of residual and recurrent neoplasms [13, 19].

The intravesical route of drug administration opens wide opportunities for scientists and practitioners to develop new and optimize existing treatments for non-invasive bladder cancer [13, 19].

However, this problem requires, first of all, large-scale preclinical studies. Just as most modern treatment methods consist in the serial administration of drugs, then changing the method of drug administration also requires serial use. In experimental studies, due to technical difficulties, a significant problem is the fulfillment of this condition - the serial introduction of drugs into the bladder without the development of complications associated with its integrity.

*The aim* of the study was to develop and evaluate the effectiveness of the method of serial intravesical administration of drugs in the experiment.

### Materials and methods

The experimental study was performed on the basis of a research laboratory of preclinical study of pharmacological substances of National Pirogov Memorial Medical University, Vinnytsya.

All experiments were performed in accordance with the "Regulations on the use of animals in biomedical experiments" with the permission of the Bioethics Committee.

The study included 49 Wistar rats, both sexes (35 females and 14 males) under 1 year of age and weighing 120 to 220 g ( $186 \pm 17$  g).

All experiments were performed with the calculation that in this way in the future it will be necessary to administer intravesically the drug of cytostatic action Doxorubicin at a dose of 5 mg/kg body weight of the experimental animal in a series of 5 injections with an interval between injections of 7 days.

In order to ensure gastric emptying, the animals were left for 12 hours without access to food, but with free access to water, before the experiments. All experiments were performed under conditionally sterile conditions under intraperitoneal ketamine anesthesia at the rate of 0.22 ml per 100 grams of body weight of the experimental animal.

In the process of developing a method of serial intravesical administration of drugs in the experiment, we

have consistently proposed a number of techniques.

The first proposed technique was retrograde catheterization of the bladder under anesthesia. This technique was performed on 14 rats (7 females and 7 males).

Catheterization was performed with a flexible catheter caliber 21G (outer diameter - 0.8 mm, inner diameter - 0.51 mm). The catheter was inserted retrogradely through the outer opening of the urethra. In case of unsuccessful catheterization, the attempt was repeated by inserting a rigid conductor into the catheter. Upon reaching the bladder, Doxorubicin was administered through a catheter. The catheter was then removed. If technically possible, the manipulation was performed 5 times with an interval of 7 days.

The second proposed method was a method of transcutaneous puncture of the bladder in rats (Patent of Ukraine for utility model № 138094 from 25.11.2019).

Clarification of the topography of the bladder and determination of the point of its transcutaneous puncture, 7 female rats under anesthesia performed retrograde catheterization of the bladder, its emptying and introduction into its cavity 0.1 ml of 60 % iodine-containing X-ray contrast agent Triombrast® (a solution of diatrizoic acid dihydrate and N-methylglucamine). Next, rats underwent review radiography in two projections. Based on the data obtained by radiography, the point of transcutaneous puncture of the bladder was determined.

Direct transcutaneous puncture of the bladder was performed in 7 female rats as follows. Rats were anesthetized and fixed in a horizontal position on the operating table. Retrograde catheterization of the bladder was performed and 1.0 ml of saline for filling was injected into it. The main landmarks were determined by palpation: right and left hip joints, left cranial ventral iliac spine. The puncture site was defined as the point at the intersection of the line connecting the right hip joint and the left cranial ventral iliac spine and perpendicular to the line connecting the hip joints at the point on the border of the first quarter of this line on the right. At this point, a 30G needle (outer diameter 0.3 mm) 13 mm long was inserted with a vacuum in the syringe perpendicular to the plane of the anterior abdominal wall until urine appeared in the syringe. The appearance of fluid in the syringe indicated that the needle was in the bladder cavity. Aspiration of fluid from the bladder was performed, followed by administration of Doxorubicin.

The third proposed technique was intraoperative puncture of the bladder, which was performed on 7 rats of both sexes. To prevent leakage of the injected drug through the puncture hole performed its sealing using the proposed method of sealing the bladder after intraoperative puncture (Patent of Ukraine for utility model № 138089 from 25.11.2019).

The manipulation was performed as follows. Under general anesthesia, a lower-middle laparotomy was performed in experimental rats. The body of the bladder

was removed into the surgical wound. The point of puncture of the bladder was determined. A 5x5 mm collagen plate was moistened with blood from the surgical wound and punctured in the center with a 30G needle (outer diameter 0.3 mm) 13 mm long. Bladder puncture was performed with urine aspiration and Doxorubicin and 0.1 ml of methylene blue solution injected into the bladder. Methylene blue was administered for intraoperative control of bladder tightness. After the puncture, the collagen plate was pressed against the wall of the bladder with fingers, which ensured their tight contact and adhesion of the plate to the wall. The puncture needle was carefully removed from the bladder cavity. Performed revision of the abdominal cavity and washing it with an antiseptic solution. The laparotomy wound was sutured tightly in layers.

The fourth proposed method was a method of subcutaneous fixation of the bladder for the next puncture in the experiment (Patent of Ukraine for utility model № 138093 from 25.11.2019), which was performed on 7 rats of both sexes and was as follows.

Under general anesthesia, a lower-middle laparotomy was performed in experimental rats. A plastic ring was fixed in the lower part of the surgical wound with separate nodal sutures so that the plane of the ring coincided with the plane of the anterior abdominal wall. A pocket was formed in the subcutaneous fat. The body of the emptied bladder was passed through the ring and placed in the formed pocket. Intraoperatively performed the first injection of the drug with a needle caliber 30G (outer diameter 0.3 mm) with a length of 13 mm with sealing the puncture hole according to the method described above. Performed revision of the abdominal cavity and washing it with an antiseptic solution. The laparotomy wound was sutured tightly in layers. Subsequent injections of the drug were performed percutaneously without anesthesia, fixing the rat in an upright position. To identify the bladder in the postoperative period, it was observed when it is partially filled with urine and will be palpable. To inject Doxorubicin into the bladder, the highest point of the subcutaneous bladder was determined by palpation and the bladder was punctured at this point with a 30G needle (outer diameter 0.3 mm) 13 mm long.

The fifth proposed method was a method of serial intravesical administration of drugs to female rats (Patent of Ukraine for utility model № 138092 from 25.11.2019) using a special catheter (Patent of Ukraine for utility model № 139761 from 27.01.2020).

The catheter is a flexible tube with a diameter of 2 Fr (0.67 mm) and a length of 20 mm, at one end of which is a balloon with a diameter of 6 Fr (2.0 mm), which does not cover the lumen of the catheter.

Catheter insertion into the bladder and subsequent drug administration were performed as follows. Under general anesthesia, a lower-middle laparotomy was performed in experimental rats. The body of the bladder was removed into the surgical wound. A longitudinal cystotomy 3 mm

long was performed. Antegradely conducted the tubular part of the catheter through the inner opening of the urethra through the urethra to the outside. The bladder was sutured. Performed revision of the abdominal cavity and washing it with an antiseptic solution. The laparotomy wound was sutured tightly in layers. To introduce liquid forms of drugs into the bladder in the postoperative period with tweezers fixed the tubular part of the catheter, which was located in the outer opening of the urethra. The syringe needle was inserted into the catheter hole and the drug was injected into the bladder. The first administration of Doxorubicin was performed on the 8th day after catheter placement, when the integrity of the bladder wall was completely restored. In the case of closing the lumen of the catheter with protein and salt layers, its patency was restored by introducing into its lumen a flexible or rigid conductor.

The obtained data were processed using the statistical software package SPSS 20.0 for Windows.

## Results

When performing the first proposed technique - retrograde catheterization of the bladder under anesthesia - we obtained the following data.

Due to the topographic features of the urethra, catheterization failed in all 7 male rats. The anatomical curves of the urethra could not be passed either by a flexible catheter or after the introduction of a rigid conductor into the catheter.

When catheterizing the bladder in female rats, the first and second injections of Doxorubicin were performed in all 7 experimental animals. Flexible catheterization was performed on 5 (71.4 %) animals at the first and 3 (42.9 %) animals at the second administration of the drug, rigid catheterization was performed at 2 (28.6 %) and 4 (57.1 %) animals, respectively.

At the third injection, only 3 (42.9 %) animals were able to instill the drug into the bladder using a catheter with a rigid conductor. In other experimental animals, the manipulation could not be performed due to swelling of the urethral mucosa, which occurred due to previous trauma and toxic effects of Doxorubicin on the injured mucosa. The manipulation stopped after the appearance of traces of blood from the outer opening of the urethra.

The fourth injection was performed with a rigid catheter with significant technical difficulties in only 1 (14.3 %) rat.

Thus, during retrograde catheterization of the bladder under anesthesia, a series of 5 intravesical injections of the drug could not be performed by any of the experimental rats.

The results of a typical contrast radiography of the bladder in two projections, which was performed to clarify its topography and determine the point of its transcutaneous puncture are shown in Figure 1.

According to the results of X-ray examination, the transcutaneous puncture site was defined as the point at the intersection of the line connecting the right hip joint and



**Fig. 1.** Contrast radiography of the bladder in two projections (A - direct projection, B - lateral projection).

the left cranial ventral iliac spine, and perpendicular to the line connecting the hip joint at the point on the border of the first quarter of this line on the right.

When performing a transcutaneous puncture, the following results were obtained. During the first administration of Doxorubicin, the puncture was successful in the first attempt in 6 (85.7 %) rats. Another 1 (14.3 %) rat needed 3 attempts to puncture the bladder. That is, the first injection according to this method was performed on all experimental rats.

During the week in 5 (71.4 %) rats developed similar symptoms. Rats were lethargic, adynamic, partially refused food, drank a lot of water, and touched the abdomen caused aggression.

Two rats under anesthesia underwent laparotomy and abdominal revision. The audit revealed perforation of the anterior wall of the bladder and signs of urinary peritonitis. Due to the development of peritonitis, it was decided to discontinue the experiment.

During the study of the effectiveness of intraoperative puncture of the bladder received the following data. During the first administration of the drug, all manipulations were performed without any technical difficulties. In no case was leakage of the methylene blue solution from the puncture hole observed. The postoperative period proceeded without features, the phenomena of peritonitis were not observed

at any animal. During the second administration of the drug in all rats at the site of the first surgery, an adhesive process of varying severity was observed. However, despite technical difficulties, the second administration of the drug was performed by all experimental rats. The third administration of the drug was carried out with great technical difficulties due to the pronounced adhesion process in the area of the postoperative scar and in the lower part of the abdominal cavity. Only 4 (57.1 %) rats were able to administer the drug intraoperatively from the lower-middle laparotomy access. In another 2 (28.6 %) rats, due to the impossibility to complete the lower-middle laparotomy, a pararectal access was performed, from which a bladder puncture was performed. In 1 (14.3 %) rat, puncture was technically impossible. During the fourth attempt to administer the drug, all rats showed a pronounced adhesion process in the lower part of the abdominal cavity with the involvement of intestinal loops. It was not possible to identify the bladder in the adhesion conglomerate.

In the study of the effectiveness of subcutaneous fixation of the bladder for its next puncture, the following data were obtained. Attempting a second injection in 2 (28.6 %) rats failed to identify the bladder in the subcutaneous tissue. A plastic ring implanted in the abdominal wall without signs of a bladder passed through it was palpated. During a revision laparotomy, it was found that in these rats, the

bladder was completely in the abdominal cavity. In our opinion, this is due to the lack of intraoperative fixation of it in the subcutaneous tissue. Suture fixation of the bladder was not performed to prevent further injury and subsequent formation of perforations in its wall. In the remaining 5 (71.4 %) rats, the second administration of the drug was performed without special features.

Attempts to administer the drug for the third time in 4 (57.1 %) rats showed accumulation of fluid in the subcutaneous tissue. At puncture, this fluid was identified as urine. Revision surgery revealed a perforation in the bladder. The bladder was fixed in the subcutaneous tissue, and the plastic ring implanted in the anterior abdominal wall was almost completely obliterated.

Another 1 (14.3 %) rat, although identified in the subcutaneous tissue, was constantly emptied, which made puncture impossible. A revision laparotomy revealed that the plastic ring was completely obliterated. Inside it were soldered loops of the intestine. The adhesion process disrupted the passage of urine through the left ureter, resulting in visual hydronephrosis of the left kidney.

When analyzing the results of the technique using intraoperative antegrade positioning of the catheter with balloon, the following data were obtained.

Thus, there were no postoperative complications from both the postoperative wound and the abdominal cavity. Intravesical administration of Doxorubicin was performed without any technical difficulties at all times of the study. No case of self-removal of the catheter in rats was observed. In the case of closing the lumen of the catheter with protein and salt layers, its patency was restored by introducing into its lumen a flexible or rigid conductor. In all cases, patency was restored without much technical difficulty.

## Discussion

Doxorubicin is a drug of the anthracycline series, which has been used for more than 40 years as an antitumor drug in various hematological and solid malignancies [12].

Although the use of this drug has shown quite good results in the treatment of malignant tumors, its potential cardiotoxicity, including life-threatening cardiomyopathy and congestive heart failure, are side effects that should be considered and prevented [7, 12, 16].

The mechanism of cardiotoxicity of Doxorubicin has not been fully studied. A number of studies have shown that oxidative stress, inflammation and apoptosis are associated with the pathogenesis of this pathological condition, with oxidative stress being the main mechanism. Mitochondrial dysfunction and calcium overload of cardiomyocytes are also important in the development of toxic heart disease. Doxorubicin-induced changes in blood biochemical parameters, such as fatty acid binding proteins, brain natriuretic peptide, and cardiac superoxide dismutase, also play a significant role in the pathogenesis [4, 8, 15, 18].

Doxorubicin has been used for many years to treat

bladder cancer [9]. If systemic chemotherapy is warranted to invade the muscle layer, superficial bladder cancer can be successfully treated by instilling the drug into the bladder. The histological structure of the epithelium due to strong intercellular connections almost completely prevents reabsorption from the bladder [11]. That is, intravesical administration of doxorubicin should prevent the toxic effects of the drug on the heart.

However, to study the systemic and local effects of the drug during its intravesical administration, it is necessary to perform serial instillations. The literature describes methods of drug administration into the bladder in rodents, but none of these methods is adapted for serial use [6, 10].

However, in the scientific literature, we have not found a technique that would be easily reproducible and meet the basic requirement - the possibility of serial injection of drugs into the bladder without the development of complications associated with its integrity.

That is why we have consistently proposed five methods that, in our opinion, could be used to achieve the purpose of this study.

All experiments were performed with the calculation that in this way in the future it will be necessary to administer intravesically the drug of cytostatic action Doxorubicin in a series of 5 injections with an interval between injections of 7 days. That is, the method had to take into account the aggressive effect of the drug on the bladder wall and any damage to it.

The first proposed technique was retrograde catheterization of the bladder under anesthesia. The disadvantage of this technique is its traumatism - each catheter insertion is accompanied by trauma to the mucous membrane of the urethra and associated pain. Therefore, this technique was performed only under general anesthesia. Although this technique is easy to perform, a series of 5 intravesical injections of the drug could not be performed by any of the experimental rats. The manipulation was prevented by swelling of the urethral mucosa, which occurred due to previous trauma and toxic effects of Doxorubicin on the injured mucosa. In our opinion, this technique can be used for serial intravesical administration of less toxic drugs than Doxorubicin, when the chemical effect on the mucous membrane of the urethra will not cause significant edema. However, this hypothesis requires further experimental research.

The second proposed technique was a method of transcutaneous puncture of the bladder in rats. This technique also requires the use of general anesthesia, as to determine the point of transcutaneous puncture, it is necessary to provide the rat with a horizontal position on its back. In addition, the reflex movements of the experimental animal in response to the puncture can lead to displacement of the main landmarks and damage to internal organs. When performing this technique, as well as the previous one, a series of 5 intravesical injections of the drug could not be performed by any of the experimental

rats. The aggressive cytostatic effect of Doxorubicin prevented the closure of the puncture hole. As in the previous case, this technique can be useful for studies of less toxic drugs, and also requires appropriate research.

The third proposed technique was intraoperative bladder puncture. Although, on the one hand, sealing the bladder with a collagen plate after a puncture prevents the drug from leaking into the abdominal cavity, the plate itself is a place of adhesion of cells and tissues of the surrounding organs and promotes the formation of adhesive conglomerate. Studies have shown that this method can be used for a series of no more than 3 intravesical injections. However, the need for repeated surgical interventions with significant trauma to the anterior abdominal wall and internal organs required the continuation of scientific research in this direction.

The fourth proposed technique was a method of subcutaneous fixation of the bladder for the next puncture in the experiment. Removal of the bladder from the abdomen, in theory, should have helped to avoid the formation of adhesive conglomerates, repeated surgery and the use of anesthesia for each administration of the drug. Fixation of the bladder in the subcutaneous tissue occurred, as planned, at the site of closure of the puncture opening of the bladder with a collagen plate. However, the second puncture was performed at a distance from the site of attachment of the collagen plate, which caused the leakage of Doxorubicin with the formation of fistula and urinary incontinence in the subcutaneous tissue. These complications could be avoided if the collagen plate was larger and covered and, accordingly, fixed the entire anterior wall of the bladder. This technique may be useful in the study of less toxic drugs than Doxorubicin, which also requires further experimental studies.

The fifth proposed technique was a method of serial

intravesical administration of drugs to female rats using a special catheter. This technique was the only one proposed, which met all the objectives. Thus, a series of 5 intravesical injections of Doxorubicin was performed. In this case, postoperative complications from both the postoperative wound and the abdominal cavity were absent in all cases. Intravesical administration of Doxorubicin did not require general anesthesia and was performed without any technical difficulties at all times of the study. No case of self-removal of the catheter in rats was observed. The only disadvantage of this technique, in our opinion, was the closure of the lumen of the catheter with protein and salt layers. However, this complication was eliminated without much technical difficulty. This technique can be used for experimental study of the effectiveness of intravesical use of chemotherapeutic drugs in superficial bladder cancer.

Further studies of the effectiveness of intravesical administration of chemotherapeutic drugs, in particular Doxorubicin, according to the proposed method for superficial bladder cancer in rats.

### Conclusions

1. The proposed method of serial intravesical administration of drugs in female rats using a special catheter is intraoperative antegrade injection into the bladder and urethra of a catheter with a diameter of 2 Fr and a length of 20 mm, at one end of which is a balloon with a diameter of 6 Fr which does not block the lumen of the catheter.

2. This technique, starting from 8 days after surgery, allows technically easy to perform serial intravesical injections of drugs without the use of general anesthesia and without the development of complications from the postoperative wound and abdominal cavity.

### References

- [1] Babjuk, M., Burger, M., Comp?rat, E. M., Gontero, P., Mostafid, A. H., Palou, J., ... & Zigeuner, R. (2019). European association of urology guidelines on non-muscle-invasive bladder cancer (TaT1 and carcinoma in situ)-2019 update. *European urology*, 76(5), 639-657. doi: 10.1016/j.eururo.2019.08.016
- [2] Cancer in Ukraine. (2020). *Бюлетень національного канцер-реєстру України № 21 [Bulletin of the National Cancer Registry of Ukraine № 21]*. K.: Національний інститут раку - K.: National Cancer Institute.
- [3] De Angelis, R., Sant, M., Coleman, M. P., Francisci, S., Baili, P., Pierannunzio, D., ... & Bielska-Lasota, M. (2014). Cancer survival in Europe 1999-2007 by country and age: results of EURO-CARE-5-a population-based study. *The lancet oncology*, 15(1), 23-34. doi: 10.1016/S1470-2045(13)70546-1
- [4] Diamanti, J., Mezzetti, B., Giampieri, F., Alvarez-Suarez, J. M., Quiles, J. L., Gonzalez-Alonso, A., ... & Battino, M. (2014). Doxorubicin-induced oxidative stress in rats is efficiently counteracted by dietary anthocyanin differently enriched strawberry (*Fragaria x ananassa* Duch.). *Journal of agricultural and food chemistry*, 62(18), 3935-3943. doi: 10.1021/jf405721d
- [5] Ferlay, J., Steliarova-Foucher, E., Lortet-Tieulent, J., Rosso, S., Coebergh, J. W. W., Comber, H., ... & Bray, F. (2013). Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *European journal of cancer*, 49(6), 1374-1403. doi: 10.1016/j.ejca.2012.12.027
- [6] Lee, S., Carrasco Jr, A., Meacham, R. B., & Malykhina, A. P. (2017). Transurethral Instillation Procedure in Adult Male Mouse. *JoVE (Journal of Visualized Experiments)*, (129), e56663. doi: 10.3791/56663
- [7] Lehmann, L. H., & Frohling, S. (2020). Mechanisms of cardiotoxicity of oncological therapies. *Der Internist*, 61(11), 1132-1139. doi: 10.1007/s00108-020-00881-2
- [8] Mandziuk, S., Gieroba, R., Korga, A., Matysiak, W., Jodlowska-Jedrych, B., Burdan, F., ... & Jozefczyk, A. (2015). The differential effects of green tea on dose-dependent doxorubicin toxicity. *Food & Nutrition Research*, 59(1), 29754. doi: 10.3402/fnr.v59.29754
- [9] Marcq, G., Jarry, E., Ouzaid, I., Hermieu, J. F., Henon, F., Fantoni, J. C., & Xylinas, E. (2019). Contemporary best practice in the use of neoadjuvant chemotherapy in muscle-invasive bladder cancer. *Therapeutic Advances in Urology*, 11, 1756287218823678. doi: 10.1177/1756287218823678
- [10] Oliveira, P. A., Pires, M. J., Nobrega, C., Arantes-Rodrigues, R.,

- Calado, A. M., Carrola, J., ... & Colaço, A. (2009). Technical report: Technique of bladder catheterization in female mice and rats for intravesical instillation in models of bladder cancer. *Scandinavian Journal of Laboratory Animal Sciences*, 36(1), 5-9. doi: 10.23675/sjlas.v36i1.163
- [11] Polák, Š., Žiaran, S., Mištinová, J., Bevizová, K., Danišovič, L., & Varga, I. (2012). Options for histological study of the structure and ultrastructure of human urinary bladder epithelium. *Biologia*, 67(5), 1018-1025. doi: 10.2478/s11756-012-0090-1
- [12] Pugazhendhi, A., Edison, T. N. J. I., Velmurugan, B. K., Jacob, J. A., & Karuppusamy, I. (2018). Toxicity of Doxorubicin (Dox) to different experimental organ systems. *Life sciences*, 200, 26-30. doi: 10.1016/j.lfs.2018.03.023
- [13] Schmidt, S., Kunath, F., Coles, B., Draeger, D. L., Krabbe, L. M., Dersch, R., ... & Meerpohl, J. J. (2020). Intravesical Bacillus Calmette-Guerin versus mitomycin C for Ta and T1 bladder cancer: Abridged summary of the Cochrane Review. *Investigative and clinical urology*, 61(4), 349-354. doi: 10.4111/icu.2020.61.4.349
- [14] Siegel, R. L., Miller, K. D., & Jemal, A. (2019). Cancer statistics, 2019. *CA: a cancer journal for clinicians*, 69(1), 7-34. doi: 10.3322/caac.21442
- [15] Sun, X. P., Wan, L. L., Yang, Q. J., Huo, Y., Han, Y. L., & Guo, C. (2017). Scutellarin protects against doxorubicin-induced acute cardiotoxicity and regulates its accumulation in the heart. *Archives of Pharmacal Research*, 40(7), 875-883. doi: 10.1007/s12272-017-0907-0
- [16] van der Zanden, S. Y., Qiao, X., & Neefjes, J. (2020). New insights into the activities and toxicities of the old anticancer drug doxorubicin. *The FEBS Journal*. doi: 10.1111/febs.15583
- [17] Zhang, D., Yao, L., Yu, S., Cheng, Y., Jiang, J., Ma, Q., & Yan, Z. (2020). Safety and efficacy of en bloc transurethral resection versus conventional transurethral resection for primary nonmuscle-invasive bladder cancer: a meta-analysis. *World Journal of Surgical Oncology*, 18(1), 1-12. doi: 10.1186/s12957-019-1776-4
- [18] Zhang, J., Cui, L., Han, X., Zhang, Y., Zhang, X., Chu, X., ... & Chu, L. (2017). Protective effects of tannic acid on acute doxorubicin-induced cardiotoxicity: involvement of suppression in oxidative stress, inflammation, and apoptosis. *Biomedicine & Pharmacotherapy*, 93, 1253-1260. doi: 10.1016/j.biopha.2017.07.051
- [19] Zhou, Z., Zhao, S., Lu, Y., Wu, J., Li, Y., Gao, Z., ... & Cui, Y. (2019). Meta-analysis of efficacy and safety of continuous saline bladder irrigation compared with intravesical chemotherapy after transurethral resection of bladder tumors. *World Journal of Urology*, 37(6), 1075-1084. doi: 10.1007/s00345-019-02628-7
-