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ORIGINAL PAPER

ORGANOMETRIC CHANGES OF RATS THYMUS AFTER XENOBIOTICS EXPOSURE

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Abstract

Background. Pollution of the environment poses major risks for human health. Chronic exposure to some well-absorbed but slowly eliminated xenobiotics can lead to their bioaccumulation in living organisms.
The objective of the study was to describe the organometric changes occurring in thymus of male rats under tryglycidyl ether of polyoxypropylenetriol exposure.
Material and methods. 40 WAG matured male rats were divided randomly into two groups. The first group served as a control included 8 animals. The second group of 32 rats, 8 rodents in each, were treated via gavage by aqueous solutions of tryglycidyl ether of polyoxypropylenetriol (TEPPT) in a dose of 1/10 LD_{50} in conversion to 5.75 g/kg during 7, 15, 30, 45 days. All animals were sacrificed on the term defined by experimental design. Thymus specimens were dissected out and measurements of the linear dimensions (length, width, height) using digital caliper were taken. The mass and volume of the thymus were counted. Limits of the thymus morphometric indices' variability in intact and experimental groups were calculated.
Results. The research indicates that tryglycidyl ether of polyoxypropylenetriol exposure caused marked organometric changes in rats' thymus. 100% effect on all morphometric indices of the thymus under impact of TEEPT in a dose of 1/10 LD_{50} on the 7th, 15th, 30th

Résumé

Changements organométriques du thymus des rats après l'influence de xénobiotiques

Contexte. La pollution de l'environnement et son influence sur le corps représentent un grand problème pour la médecine, car elles sont accompagnées d'une augmentation de l'incidence parmi la population. L'exposition chronique à certains xénobiotiques bien absorbés mais éliminés lentement peut entraîner leur bioaccumulation dans l'organisme vivant.
Objectifs. L'étude a été menée pour décrire les modifications et les altérations organométriques survenant dans le thymus de rats mâles exposés au tryglycidyl éther ou au poly oxypropylènetriol.
Méthodes. 40 rats mâles matures WAG ont été divisés de manière aléatoire en deux groupes. Le premier groupe a servi de témoin constitué de 8 animaux. Le deuxième groupe de 32 rats, chacun composé de 8 rongeurs, a été traité par gavage à l'aide de solutions aqueuses de TEPPT au 1/10 LD_{50} converties à 5,75 g kg pendant 7, 15, 30 et 45 jours. Tous les animaux ont été sacrifiés au terme défini par le plan expérimental. Les échantillons de thymus ont été disséqués et des mesures des dimensions linéaires (longueur, largeur, hauteur) à l'aide d'un pied à coulisse numérique ont été prises. La masse et le volume du thymus ont été comptés. Les limites de la variabilité des indices

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and 45th day was noted. However, more pronounced changes were observed on 7th and 30th days. The study revealed that IndT of the control group, which is related to the length and width of the thymus, has the greatest limits of the parameters' fluctuations and their significant variability. IndHL of the control group, which is associated with the height and length of the thymus, has the lowest fluctuation limits of the parameters. In our opinion, this is connected, first of all, with the peculiarities of the structure and form of the rats' thymus.

Conclusions. Thymus shows active response on the induced xenobiotic and these data can be successfully extrapolated from experimental animals to humans.

Keywords: thymus, rats, xenobiotics, organometry, polyethers.

morphométriques du thymus dans les groupes intact et expérimental ont été calculées.

Résultats. Les recherches indiquent que l'exposition à l'éther de glycidyle de polyoxypropylènetriol a causé des modifications organométriques marquées du thymus chez le rat. Un effet de 100% sur tous les indices morphométriques du thymus sous l'impact du TEEPT à une dose de 1/10 LD_{50} les 7ème, 15ème, 30ème et 45ème jours a été noté. Cependant, des changements plus prononcés ont été observés les 7-ème et 30-ème jours. L'étude a révélé que l'IndT du groupe de contrôle, qui est lié à la longueur et à la largeur du thymus, est celle qui limite le plus les fluctuations des paramètres et leur variabilité significative. IndHL du groupe de contrôle, qui est associé à la hauteur et à la longueur du thymus, a les limites de fluctuation les plus basses des paramètres. À notre avis, cela est liée, tout d'abord, aux particularités de la structure et de la forme du thymus du rat.

Conclusions. On déduit que le thymus en étude a une réponse active au xénobiotique induit et ces données peuvent être extrapolées avec succès d'animaux expérimentaux à l'être humain.

Mots-clés: thymus, rats, xénobiotiques, organométrie, polyéthers.

Introduction

Pollution of the environment is, undoubtedly, one of the most pressing problems of our time. The number of new chemicals increases annually, and the prevalence of harmful effects of previously known pollutants is rapidly increasing[1]. Along with already existing occupational diseases, new terms arise – ecological pathology and environmental diseases. It is known that under the influence of xenobiotics initially there are imperceptible changes in organs and tissues, and subsequently they explicit pathology at the molecular and cellular levels and, finally, at the macro level. Adaptation capabilities of the human body are not limitless, therefore, there is an urgent need for the immediate solution of these global problems of mankind.

Every day we encounter various chemicals that are in household chemicals, used as dyes in food, accumulate in soil and water, even included in the pharmaceutical products[2,3]. Therefore, xenobiotics enter the body in all possible ways: by inhalation, percutaneously, enterally.

Some of the most common and toxic substances that lead to significant pathological changes are simple polyesters[4]. Quite a number of studies relates to the study of the xenobiotics effects, occurring in the conditions of their influence on the body, in particular on the organs of the immune system. It is known that the immune system is particularly sensitive to the concentration of chemicals that are not yet toxic to other systems of the body[5,6].

However, the morphological aspects remain virtually unheard of. Only in certain scientific papers were studied the patterns of morphological changes in the thymus and spleen when polyesters were introduced into drinking water[7]. It should be noted that the severity of this problem is confirmed by the newly formed science – immunotoxicology, which deals with the problems of the influence of xenobiotics on the organs of the immune system.

Widespread xenobiotics include simple polyesters, which are produced by the process of polycondensation of cyclic oxides or polycondensation of glycols. According to the physical and chemical properties and features of the molecular structure, they belong to non-ionogenic surfactants. Simple polyesters are characterized by quite significant amounts of synthesis, widely used in the national economy, especially in automotive, mechanical engineering, chemical industry, electrical engineering and furniture industry[8]. Annually, industrial production introduces dozens of new polyols, which carry a potential and real danger to the population and are characterized by significant amounts of synthesis.

The objective of the study was to describe the organometric changes occurring in thymus of male rats under tryglycidyl ether of polyoxypropylenetriol exposure. To detect pathological changes in the thymus we selected xenobiotic that requires a detailed study of its effects through widespread prevalence. Tryglycidyl ether of polyoxypropylenetriol (TEPPT) is used as a basis for the industrial release of plastics, polyurethane foam, paint and varnish materials, even wet wipes made from fibers of this chemical.

Materials and methods

Animals

The following experimental work was conducted on 40 WAG matured male rats with initial body weight of 180-200 g. White rats were used in the experiment because the structure of their organs of the immune system is not fundamentally different from that of humans. Keeping and manipulation of animals were carried out in compliance with European Convention for the Protection of Vertebrate Animal (Strasbourg, 18.03.1986), principles of Ukrainian law No 3447-IV about the protection of animals from cruel treatment. Experimental protocols were approved by the ethical committee of the Faculty of Medicine, Kharkiv National Medical University.

Experimental design

Rodents were randomly divided into two groups. The first group served as a control (8 animals) and was fed a regular diet and received appropriate amount of water. The second group of rats (32 animals divided into 4 subgroups by 8 in each) was treated via gastric gavage during 7, 15, 30, 45 days by aqueous solutions of TEPPT in does 1/10 LD_{50} in conversion to 5.75 g/kg. Intact and experimental rats used in the study were culled by cervical dislocation of the neck on the day determined by experiment using invented instrument reported in patent[9]. The thymus was dissected with adjacent adipose and connective tissue in order to save its structure for further investigation with the help of original instrument which has been described in detail in patent[10].

Organometry

According to the term determined by experimental design, in each group of animals was simultaneously isolated the entire complex of mediastinum with the primary lymphoid organ – thymus. Initially, organ, without its separation, in order to preserve the original topographic anatomical features, underwent the measurement of the linear dimensions using a digital caliper. During the experiment, photographing of its individual stages was carried out. Further, the animal's immune organ was carefully extracted from adipose tissue and weighed using laboratory weights with an accuracy of 0.25 mg. The volume of organs was calculated using a graduated tube (by displaced fluid volume). The data obtained as a result of morphometric studies, were recorded in protocols, and the average values were noted into consolidated individual cards.

To determine the limits of the variability of the morphometric indices of the thymus method of calculating its indexes using the formulas is used:

$$\mathbf{Ind\ T = \frac{W\ of\ thymus}{L\ of\ thymus} \times 100};$$

$$\mathbf{Ind\ HW = \frac{H}{W} \times 100};\ \mathbf{Ind\ HL = \frac{H}{L} \times 100},$$

where «W» – width, «L» – length, H – height.

The determination of the length, width and height of the organ by means of a digital caliper was counted with an accuracy of 0.05 mm. The data of the organometry were exported to MS office professional 2010 s/n 02260-556-0110075-48150 and Excel, Word s / n 02260-556-0110075-48150 (owned by the Kharkov National University of Radio Electronics) for further evaluation of the reliability of the differences was used nonparametric criteria of Mann-Whitney. Under the width was understood the small axis of the projection of the organ in the frontal plane. The organ length corresponded to the large axis of the thymus projection in the frontal plane. Under the height was understood the small axis of the projection of the gland in the sagittal plane.

Results

During the investigation of the thymus of mature male rats of the WAG line, it was found that the thymus consists of two lobules of different sizes – the right and left, connected by a loose connecting tissue. Sometimes between the major lobules occurs an intermediate one. In rare cases thymus may consist of one or three lobules and very rarely from a larger number of particles (up to 6). The mass and volume of the control group are described in Table 1, Table 2.

Under the impact of TEPPT at a dose of 1/10 LD_{50}, stable decrease in the thymus mass is considered to be significant compared to the control group of rats. The thymus mass index varies from min = 140×10^{-6} kg to max = 210×10^{-6} kg, with an average value of 173.5×10^{-6} kg up to 184.5×10^{-6} kg. So, we noticed the largest change in the thymus mass on the 7th and

Table 1. Indicators of thymus mass of control group on 7, 15, 30, 45 days of the experiment ($\times 10^{-6}$ kg)

mass	*7 day*	*15 day*	*30 day*	*45 day*
max	310.00	340.00	370.00	320.00
min	210.00	220.00	210.00	230.00
average value	265.00	287.50	304.38	295.13

Table 2. Indicators of thymus volume of control group on 7, 15, 30, 45 days of the experiment ($\times 10^{-9}$ m^3)

volume	*7 day*	*15 day*	*30 day*	*45 day*
max	681.81	1031.18	803.33	1117.85
min	201.60	187.19	311.04	190.00
average value	354.25	463.70	546.77	592.70

Table 3. Comparative analysis of rats' thymus mass of control and experimental groups under the influence of TEPPT in a dose 1/10LD_{50} ($\times 10^{-6}$ kg)

Indicator	*Control group 7th day*	*Experi mental Group 7th day*	*Control group 15th day*	*Experi mental Group 15th day*	*Control group 30th day*	*Experi mental Group 15th day*	*Control group 45th day*	*Experi mental Group 15th day*
%		34.43		35.83		40.62		39.90
max	310.00	200.00	340.00	220.00	370.00	230.00	320.00	210.00
min	210.00	140.00	220.00	150.00	210.00	140.00	230.00	160.00
average value	265.00	173.75	287.50	177.38	304.38	180.75	295.13	184.50

Table 4. Comparative analysis of rats' thymus width of control and experimental groups under the influence of TEPPT in a dose 1/10LD_{50} ($\times 10^{-3}$ m)

Indicator	*Control group 7th day*	*Experi mental group 7th day*	*Control group 15th day*	*Experi mental group 15th day*	*Control group 15th day*	*Experi mental group 30th day*	*Control group 15th day*	*Experi mental group 45th day*
%		34.91		32.61		11.67		36.47
max	20.00	14.60	20.70	15.60	20.60	60.00	21.30	109.00
min	8.50	4.60	9.10	4.30	8.90	5.40	9.50	5.80
average value	13.71	8.93	14.91	10.05	16.18	18.06	16.86	23.01

30th days of the experiment, with an average value of 136,13$\times 10^{-6}$ kg and 180,75$\times 10^{-6}$ kg, respectively. On 7th day, the change in the thymus mass was equal to 34.43%, and 40.62% on the 30th day (Fig. 1, Table 3).

The lowest indicator of the thymus volume was recorded on the 7th and 15th day of the experiment and amounted to an average of 83.63$\times 10^{-9}$ m^3 and 137.43$\times 10^{-9}$ m^3, respectively. It should also be noted that the highest rates of both mass and thymus volume in the experimental group of rats were on the 45th day and had an average value of 184.5$\times 10^{-6}$ kg and 317.32$\times 10^{-9}$ m^3 respectively. It is found that these parameters on the 45th day of the experiment are most closely related to the minimal normal parameters of the rats' control group, but nevertheless much less than them (Fig. 2).

The study of the morphometric parameters of the thymus in the experimental groups of rats has established a significant reduction of all parameters and their deviation from the parameters of those in control group.

Thus, the lowest indicators of the thymus width were noted on the 7th and 15th days of the experiment and amounted to an average of 8.93$\times 10^{-3}$ m and 10.05$\times 10^{-3}$ m, respectively. On the 7th day, the change in the thymus width was equal to 34.91% on average and 32.61% on the 30th day. These values ranged from min = 4.3$\times 10^{-3}$ m to max = 109.0$\times 10^{-3}$ m, the average value was from 8.93$\times 10^{-3}$ m to 23.01$\times 10^{-3}$ m. It was established, that these parameters on the 45th day of the experiment are most closely related to the minimum

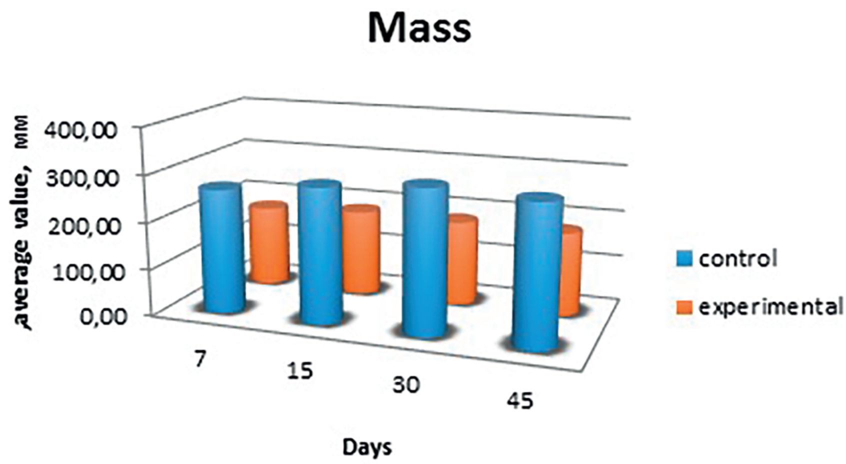

Fig 1. Indicators of thymus mass in control and experimental groups under the influence of TEPPT in a dose of 1/10 LD_{50} on 7, 15, 30, 45 days of the experiment

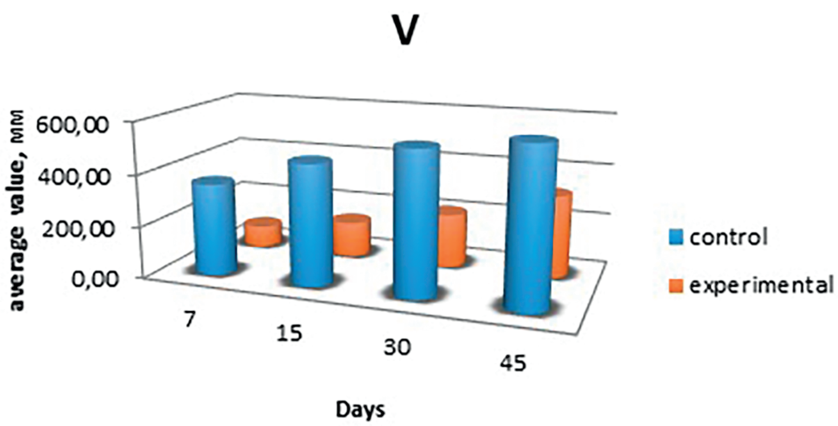

Fig. 2. Indicators of thymus volume in control and experimental groups under the influence of TEPPT in a dose of 1/10 LD_{50} on 7, 15, 30, 45 days of the experiment (×10^{-9} m^3).

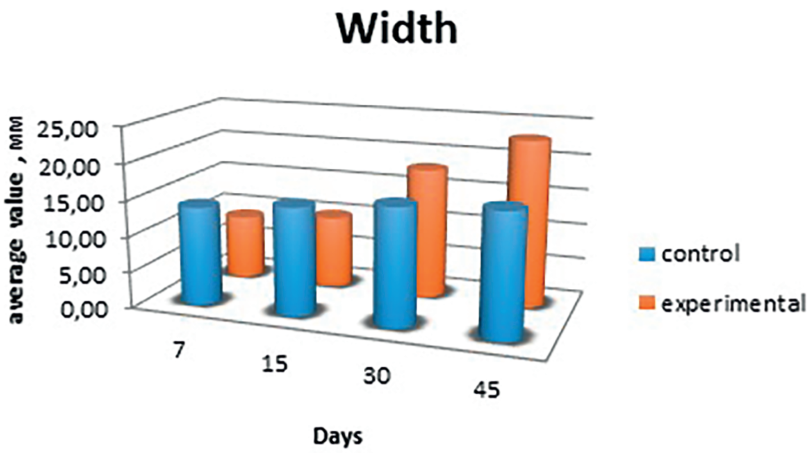

Fig. 3. Indicators of thymus width in control and experimental groups under the influence of TEPPT in a dose of 1/10 LD_{50} on 7, 15, 30, 45 days of the experiment (×10^{-3} m).

parameters of the norm of the control group of rats – the average value is 23,01×10^{-3} m (Fig. 3, Table 4).

The lowest indicators of thymus length were noted on the 7th and 30th days of the experiment and amounted to an average of 10.23×10^{-3} m and 18.13×10^{-3} m, respectively. On 7th day, the change in the thymus length was equal to an average of 41.99%, and on the 30th day 34.28%. These indicators ranged

Table 5. Comparative analysis of rats' thymus length of control group and experimental under the influence of TEPPT in a dose 1/10LD_{50} ($\times 10^{-3}$ m)

Indicator	*Control group 7th day*	*Experi mental group 7th day*	*Control group 15th day*	*Experi mental group 15th day*	*Control group 30th day*	*Experi mental group 30th day*	*Control group 45th day*	*Experi mental group 45th day*
%		41.99		35.91		34.28		28.70
max	22.00	13.40	22.50	15.20	21.80	15.60	23.60	16.30
min	14.00	8.10	16.50	10.30	15.70	9.80	14.80	10.20
average value	17.63	10.23	19.74	12.65	18.13	11.91	19.16	13.66

Table 6. Comparative analysis of rats' thymus height of control group and experimental under the influence of TEPPT in a dose 1/10LD_{50} ($\times 10^{-3}$ m)

Indicator	*Control group 7th day*	*Experi mental group 7th day*	*Control group 7th day*	*Experi mental group 7th day*	*Control group 7th day*	*Experi mental group 7th day*	*Control group 7th day*	*Experi mental group 7th day*
%		41.10		27.37		43.07		35.00
max	6.50	3.90	7.20	4.20	7.60	4.80	8.40	5.20
min	2.50	1.80	2.80	2.00	3.20	2.10	3.00	1.90
average value	4.56	2.69	4.48	3.25	5.78	3.29	5.25	3.41

from min = 8,10×10^{-3} m to max = 16,30×10^{-3} m, the mean value was from 10,23×10^{-3} m to 13,66×10^{-3} m. It was established, that these parameters on the 45th day of the experiment most closely approximated to the minimal normal parameters of the rats' control group – the average value of 13.66×10^{-3} m, respectively. At the same time, the smallest changes in length are noted on the 45th day of the experiment, which amounted to 28.7% (Fig. 4, Table 5).

The lowest indicators of thymus height were noted on the 7th day of the experiment and amounted to an average of 2.69×10^{-3} m. On the 7th day, the change in the thymus height was equal to an average of 41.10%. These values ranged from min. 1.8×10^{-3} m to max. 5.2×10^{-3} m, the mean value was from 2.69×10^{-3} m to 3.41×10^{-3} m. It was noted that the reduction of thymus height on the 15th, 30th and 45th days of the experiment was almost the same, but significantly lower than the mean value of the thymus height of the control group. Simultaneously, among the experimental rats, the greatest approximation of the height parameters to the minimum indicators of the control group was marked on 45th day (Fig. 5, Table 6).

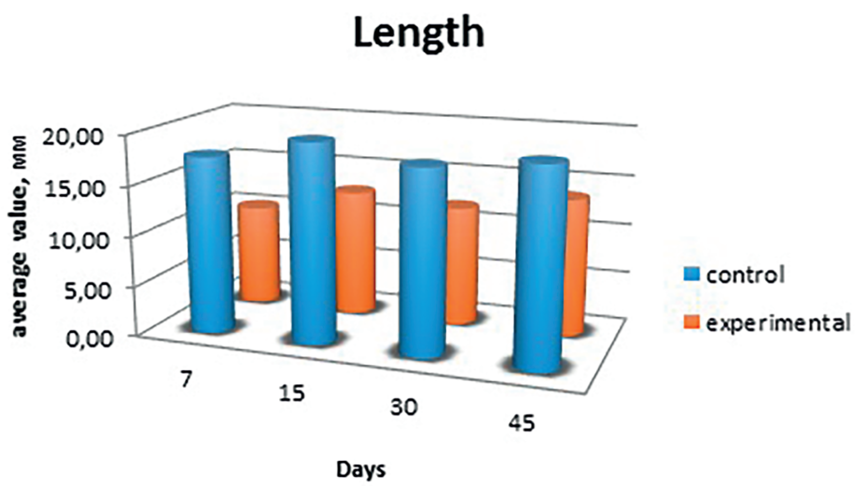

Fig. 4. Indicators of thymus length in control and experimental groups under the influence of TEPPT in a dose of 1/10 LD_{50} on 7, 15, 30, 45 days of the experiment ($\times 10^{-3}$ m).

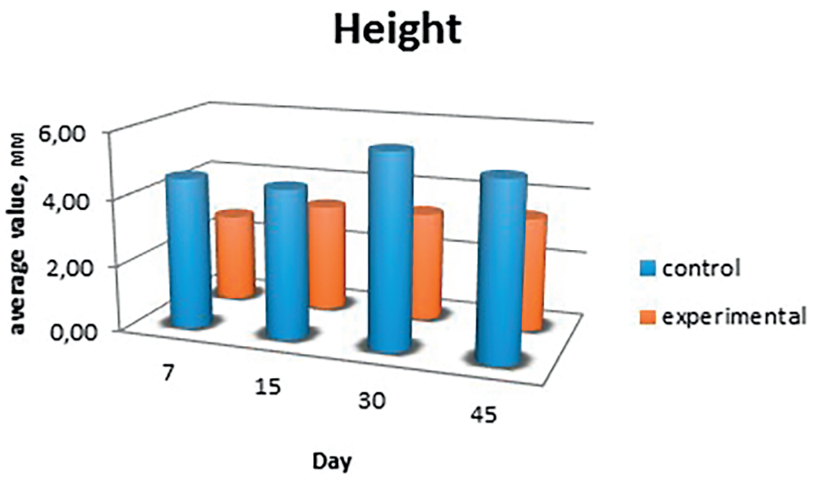

Fig. 5. Indicators of thymus height in control and experimental groups under the influence of TEPPT in a dose of 1/10 LD_{50} on 7, 15, 30, 45 days of the experiment ($\times 10^{-3}$ m).

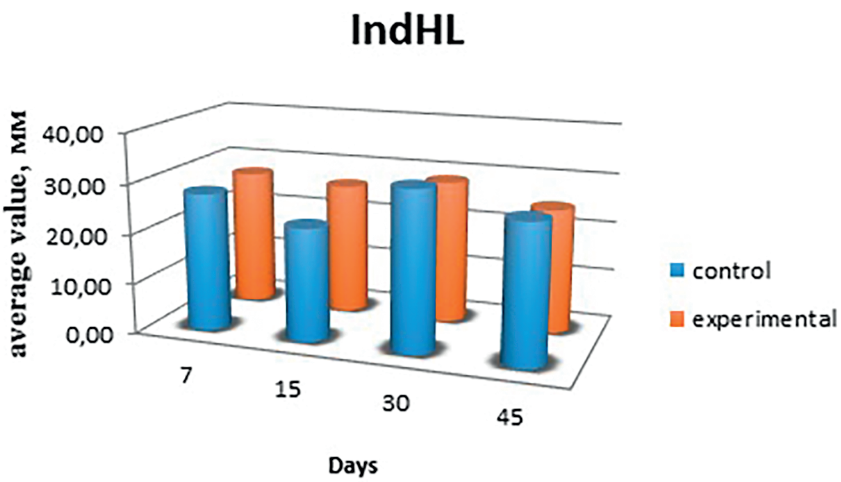

Fig. 6. The indices of IndHL of the rats' thymus of control group and under the influence of TEPPT in a dose of 1/10 LD_{50} on 7, 15, 30, 45 days of the experiment.

It should also be noted that the largest parameters of width, length and height of thymus in the experimental group of rats were on 45th day and had the average value: width 23.01×10^{-9} m^3, length 13.66×10^{-9} m^3 and height 3.41×10^{-9} m^3. It was established that these data on the 45th day of the experiment are close to the minimal normal parameters of the rats' control group.

When studying the peculiarities of the variability of the morphometric indices of thymus under the influence of TEPPT at a dose of 1/10 LD_{50}, we established the following limits of their oscillations: IndHL of the thymus of the experimental group ranged from min = 13.43 to max = 46.6, the average value was from 24.83 to 28.63; IndHW of the thymus of the experimental group ranged from min = 12.75 to max = 91.3, the average value was from 30.13 to 40.96; IndT of the experimental group ranged from min = 34.33 to max = 600.0, the average value was from 78.39 to 191.61 (Fig. 6, 7, 8).

While studying the peculiarities of the variability of the morphometric indices of the thymus in norm, were established the following limits of their oscillations: Ind HL of the control group ranged from min. 12.73 to max. 44.21, the average value was from 22.54 to 31.88; Ind HW of the thymus of the control group ranged from min. 17.54 to max. 85.39, the average value was from 31.30 to 39.52; Ind of the control group ranged from min. 43.0 to max. 143.93, the mean value ranged from 75.99 to 91.44.

Discussion

Thus, we detected organometric features of the thymus structure under the influence of tryglycidyl ether polyoxypropylene tryol in a dose of 1/10 LD_{50},

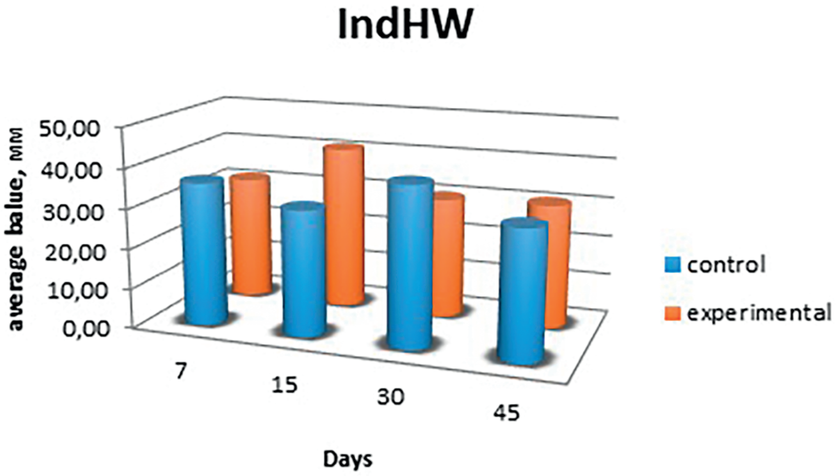

Fig. 7. The indices of IndHW of the rats' thymus of control group and under the influence of TEPPT in a dose of 1/10 LD_{50} on 7, 15, 30, 45 days of the experiment.

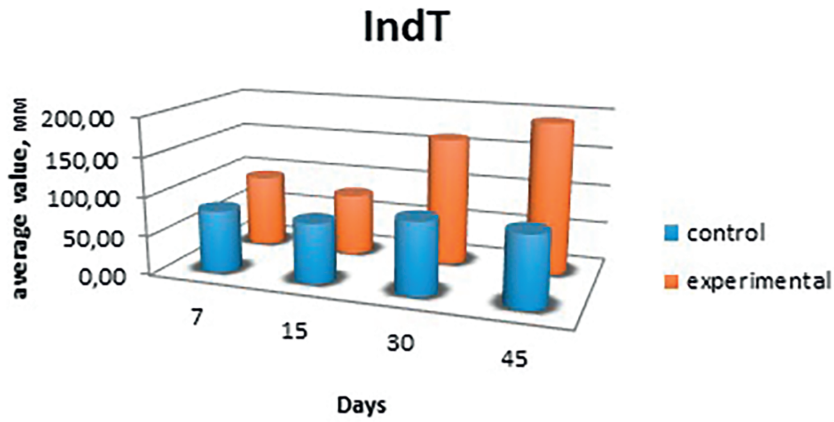

Fig. 8. The indices of IndT of the rats' thymus of control group and under the influence of TEPPT in a dose of 1/10 LD_{50} on 7, 15, 30, 45 days of the experiment.

at different duration of the experiment (7, 15, 30, 45 days).

The method of indices calculation according to the classical formulas used to determine the variability fluctuations of the thymus morphometric parameters was used. These indexes can be used in additional instrumental studies (ultrasound, CT, MRI) to determine the limits of the norm and the likely limits of the influence of xenobiotics on the thymus in human.

Study uncovered that IndT of the control group, which is related to the length and width of the thymus, has the greatest limits of the parameters fluctuations and their significant variability. Ind HW of the thymus of the control group, which is associated with the height and length of the thymus, has the lowest fluctuation limits of the parameters. In our opinion, this is connected, first of all, with the peculiarities of the structure and form of the rats' thymus.

Literature analysis revealed that to evaluate the impact of xenobiotic on such a complex system as immune, a multi-stage approach was proposed, where it was determined that a change in the weight of the thymus could be the best indicator of systemic direct immunotoxicity. A reliable indicator of local and systemic immunotoxicity other than body weight is the microscopic evaluation of these organs[11]. Based on these data, we can state that it is precisely that the discovery of morphological changes in the preclinical stages of the formation of transformations in target organs is also an important task for researchers.

Obtained data about reducing the organometric indices of the thymus throughout the experiment coincide with the data of Smirnov (2014), who studied the effects of tartrazine (included as additive in many foods) on the thymus structure and found that the mass, length, width and, to a lesser extent, thickness were reduced on 3, 10, 15, 24, 45 days of the experiment. During our study, we noted a 100% effect on all morphometric indices of thymic TEPPT in a dose of 1/10 LD_{50} on 7, 15, 30, and 45 days.

Many works are dedicated to the study of the effects of substances on the body of aging animal and in newborns[13,14], and no sufficient data regarding mature animals. In our research, we consider it expedient to study the influence of polyester precisely on mature animals, which corresponds to 20-30 years in recounting animals age on the human.

Conclusions

The obtained results suggest that there are changes at all levels of the organization of the thymus under the impact of widely used xenobiotic TEPPT in a dose 1/10 LD_{50} with the variable severity depending on the term of experiment.

The most significant changes of the mass, volume, length, width, height of organ were noted on 7th and 30th days of research, which was due to the active initial reaction of thymus on exogenous impact.

Variability forms of the thymus under the influence of simple polyesters were revealed, analyzed obtained statistical data and derived indices, which can be used in additional instrumental studies.

The fluctuations of the individual variability of the indices of the thymus mass of laboratory rats are established in the norm and under the xenobiotics influence.

Acknowledgments

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Authors' contributions

Authors were the main researchers and performed the experimental work, as well as analyzed the data and prepared the manuscript.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article“

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law.“

„All institutional and national guidelines for the care and use of laboratory animals were followed“

„No funding for this study“

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