

Lutsiv I., Hudyma A., Halnykina S., Deneffil O. The role of bilateral ovariectomy in the impairments of prooxidant-antioxidant balance and renal concentrating capacity in rats in the period of late manifestations of the traumatic disease and the efficacy of hormone replacement therapy. *Journal of Education, Health and Sport*. 2021;11(2):277-289. eISSN 2391-8306. DOI <http://dx.doi.org/10.12775/JEHS.2021.11.02.027> <https://apcz.umk.pl/czasopisma/index.php/JEHS/article/view/JEHS.2021.11.02.027> <https://zenodo.org/record/5153923>

The journal has had 5 points in Ministry of Science and Higher Education parametric evaluation. § 8.2) and § 12.1.2) 22.02.2019.

© The Authors 2021;

This article is published with open access at Licensee Open Journal Systems of Nicolaus Copernicus University in Torun, Poland provided the original author(s) and source are credited. This is an open access article licensed under the terms of the Creative Commons Attribution Non commercial license Share alike. (<http://creativecommons.org/licenses/by-nc-sa/4.0/>) which permits unrestricted, non commercial use, distribution and reproduction in any medium, provided the work is properly cited.

The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 11.01.2021. Revised: 10.02.2021. Accepted: 26.02.2021.

THE ROLE OF BILATERAL OVARIECTOMY IN THE IMPAIRMENTS OF PROOXIDANT-ANTIOXIDANT BALANCE AND RENAL CONCENTRATING CAPACITY IN RATS IN THE PERIOD OF LATE MANIFESTATIONS OF THE TRAUMATIC DISEASE AND THE EFFICACY OF HORMONE REPLACEMENT THERAPY

I. I. Lutsiv, A. A. Hudyma, S. O. Halnykina, O. V. Deneffil

Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine

Abstract

Introduction. Traumatic events are currently considered to be one of the topical issues. The progression of renal failure plays an important role in the pathogenesis of traumatic disease. It is essential to evaluate the ability of renal tubular epithelium to the urine osmotic concentration in order to indicate the direct renal tubular damage. A sodium-free water clearance (S-CH₂O) is a sensitive indicator reflecting the kidneys ability to concentrate the urine. It is established that the renal functional state, the resistance of the kidneys to the development of various disorders depends on the estrogen concentration. The key mechanism of the indirect effect of estrogens on the kidneys is via their direct antioxidant action. However, the role of estrogens in the pathogenesis of oxidative and functional impairments of the kidneys in the presence of cranioskeletal trauma is insufficiently studied. There is no data available on the efficacy of hormone replacement therapy under those circumstances.

The objective of research: to determine the pro-oxidant-antioxidant balance and renal concentrating capacity following the cranioskeletal trauma model in rats with bilateral

ovariectomy in the period of late manifestations of the traumatic disease and evaluate the efficacy of hormone replacement therapy.

Material and methods: The experimental studies were conducted on 64 white non-linear female rats weighing 200-220g. The experimental model of hypoestrogenism was performed through the surgical removal of gonads. The rats were subjected to the cranioskeletal model one month after removal of the gonads. Hormone replacement therapy (HRT) was used as a corrective treatment in the separate subgroup of gonadectomized rats subjected to the cranioskeletal trauma. The control groups consisted of intact animals and rats 1 month after removal of the gonads that were not injured. The renal functional state was determined via a water loading test in the control groups of animals and after 1 and 2 months of posttraumatic period. The sodium concentration, as well as the S-CH₂O value was measured in serum and urine. The content of thiobarbituric acid reactive substances (TBARs) and catalase activity were determined in renal cortex and medulla. The prooxidant/antioxidant ratio (ProAntidex) was calculated based on the above data.

The results and discussion. The conducted studies indicated the considerable decrease in ProAntidex value in renal cortex and medulla in the group of gonadectomized rats compared to the animals without gonadectomy after 1 month of the posttraumatic period, confirming the protective antioxidant role of estrogens in adequate renal function. The cranioskeletal trauma model led to the declined parameter value in renal cortex and medulla in both experimental groups after 1 month of the posttraumatic period. The prooxidant/antioxidant ratio was significantly decreased in the first month following the trauma in the gonadectomized rats as compared to the rats without the gonadectomy, and remained at the same level up to the 2nd month of the posttraumatic period. The identified abnormal value of ProAntidex in the posttraumatic period clearly affected the dynamics of S-CH₂O. This parameter was reported as decreased in both experimental groups compared to the control. However, the parameter was substantially decreased 1 and 2 months after trauma under conditions of the gonadectomy.

The administration of hexestrol and progesterone to the gonadectomized rats with trauma model resulted in the considerable increase in value of ProAntidex in renal cortex and medulla starting from the 1st day of the posttraumatic period compared to the animals without corrective medication. Moreover, it was accompanied by a statistically significant increase in S-CH₂O rate, indicating the enhancement in the functional capacity of the renal tubules.

Conclusions. The value of ProAntidex decreases in renal cortex and medulla in the group of gonadectomized female rats after 1 month of the posttraumatic period, and is

significantly lower compared to the animals without removal of the gonads. The cranioskeletal trauma model leads to the substantially declined prooxidant/antioxidant ratio value in renal cortex and medulla, which is reported as considerably greater in the group of gonadectomized animals after 1 month of the posttraumatic period, showing no tendency to enhance after 2 months of the experiment. The administration of hormone replacement therapy to the gonadectomized rats is accompanied by the increase in ProAntidex value and S-CH₂O rate compared to the animals without corrective medication.

Key words: gonadectomy; cranioskeletal trauma; kidney; prooxidant/antioxidant ratio; sodium-free water clearance.

Introduction. Traumatic events are currently considered to be one of the topical issues. Its structure is predominated by the increased incidence of combined injuries and greater frequency of cranioskeletal injuries, which are followed by the development of traumatic disease and high mortality rate [1, 2].

The development of renal failure plays a major role in the pathogenesis of traumatic disease. As described by some authors [3, 4], the early period of experimental cranioskeletal trauma is characterized by a reduced diuresis, decreased glomerular filtration rate with the abnormally high levels of nitrogen-containing compounds in the background of reduced sodium and water reabsorption as well as significantly increased protein excretion.

Prerenal failure, which, is accompanied by a reduced diuresis and decreased glomerular filtration, occurs as a result of impaired renal perfusion under conditions of the severe trauma. It is essential to evaluate the ability of renal tubular epithelium to the urine osmotic concentration in order to indicate the direct renal tubular damage [5]. A sodium-free water clearance (S-CH₂O), defined as the amount of final urine volume filtered by nephrons, is a sensitive indicator reflecting the kidneys ability to concentrate the urine. The measurement of S-CH₂O is suggested as a criterion for the early detection of acute renal impairment and the differentiation of renal and non-renal causes of renal dysfunction development [6].

It is established that the renal functional state, the resistance of the kidneys to the development of various disorders depends on the estrogen concentration [7]. The key mechanism of the indirect effect of estrogens on the kidneys is via their direct antioxidant action, which reduces the severity of free radical processes in the kidney as well as the degree of damage to renal tubular epithelial cells. [8]. However, the role of estrogens in the pathogenesis of oxidative and functional impairments of the kidneys in the presence of

cranioskeletal trauma is insufficiently studied. There is no data available on the efficacy of hormone replacement therapy under those circumstances.

The objective of research: to determine the pro-oxidant-antioxidant balance and renal concentrating capacity following the cranioskeletal trauma model in rats with bilateral ovariectomy in the period of late manifestations of the traumatic disease and evaluate the efficacy of hormone replacement therapy.

Material and methods: The experimental studies were conducted on 64 white non-linear female rats weighing 200-220g. The experimental model of hypoestrogenism was performed through the surgical removal of gonads [9]. The rats were subjected to the cranioskeletal model through a sequential measured strike to the skull with subsequent receiving of moderately severe closed craniocerebral injury and measured blow to the femur resulted in a closed femoral fracture one month after removal of the gonads [10]. The above-mentioned traumas were modeled in healthy rats in the comparison group. Hormone replacement therapy (HRT) was used as a corrective treatment via the intraperitoneal injections of hexestrol («SynestroL», LLC «Biopharma», Ukraine) at a dose of 0,1 mg·kg⁻¹ and progesterone («Progesterone», JSC «Farmak». Ukraine) at a dose of 5 mg per animal in the separate subgroup of gonadectomized rats subjected to the cranioskeletal trauma [11]. The drugs were diluted in oil for daily administration within the experimental period after cranioskeletal trauma.

The control groups consisted of intact animals and rats 1 month after removal of the gonads that were not injured

The renal functional state was determined via a water loading test in the control groups of animals and after 1 and 2 months of posttraumatic period [12]. A tap water heated to 30 °C in the volume 5% of body weight was administered intragastrically via a metal gastric tube. The urine samples were collected within two hours and the diuresis was measured. After urine collection, the rats were removed from the experiment by means of complete exsanguination from the heart. The sodium concentration in the urine and serum samples was determined using biochemical analyzer “Humalyzer-2000” and the value of S-CH₂O was subsequently measured based on the above data [12]. Furthermore, the content of thiobarbituric acid reactive substances (TBARs) [14] and catalase activity [13] were determined in renal cortex and medulla of the experimental animals and the prooxidant/antioxidant index (ProAntidex) [15] was calculated by the ratio of the values.

The international standards for the humane treatment of animals were followed in compliance with the regulations of «The European Convention for the protection of vertebrate

animals used for experimental and other scientific purposes» (European Convention, 1986) in experimental work with laboratory rats. All surgical procedures and trauma models were performed under thiopental sodium anesthesia (40 mg·kg⁻¹). The test animals were euthanized through complete exsanguination from the heart.

All obtained data were processed to the statistical analysis by the nonparametric The Mann–Whitney U test using STATISTICA 10.0 software («StatSoft Inc.», USA).

The results and discussion. Table 1 illustrates that the value of ProAntidex in the control group without gonadectomy was nearly the same in both renal cortex and renal medulla ($p > 0,05$).

Table 1 – The value of ProAntidex in renal cortex and medulla (CU) in the dynamics of cranioskeletal trauma model in rats with bilateral ovariectomy (Me (LQ; UQ)) – median (lower and upper quartiles)

Experimental conditions	Kidney layers	Control group	Observation period	
			1 month	2 months
Preserved gonads	Renal cortex	1,02 (0,87; 1,22) (n=6)	0,41* (0,40; 0,42) (n=6)	0,90^ (0,80; 1,05) (n=9)
	Renal medulla	1,00 (0,94; 1,08) (n=6)	0,25*# (0,20; 0,27) (n=6)	0,75*^ (0,68; 0,81) (n=9)
Gonadectomy	Renal cortex	0,66 (0,65; 0,82) (n=6)	0,24* (0,21; 0,26) (n=7)	0,23* (0,13; 0,28) (n=6)
	Renal medulla	0,54# (0,53; 0,56) (n=6)	0,14*# (0,14; 0,16) (n=7)	0,16*# (0,11; 0,18) (n=6)
p ₁		<0,05	<0,05	<0,05
p ₂		<0,05	<0,05	<0,05

Notes:

- * – the differences concerning the control group are statistically significant ($p < 0,05$);
- # – the differences in value of parameter between the renal and cortex medulla in the groups of animals with preserved gonads and gonadectomy are statistically significant ($p < 0,05$);
- ^ – the differences in the value of parameter between the 1st and 2nd month of the experiment are statistically significant ($p < 0,05$);
- p₁ – the probability of differences in the value of parameter in renal cortex between the groups of animals with preserved gonads and gonadectomy.;
- p₂ – the probability of differences in the value of parameter in renal medulla between the groups of animals with preserved gonads and gonadectomy.

The parameter was substantially decreased in renal medulla under conditions of the surgical removal of gonads (by 18,2 %. $p < 0,05$). A combined craniocerebral and femur trauma resulted in the reduced ProAntidex after 1 month among all experimental groups: in renal cortex of rats without gonadectomy by 59,8 % ($p < 0,05$), in renal medulla of rats with preserved gonads by 75,0 % ($p < 0,05$), in renal cortex of rats with gonadectomy by 63,6 % ($p < 0,05$) and in renal medulla of rats with the surgical removal of gonads by 74,1 % ($p < 0,05$). The value of studied parameter in renal medulla was substantially lower than the corresponding value in renal cortex in rats without gonadectomy after 1 month of the posttraumatic period (by 39,0 %, $p < 0,05$).

The ProAntidex appeared increased after 2 months of the posttraumatic period compared to the previous observation period in both renal cortex and medulla of the animals without gonadectomy (by 2,20 times, $p < 0,05$ and by 2,00 times, respectively, $p < 0,05$). It should be emphasized that the parameter almost reached the level of the control group in renal cortex ($p > 0,05$), but remained considerably decreased in renal medulla compared to the control (by 25,0 %, $p < 0,05$).

The results of ProAntidex comparison in the experimental group of rats with gonadectomy indicated that the value in both kidney layers continued to be at the level of the previous observation period ($p > 0,05$). The parameter remained considerably lower in renal medulla than in renal cortex at all observation periods (by 41,7%, $p < 0,05$ and 74,4 %, $p < 0,05$ respectively, $p_1 < 0,05$).

The intergroup comparison of the parameter demonstrated a statistically significantly lower value of ProAntidex in renal cortex in the group of animals with removed gonads than in rats with preserved gonads within the posttraumatic period (by 41,4 and 74,4 %, respectively, $p_1 < 0,05$). Moreover, the parameter also appeared considerably decreased in renal medulla in the group of animals with gonadectomy (by 44,0 and 78,7 %, respectively, $p_2 < 0,05$).

The S-CH₂O determination evidenced a nearly the same parameter value in the intact animals and gonadectomized rats of the control group (Table 2) ($p > 0,05$). The studied parameter was found to be lower in the group of animals without gonadectomy compared to the control group 1 month after the cranioskeletal trauma (by 23,1 %, $p < 0,05$). The parameter appeared increased by 21,6% ($p < 0,05$) after 2 months of the posttraumatic period compared to the previous observation period, but was substantially decreased in contrast to control level not reaching the level of this group ($p < 0,05$).

Table 2 – The dynamics of sodium-free water clearance ($\text{ml}\cdot 2\text{hr}^{-1}\cdot 100\cdot \text{g}^{-1}$) in the course of cranoskeletal trauma model in rats with bilateral ovariectomy (Me (LQ; UQ)) – median (lower and upper quartiles)

Experimental conditions	Control group	Observation period		p
		1 month	2 months	
Preserved gonads	4,16 4,06; 4,16 (n=6)	3,20* 3,09; 3,55 (n=6)	3,89* 3,68; 3,89 (n=6)	<0,05
Gonadectomy	3,97 3,90; 4,05 (n=6)	2,44*# 2,27; 2,61 (n=6)	2,39*# 2,26; 2,51 (n=6)	>0,05

Notes:

1. * – the differences concerning the control group are statistically significant ($p < 0,05$);
2. # – the differences in value of parameter between the groups of animals with preserved gonads and gonadectomy are statistically significant ($p < 0,05$ ($p < 0,05$));
3. p – the probability of differences in the value of parameter between the 1st and 2nd month of the post-operative period.

The results obtained from the analysis on the S-CH₂O value in the group of gonadectomized rats indicated that the parameter decreased compared to the control after 1 month and 2 months of the posttraumatic period by 38,5% ($p < 0,05$) and 39,8% ($p < 0,05$), respectively. As can be seen, the differences between the results of the 1st and 2nd months of the posttraumatic period were not significant in this experimental group ($p > 0,05$). The intergroup comparison of the S-CH₂O within the posttraumatic period evidenced that after either 1 month or 2 months the gonadectomy resulted in statistically significantly decreased value of the parameter compared to the group without gonadectomy (by 23,8 and 38,6 %, respectively $p < 0,05$).

The administration of hormone replacement therapy (Table 3) contributed to the statistically significant increase in ProAntidex value in renal cortex of the gonadoectomized rats compared to the animals without corrective medication (by 2,39 times, $p_2 < 0,05$), but the parameter was substantially decreased in contrast to both the control level (by 46,1 %, $p < 0,05$) and the animals with preserved gonads (by 38,9 %, $p_1 < 0,05$).

Furthermore, the hormone replacement therapy led to the increase in the value of ProAntidex in renal medulla of the rats subjected to the removal of the gonads compared to the animals without corrective medication (by 2,75 times, $p_2 < 0,05$), but the parameter was substantially decreased in contrast to both the control level (by 56,0 %, $p < 0,05$) and the

animals with preserved gonads (by 41,3 %, $p_1 < 0,05$).

Table 3 – The effect of combined hormone replacement therapy on the value of ProAntidex of renal cortex and medulla (CU) and the sodium-free water clearance ($\text{ml} \cdot 2\text{hr}^{-1} \cdot 100 \cdot \text{g}^{-1}$) 2 months after combined cranioskeletal trauma model in rats with bilateral ovariectomy ((Me (LQ; UQ)) – median (lower and upper quartiles)

Parameter	Control group. Preserved gonads (n=6)	2 month after trauma		
		Without corrective medication		Gonadectomy + replacement therapy (n=6)
		Preserved gonads (n=6)	Gonadectomy (n=6)	
ProAntidex of renal cortex	1,02 0,87; 1,22	0,90 0,80; 1,05	0,23* 0,13; 0,28 $p_1 < 0,05$	0,55* 0,51; 0,64 $p_1 < 0,05$ $p_2 < 0,05$
ProAntidex of renal medulla	1,00 0,94; 1,08	0,75* 0,68; 0,81	0,16* 0,11; 0,18 $p_1 < 0,05$	0,44* 0,38; 0,46 $p_1 < 0,05$
Sodium-free water clearance	4,16 4,06; 4,16	3,89* 3,68; 3,89	2,37* 2,26; 2,51 $p_1 < 0,05$	3,03* 2,96; 3,14 $p_1 < 0,05$ $p_2 < 0,05$

Notes:

- * – the differences concerning the control group are statistically significant ($p < 0,05$);
- p_1 – the probability of differences in the value of parameter concerning the group without corrective medication with preserved gonads after 2 months of the posttraumatic period;
- p_2 – the probability of differences in the value of parameter concerning the group without corrective medication with gonadectomy after 2 months of the posttraumatic period.

The results obtained from the analysis on the S-CH₂O value under conditions of the administration of HRT in the group of gonadectomized rats indicated that the parameter increased compared to the animals without corrective medication by 27,8 % ($p_2 < 0,05$), but was substantially decreased not reaching the level of both the control group and animals with preserved gonads (by 27,2 %, $p < 0,05$ and by 24,5 %, $p_1 < 0,05$, respectively).

The obtained results evidenced that after 1 month of the posttraumatic period the gonadectomy is followed by a marked decrease in the value of ProAntidex in renal cortex and medulla compared to the animals with preserved gonads. The acquired data indicates the dominance of the pro-oxidant mechanisms in the group of gonadectomized rats and confirms

the protective antioxidant role of estrogens in adequate renal function, as reported in a number of publications [16].

The cranioskeletal trauma model led to a decrease in the studied parameter after 1 month of the posttraumatic period in renal cortex and medulla of both experimental groups. The value of ProAntidex considerably increased after 2 months of the posttraumatic period in animals without gonadectomy, reaching the level of control group in renal cortex. The prooxidant/antioxidant ratio was significantly decreased in the first month following the trauma in the gonadectomized rats as compared to the rats without the gonadectomy, and remained at the same level up to the 2nd month of the posttraumatic period. Consequently, the pathogenic factors of cranioskeletal trauma exert a pronounced prooxidant effect on the kidney, even after 1 month of the posttraumatic period, but the hypoestrogenism leads to more marked abnormalities, which do not subside up to 2 months of the posttraumatic period. It can be substantiated that in conditions of estrogen deficiency the failure of physiological antioxidant defense mechanism in kidney occurs rapidly and is not compensated up to 2 months of the experiment, which, once more, proved the fundamental role of estrogens in antioxidant defense mechanism in kidney in the presence of traumatic disease.

The intensification of prooxidant mechanisms in the kidney initially affects the renal tubules under trauma conditions [17]. Therefore, the identified abnormal value of ProAntidex in the posttraumatic period clearly affected the dynamics of S-CH₂O. Our findings revealed the decrease of the studied parameter in both experimental groups compared to the control. However, the ProAntidex value was considerably decreased under conditions of gonadectome after 1 and 2 months following the trauma.

The acquired data suggest that the cranioskeletal trauma conduces to the development of renal cause of an impaired renal function, which appeared long-lasting without the tendency to the enhancement in gonadectomized rats, while in rats without surgical removal of the gonads the renal concentrating capacity considerably increases reaching the control level 2 month after posttraumatic period.

The obtained results, additionally, evidences a strong nephroprotective role of estrogens in the presence of cranioskeletal trauma prompting us to study the efficacy of combined hormone replacement therapy. The administration of hexestrol and progesterone to the gonadectomized rats subjected to trauma model resulted in the considerable increase in value of ProAntidex in renal cortex and medulla starting from the 1st day of the posttraumatic period compared to the animals without corrective medication. Moreover, it was accompanied

by a statistically significant increase in S-CH₂O rate, indicating the enhancement in the functional capacity of the renal tubules.

Consequently, estrogens play a fundamental role in the ensuring adequate antioxidant-prooxidant balance in renal cortex and medulla as well as in the regulation of renal concentrating capacity, which indicates the normal renal tubular transport function. It can be substantiated that a short-course of combined hormone replacement therapy will increase the body's resistance to the pathogenic factors of traumatic disease and reduce the risk of the development of multiple organ dysfunction syndrome in female trauma-patients in the postmenopausal period, which substantiate the pertinence of further in-depth preclinical study.

Conclusions.

1. The value of ProAntidex significantly decreases in renal cortex and medulla in the group of gonadectomized female rats compared to the animals without gonadectomy after 1 month of the posttraumatic period.

2. The cranioskeletal trauma model leads to the substantially declined prooxidant/antioxidant ratio value in renal cortex and medulla, which is reported as considerably greater in the group of gonadectomized animals after 1 month of the posttraumatic period, showing no tendency to enhance after 2 months of the experiment.

3. The administration of hormone replacement therapy to the gonadectomized rats is accompanied by the increase in ProAntidex value and S-CH₂O rate compared to the animals without corrective medication. Notwithstanding that the studied parameters do not reach the level of both the control group and animals with preserved gonads, the obtained results indicate the prospects of combined hormone replacement therapy in the correction of pathogenic manifestations of traumatic disease in hypoestrogenic state.

Perspectives of further research. The prospect of being able to increase the number of studies on the functional status of internal organs under the conditions of trauma in the background of hypoestrogenism and the administration of hormone replacement therapy serves as a rational incentive for future research.

References

1. Huriev S. O., Fil A. Yu., Tanasiienko O. M. (2015) Analiz bezposeredikh prychnyn smerti u postrazhdalykh iz politravmoiu ta poshkodzhenniam skeleta. [Analysis of the immediate causes of death of the injured affected by polytrauma and skeletal damage]. *Travma*. vol.16, no. 4, pp. 7-10. [in Ukrainian].

2. Ziablitsev S. V., Yelskyi V. M. (2020) Syndromy travmatychnoi khvoroby pry cherepno-mozkovii travmi [Syndromes of traumatic disease in traumatic brain injury] Kramatorsk: Kashtan, 2020. – 264 p. [in Ukrainian].
3. Merliev D. I., Hudyma A. A. (2014) Dynamika funktsionalnoho stanu nyrok v umovakh skeletnoi, cherepno-mozkovoï ta poïednanoi travmy u period rannikh proïaviv travmatychnoi khvoroby [The dynamics of functional state of the kidneys under conditions of skeletal, craniocerebral and combined trauma in the period of early manifestations of the traumatic disease]. *Visnyk naukovykh doslidzhen - Bulletin of Scientific Research*. no.2, pp. 90-93. [in Ukrainian].
4. Kovalov V. V., Popovych D. V. (2018) Dynamika funktsionalnoho stanu nyrok u rannii period pislia nanesennia skeletnoi travmy riznoi tiazhkosti, uskladnenoï krovovtratoiu [Dynamics of functional condition of kidneys in the early period after skeletal injury of different severe complicated by blood loss]. *Visnyk naukovykh doslidzhen - Bulletin of Scientific Research*. no.4, pp. 184-189. [in Ukrainian].
5. Kolesnikova T.I. (2008) Osmoticheskiy gomeostaz u bolnykh v kriticheskikh sostoyaniyakh razlichnogo geneza [Osmotic homeostasis in patients in critical conditions of various genesis]. Donetsk: *Pytannia eksperymentalnoi ta klinichnoi medytsyny*. vol.2, no.12, pp. 22-25. [in Russian].
6. Cherniy V. I., Kolesnikova T. I., Gorodnik G. A. (2008) Klirens svobodnoy vodyi kak kriteriy ranney diagnostiki porazheniya vyidelitelnoy funktsii pochek u neyroreanimatsionnykh bolnykh s ugrozoy razvitiya ostroy pochechnoy nedostatochnosti [Free water clearance as a criterion for the early diagnosis of impairment of renal excretory function in neurocritical patients with the risk of development of acute renal failure]. *Ukrainskyi zhurnal ekstremalnoi medytsyny imeni H. O. Mozhaieva*. no.4, pp. 31-35. [in Russian].
7. Gluhovschi G., Gluhovschi A., Anastasiu D. (2012) Chronic kidney disease and the involvement of estrogen hormones in its pathogenesis and progression / [et al.]. *Rom. J. Intern. Med.* vol. 50 (2), pp. 135–144.
8. Muller V., Losonczy G., Heemann U. (2002) Sexual dimorphism in renal ischemia-reperfusion injury in rats: possible role of endothelin / [et al.]. *Kidney Int.* vol. 62 (4), pp. 1364–1371.
9. Kamyshnikov V. S., Kovalenko Yu. D., Kohnovich N. N. (2003) Kliniko-laboratornyi monitoring biokhimicheskikh markerov osteoporoza v eksperimente [Clinical and laboratory monitoring of the biochemical markers of osteoporosis in the experiment].

Dostyzhennia medytsynskoi nauky Belarusy.no.7. Retrieved from http://med.by/dmn/book.php?book=03-18_10. [in Russian].

10. Borys R. M., Hozhenko A. I., Hudyma A. A. (2013) Dynamika pokaznykiv peroksydnoho okysnennia lipidiv u period rannikh i piznikh proiaviv travmatychnoi khvoroby za umov eksperymentalnoi kranioskeletnoi travmy ta yii korektsii klitynnoiu terapiieiu [The dynamics of indicators of lipid peroxidation in the period of early and late manifestations of traumatic disease in the presence of cranoskeletal trauma and its correction by cell therapy]. *Klinichna ta eksperymentalna patolohiia - Clinical & experimental pathology*, vol. XII, no. 2 (44), pp. 31–34. [in Ukrainian].

11. Mashevskiy A. S., Lukovych I. A., Datsko V. A. (2016, October 20-21) Strukturni porushennia pechinky, holovnoho mozku ta kistky shchuriv pry kranioskeletnii travmi na foni eksperymentalnoho hipoestrogennoho osteoporozu cherez 2 misiatsi posttravmatychnoho periodu ta yikh korektsiia zamisnoiu hormonalnoiu terapiieiu [Structural abnormalities of the liver, brain and bone of rats with cranoskeletal trauma model in the background of experimental hypoestrogenic osteoporosis after 2 months of the post-traumatic period and their correction by hormone replacement therapy] *Prykladni aspekty morfolohii: zb. mater. nauk.-prakt. konf.* Ternopil, pp. 107-109. [in Ukrainian].

12. Rohovyi Yu. Ye. (2012) Patofiziolohiia hepatorenalnoho syndromu na poliurychnii stadii sulemovoi nefropatii [Pathophysiology of during the polyuric phase of sublimate nephropathy]. Chernivtsi, Misto. 200 p. [in Ukrainian].

13. Korolyuk M. A., Ivanova L. I., Mayorova I. G., Tokarev V. E. (1988) Metod opredeleniya aktivnosti katalazyi [Method for determination of catalase activity]. *Laboratornoe delo*. no.1, pp. 16–19. [in Russian].

14. O. V. Stefanov (Eds.). (2001) Doklinichni doslidzhennia likarskykh zasobiv: metodychni rekomendatsii [Preclinical researches of drugs: guidelines]. K. : Avitsenna, 528 p. [in Ukrainian].

15. A. P. Levytskyi, V. M. Pochtar, O. A. Makarenko, L. I. Hrydina (2006) Antyoksydantno-prooksydantnyi indeks syrovatky krovi shchuriv z eksperymentalnym stomatytom i yoho korektsiia zubnymy eliksiyamy [The serum prooxidant-antioxidant balance of rats with an experimental stomatitis and its correction by dental elixirs]. *Odeskyi medychnyi zhurnal*. no.1, pp.22-25. [in Ukrainian].

16. Lee H. R., Kim T. H., Choi K. C. (2012) Functions and physiological roles of two types of estrogen receptors, ERalpha and ERbeta, identified by estrogen receptor knockout mouse. *Lab Anim Res*. vol. 28, pp. 71—76.

17. Shatskyi V.V. (2019) Osoblyvosti proksymalnoho i dystalnoho transportu natriiu za umov hostroi krovovraty, uskladnenoj ishemiiu-repefuziieu kintsivky, ta yikh korektsiia v eksperymenti [Features of proximal and distal transport of sodium in acute blood loss, complicated by ischemia-reperfusion of extremity, and their correction in the experiment]. *Medychna ta klinichna khimiia - Medical and Clinical Chemistry*. vol.21, no.4, pp.127-133. [in Ukrainian].