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EXPERIMENTAL STUDY OF THE CHRONIC TOXICITY OF THE TRIBULEPIL COLLECTION

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Abstract:

The study of the chronic toxicity of the Tribulepil collection developed at the Tashkent Pharmaceutical Institute was carried out. Tribulepil collection was administered orally daily for 3 months, in single doses of 25 ml/kg, 50 ml/kg and 100 ml/kg. During the study, the body weight of the animals was determined, and a clinical blood test was performed. During the autopsy, the mass coefficients of the heart, liver, kidneys, stomach, testes and uterus were determined, and a pathomorphological examination of the internal organs was performed. It is shown that with prolonged administration, the collection of "Tribulepil" in 50 ml / kg and 100 ml / kg doses causes a delay in body weight gain. The pathomorphological examination established the absence of toxic effects on parenchymal organs and, in particular, on the genitals, only the presence of signs of circulatory disorders is noted only in the group with the use of the maximum (250 ml/kg) dose and only in females.

Keywords: Tribulepil, chronic toxicity, preclinical studies, mice

INTRODUCTION.

Scientists of the Tashkent Pharmaceutical Institute (Professor H.M. Komilov and G.K. Rakhimova) have developed a collection composition "Tribulepil" based on the aboveground part of narrow-leaved ivan tea, which has high antibacterial, anti-inflammatory activity for the treatment of prostate diseases [1]. After the development of the composition of the collection "Tribulepil", pharmacological studies were carried out, which made it possible to evaluate the effectiveness of the selected composition. In the future, the chronic toxicity of the Tribulepil collection was studied.

The aim of the study is to study the chronic toxicity of the Tribulepil collection at the stage of preclinical study.

MATERIALS AND METHODS OF RESEARCH.

The experiments were conducted in accordance with the "Rules for Laboratory Work using Experimental Animals", as well as the rules given in the European Convention for the Protection of Vertebrate Animals Used for Experimental Research or for Other Scientific Purposes (ETS No. 123) Strasbourg, 03/18/1986. [2].

The chronic toxicity of the Tribulepil collection was carried out on clinically healthy and sexually mature animals that had been quarantined for at least 10-14 days. In the experiment, white mice with a weight of 18-22 g were used. The animals were kept in vivarium conditions (with natural lighting conditions, at a temperature of 22-24 ° C, relative humidity of 55-60%) under standard conditions of keeping and feeding [3]. The control group of animals were kept in the same conditions.

The experimental groups of the Tribulepil collection were administered orally, once a day, for three months at a dose of 25 ml / kg (in accordance with the experimental therapeutic dose), 50 ml / kg (in accordance with the intermediate dose, which was 2 times higher than the therapeutic dose) and 250 ml / kg (in accordance with the toxic dose, which exceeded the therapeutic dose by 10 times). The control animals were injected with distilled water in similar volumes, a course of 3 months.

Weighing of animals was carried out on an electronic scale MW - 120 (Korea) every seven days from the beginning of the experiment.

During the study, the general clinical condition, behavior, intensity and nature of motor activity, changes in the frequency of respiratory movements, preservation of position reflexes were evaluated, toxic effects on the central nervous system (CNS) were noted: convulsive phenomena, lethargy, hemiparesis or paraparesis, hyperactivity.

At the end of the study, mice were removed from the experiment in compliance with the rules of humane treatment of laboratory animals [4]. Internal organs (heart, liver, stomach, kidney, testes, uterus) were weighed to determine their mass coefficients. Further, all organs were fixed in a 10% formalin solution for pathomorphological examination.

The research results were subjected to statistical processing in Microsoft Excel and Statistica 6.0. using the Student's t-test. The following elements of descriptive statistics were calculated: arithmetic

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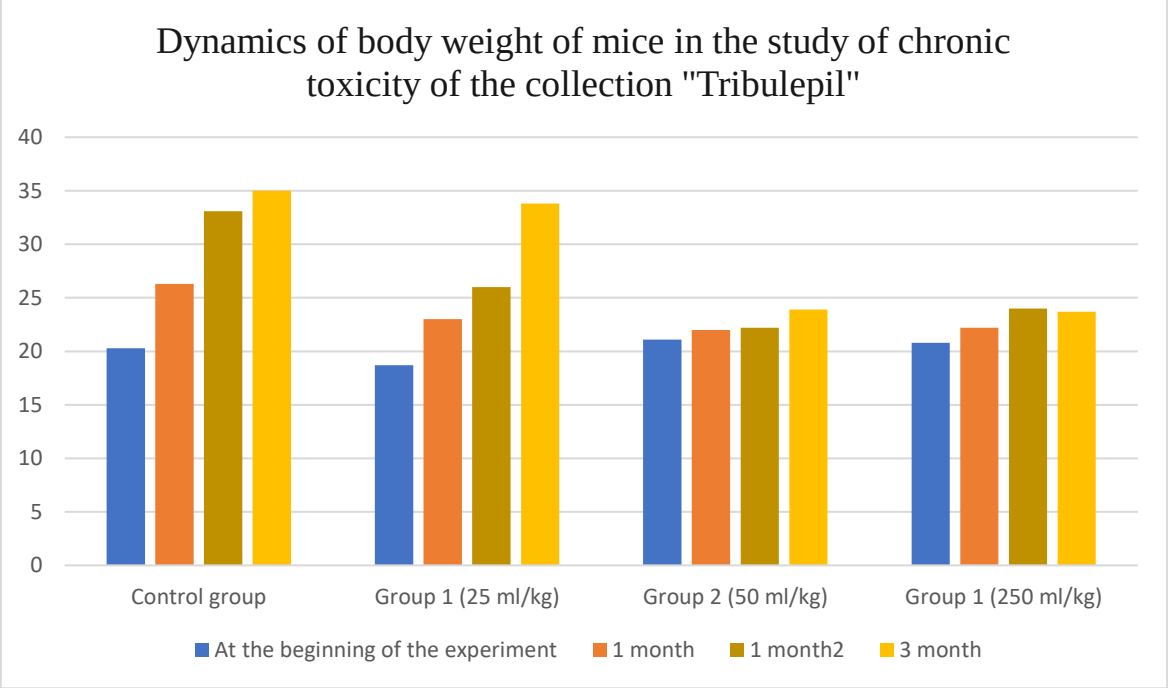
mean (M), standard error of the mean (m), standard deviation.

RESULTS AND THEIR DISCUSSION

According to the results of observations of the condition of animals receiving Tribulepil in doses of 25; 50; and 250 ml / kg (respectively, experimental groups 1; 2 and 3) for three months, relatively no special changes were found. All the experimental groups of mice were almost identical in terms of the condition of the coat, mucous membranes, food and water consumption, as well as behavior. It was revealed that both males and females who received the drug in a single dose 10 times higher than the therapeutic dose significantly gained body weight more slowly compared to the control.

Under the conditions of a 3-month introduction of the Tribulepil collection, the death of mice was not noted. The mass of mice during the experiment is shown in Diagram 1.



During the period of administration of the substance, the dynamics of changes was positive in all animals included in the experiment. There were only insignificant trends of lower dynamics of body weight gain in female mice in all experimental groups.

The functional state of internal organs as a whole is reflected by their mass coefficients. The results are presented in Table 2.

Table-2

The mass coefficients of the internal organs of mice in the study of the chronic toxicity of the collection "Tribulepil"

№	Internal organs	Mass of internal organs			
		Control	25 мл/кг	50 мл/кг	250 мл/кг
1.	Heart	0,21±0,03	0,18±0,01	0,20±0,01	0,22±0,01
2.	Liver	1,46±0,22	1,63±0,10	1,11±0,06	1,48±0,08
3.	Stomach	0,59±0,03	0,49±0,08	0,34±0,06	0,36±0,08
4.	Kidney (right)	0,19±0,03	0,16±0,07	0,19±0,02	0.19±0.04
5.	Kidney (left)	0,21±0,06	0,15±0,04	0,19±0,07	0,19±0,03
6.	Uterus	0,251±0,03	0,191±0,06	0,227±0,04	0,204±0,02
7.	Testes	0,385±0,02	0,241±0,02	0,242±0,08	0,263±0,04

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As can be seen from the data presented in Table 2, the relative mass of most internal organs in experimental mice does not statistically differ from the control. The experimental group who received the Tribulepil collection at a dose of 25 ml/kg had an increased liver mass coefficient compared to the control.

With prolonged administration of Tribulepil collection in the above-mentioned doses to laboratory animals, there were no significant differences between hematological parameters (erythrocytes, hemoglobin, leukocytes), relative to those in animals of the control group. The level of the studied indicators corresponded to the parameters of the physiological norm for these animal species.

The peripheral effect of the Tribulepil collection on blood parameters during its prolonged administration (M ± m; n = 6)

Indicators	The norm	Indicators before the experiment				Peripheral blood parameters during the experiment							
						On the 44th day of the experiment				On the 88th day of the experiment			
		control	25 ml/kg	50 ml/kg	250 ml/kg	control	25 ml/kg	50 ml/kg	250 ml/kg	control	25 ml/kg	50 ml/kg	250 ml/kg
Hemoglobin (HGB) g/l	122-162	148,00±5,00	152,00±2,55	155,00±4,08	150,00±1,35	152,00±4,55	141,00± 1,45	140,00± 0,50	137,00± 1,00	155,00±2,97	139,00± 0,45	136,00± 3,05	132,00± 4,60
Erythrocytes (RBC) 1012 /l	7-12	10,74 ±.0,25	11,09 ±0,17	10,98 ± 0,19	10,77 ±0,32	10,87 ± 0,28	10,74 ±0,25	10,61 ±0,95	10,25 ±0,01	10,85 ±0,45	10,70 ±0,15	10,35 ±0,56	10,12 ±0,25
White blood cells (WBC) 109 /l	6-15	5,99± 0,02	6,55± 0,12	7,01± 0,15	6,94± 0,21	8,64± 0,25	7,45± 0,13	7,01± 0,03	6,98± 0,05	9,25± 0,09	8,78± 0,06	7,02± 0,02	6,85± 0,31

Note: * - the changes are significant relative to the control group at P <0.05.

As a result of the histological examination, it was found that there were no significant differences in the internal organs in the experimental group compared with the control group in both females and males. In the heart, liver and kidneys of females of the IV experimental group, stagnant phenomena were noted, characterized by the expansion of the vascular lumen and the development of focal hemorrhages, minor chronic inflammatory infiltration in the kidney, in two cases. At the same time, no such changes were observed in the group of males. In the liver, especially in females of the IV experimental group, signs of a mesenchymal reaction were determined, mainly along the periportal tracts associated with stagnant circulatory phenomena, possibly due to the shock concentration of the above drug. In the myocardium, the fibrous structure, transverse striation and nuclei of cardiomyocytes in all experimental groups are preserved, there are individual cases of vascular hyperemia and under the endocardium. In the liver, kidneys, and stomach, the histological picture also corresponds to the picture as in the control, which indicates the absence of signs of toxic effects and other significant pathological changes after the application of the Tribulepil collection. Genitals: in the gonads of males and in the uterus of females, no morphological changes were detected under the influence of this collection. In the testes, the spermatogenic epithelium is determined in the reproduction phase, which gives us the opportunity to judge the preservation of the gonad function. In the uterus, the endometrium is of normal thickness, the number of glands is not changed, signs of hyper- and hypoplastic process, as well as nodular changes in the myometrium were not observed.

Thus, the above morphological changes, both in the control and in the experimental groups, are of a general pathological nature. Signs of congestive

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circulatory disorders in individual organs are associated with the maximum therapeutic dose, since it is observed only in the fourth experimental group of females, whereas in males of the same group no significant changes were noted. The result of the morphological study on the use of the Tribulepil collection indicates the absence of toxic effects on parenchymal organs and, in particular, on the genitals, only the presence of signs of circulatory disorders is noted only in the group with the use of the maximum dose and only in females.

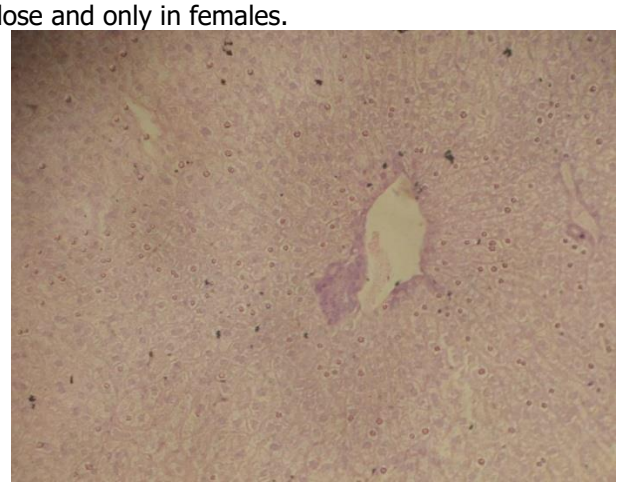

Fig. 1. Liver lobule - proliferation of cells of histogenic nature is noted around the central vein, bilirubin grains in hepatocytes (female experimental group II) Uv. Vol. 10.0 Color: hematoxylin and eosin.

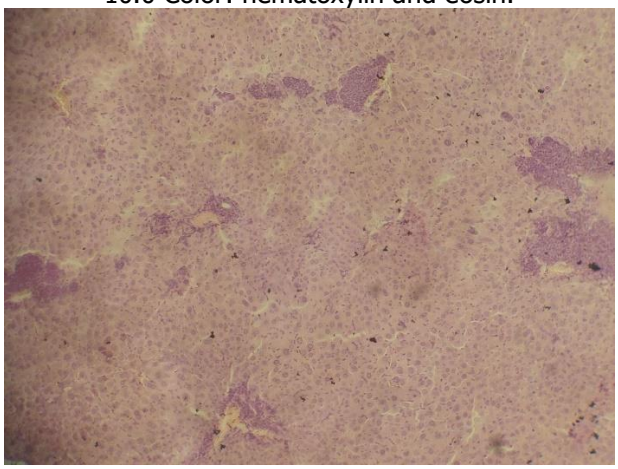

Fig. 2. Liver in the periportal tracts there is a focal cellular-mesenchymal reaction, the beam structure is destroyed in places (female experimental group II) Uv. About 10.0. Color: hematoxylin and eosin.

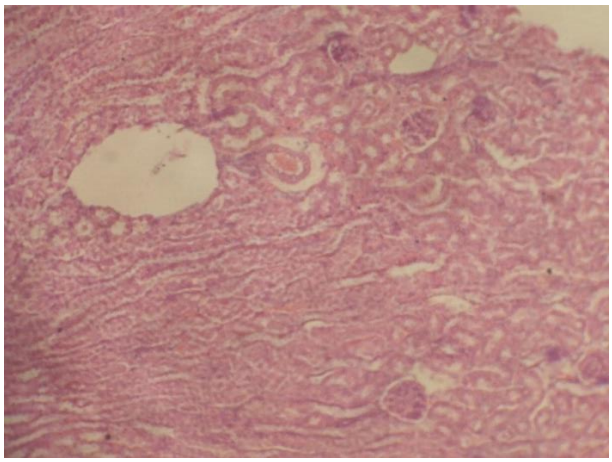

Fig. 3. Kidney-right - a blood vessel with an expanded lumen is determined in the stroma, an uneven distribution of glomeruli (female experimental group II) Uv. 10.0. Vol. Color: hematoxylin and eosin.

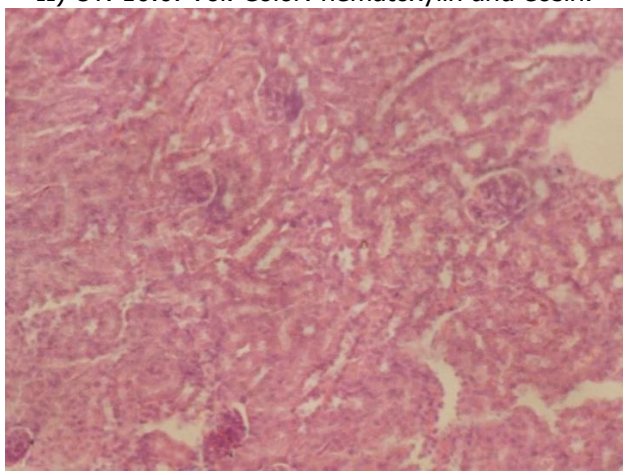

Fig. 4. Left kidney - oval-rounded glomeruli, tubules without signs of damage (female experimental group II) Uv. 10.0. Vol. Color: hematoxylin and eosin

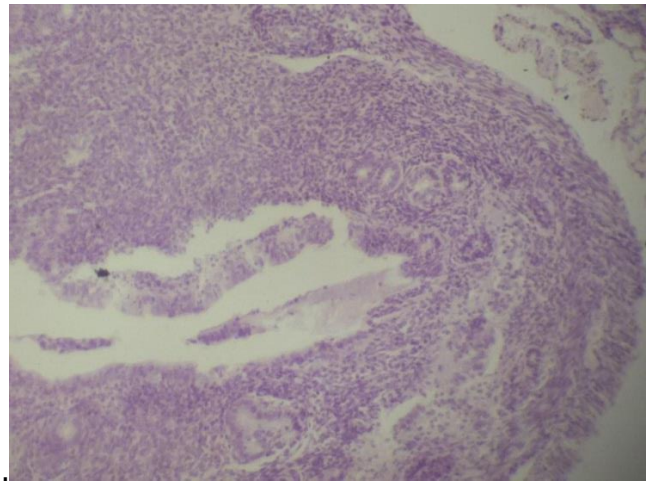

Fig. 5. Uterus- the epithelium of the endometrial mucosa is not thickened, several glands under the epithelium (female experimental group II) Uv. 10.0.

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Vol. Color: hematoxylin and eosin.

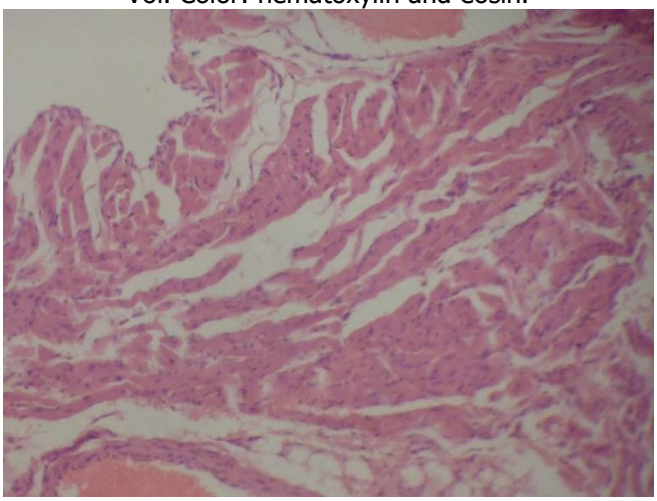

Fig. 6. Heart - there is a slight accumulation of fatty tissue in the intermuscular layers (male experimental group III) Uv. Vol.4.0. Color: hematoxylin and eosin.

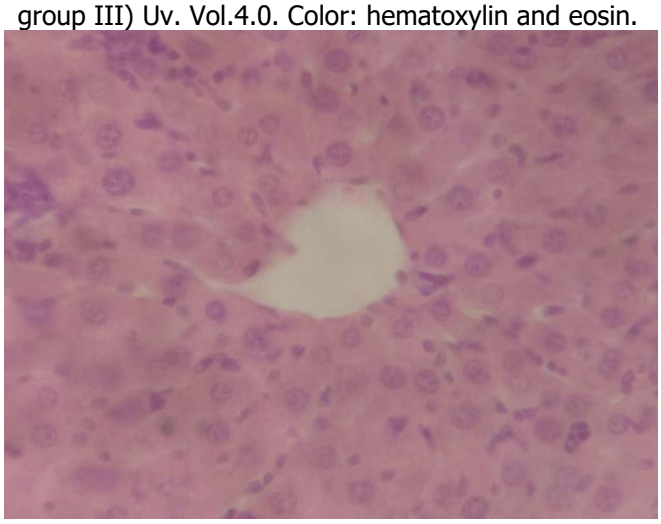

Fig. 7. Liver hepatocytes depart from the central vein in the form of beams, their nuclei are preserved. (male experimental group III) Uv. Vol.4.0. Color: hematoxylin and eosin.

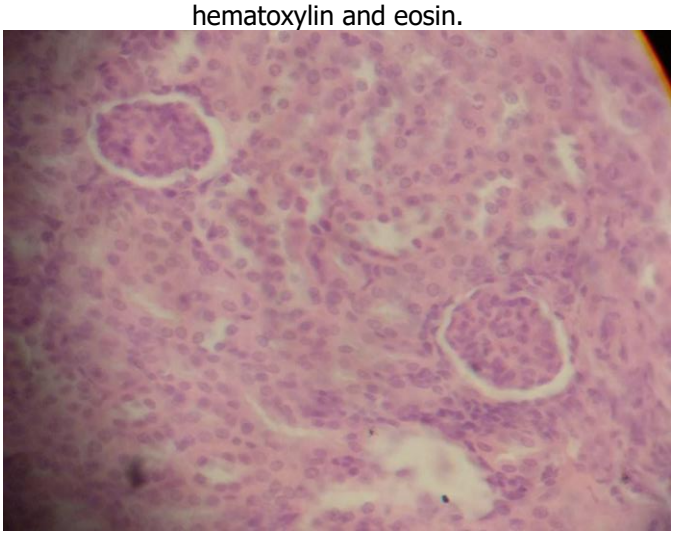

Fig. 8. Kidney lev. - two glomeruli are equally large in the field of view under high magnification in the epithelium of the tubules of the nucleus of a clear configuration (male experimental group III) Uv. Vol.4.0. Color: hematoxylin and eosin.

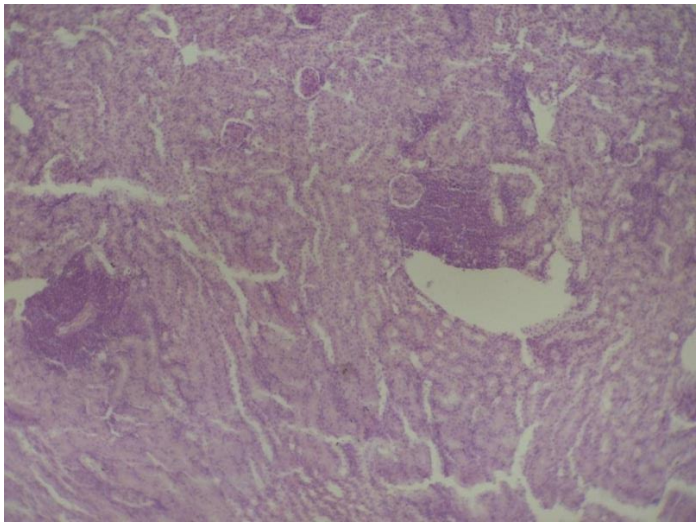

Fig. 9. Right kidney - focal lymphocytic cell infiltration in the stroma, glomeruli and tubular apparatus unchanged. (male experimental group III) Uv. Vol.4.0. Color: hematoxylin and eosin.

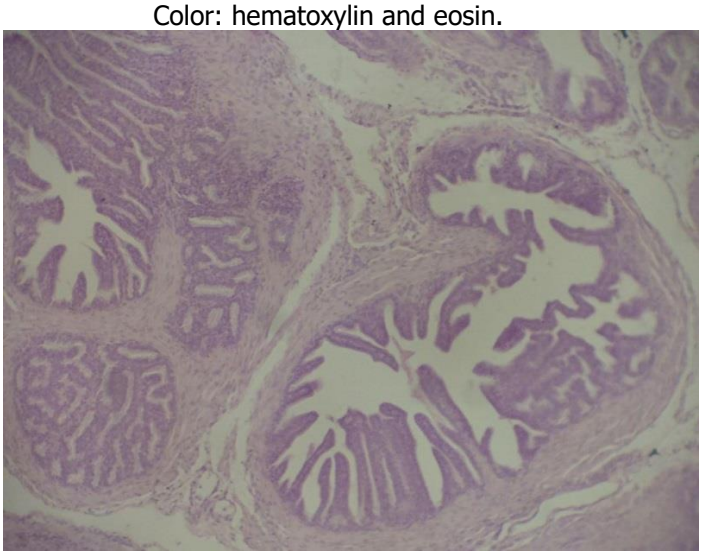

Fig. 10. Testis-epithelium of tubules with papillary overgrowth, in lobules the spermatogenic epithelium contains Sertolli cells of the 1st and 2nd order. (male experimental group III) Uv. Vol.4.0. Color: hematoxylin and eosin.

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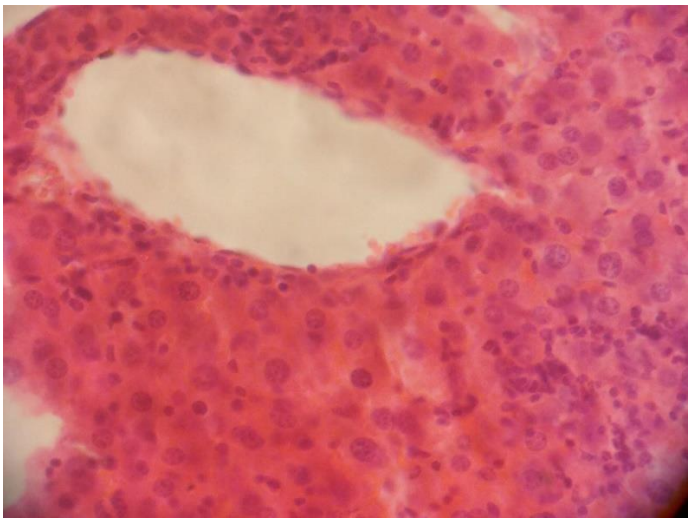


Fig. 11. Liver - under a large magnification of the lens, the central vein and hepatocytes retain the beam structure, the nuclei are not changed. (female experimental group III) Uv. Ob.40.0. Coloration:

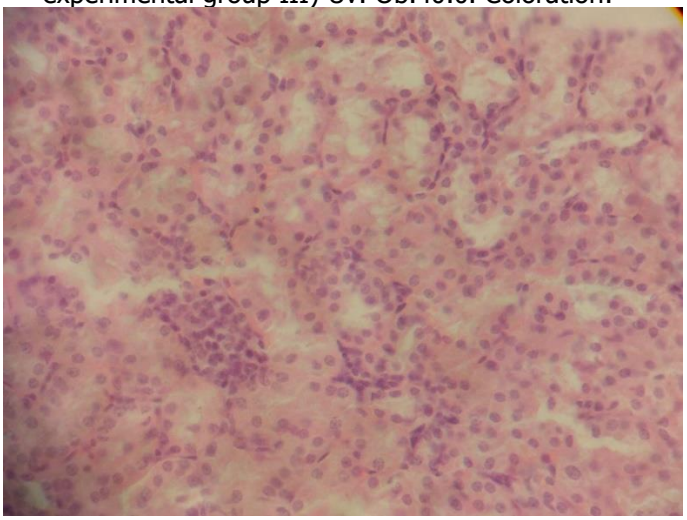


Fig. 12. The kidney is right-the nuclei in the tubules have clear contours, the lumen is free. (female experimental. group III) Uv. About.40.0. Color: hematoxylin and eosin

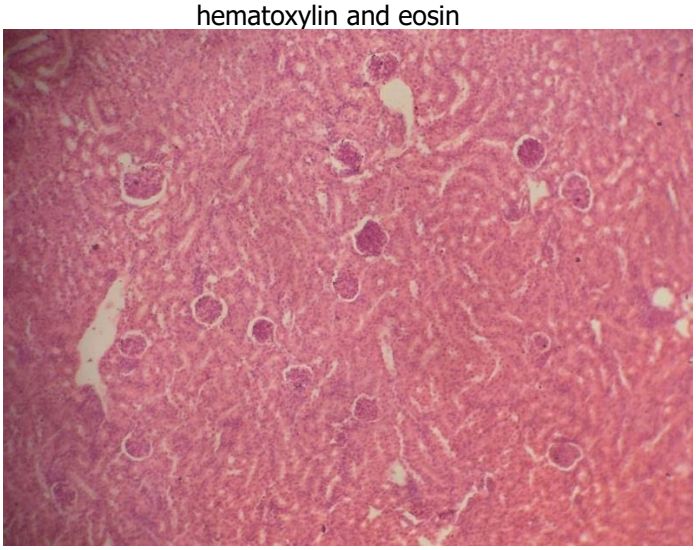
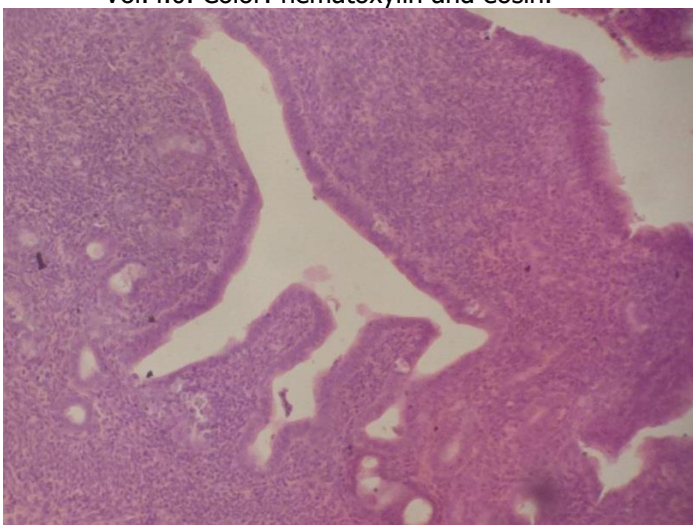


Fig. 13. Kidney left -equidistant distribution of round-shaped glomeruli, convoluted and straight tubules preserved. (female experimental group III) Uv. Vol.4.0. Color: hematoxylin and eosin.



14. Uterus - the cavity is not expanded, the epithelium is not thickened, there is a focal villous growth, the prismatic epithelium is unchanged. The glands are defined under the epithelium. Myometrium is unchanged. (female experimental group III) Uv. Vol.4.0. Color: hematoxylin and eosin.

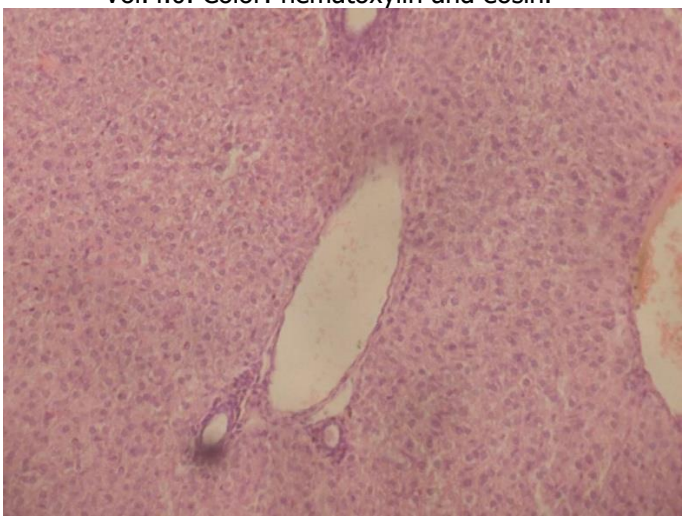


Fig. 15. Liver the lumen of the vessels is expanded, the nuclei in hepatocytes with clear contours. (male group IV) Uv. About.10.0. Color: hematoxylin and

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eosin experimental.

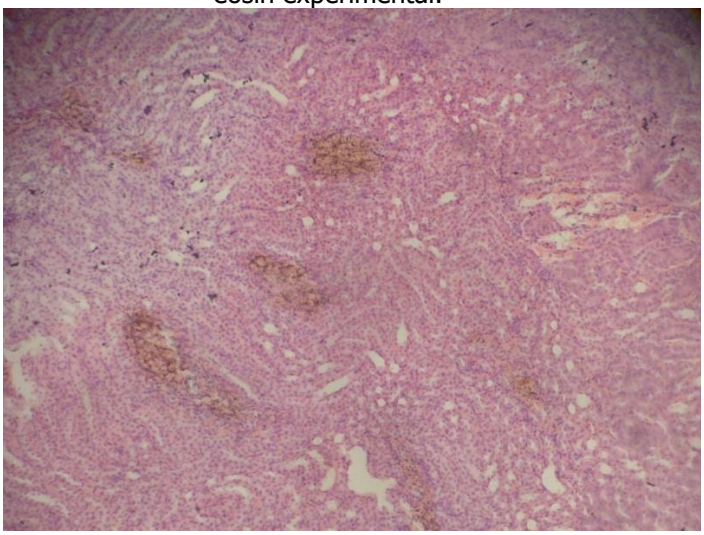

Fig. 16. Kidney -right general view, hemorrhage in the stroma of a focal nature (male experimental group IV) Uv.
Volume 4.0. Color: hematoxylin and eosin

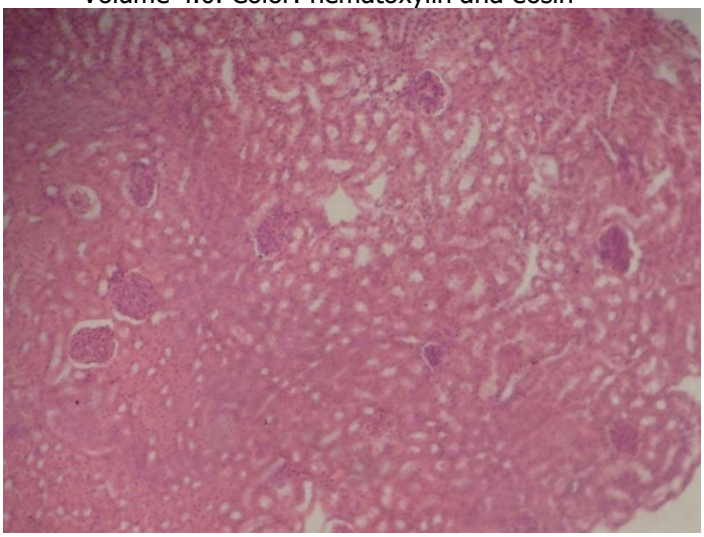

Fig. 17. Right kidney. Several round-shaped glomeruli, tubules unchanged. (male experimental group IV) Uv. About.10.0. Color: hematoxylin and eosin

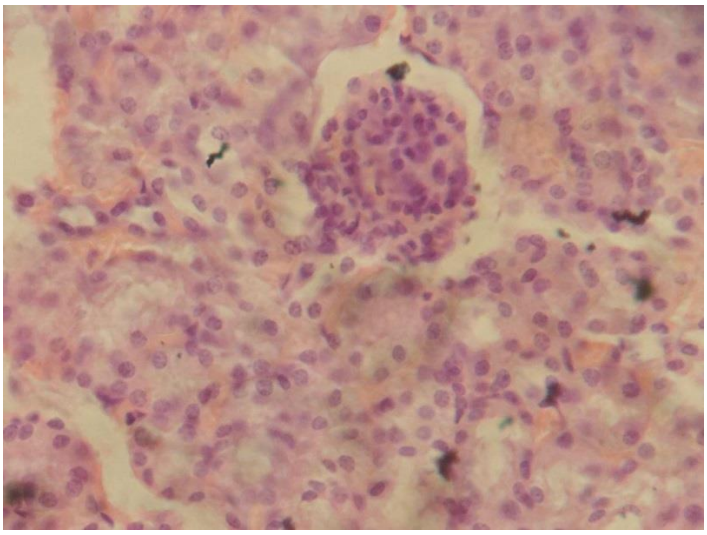

Fig. 18. Right kidney. – under a large increase, the glomerulus of a rounded shape, tubules with the preservation of epithelial nuclei and erythrodiapedesis into the stroma (male experimental group IV) Uv. Vol.4.0. Color: hematoxylin and eosin.

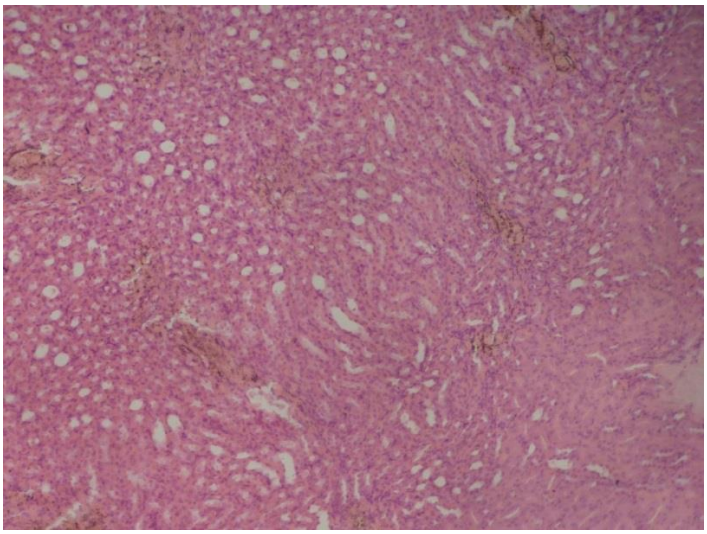

Fig. 19. Left kidney with foci of hemorrhages in the stroma (male experimental group IV) Uv. Vol.4.0. Color: hematoxylin and eosin.

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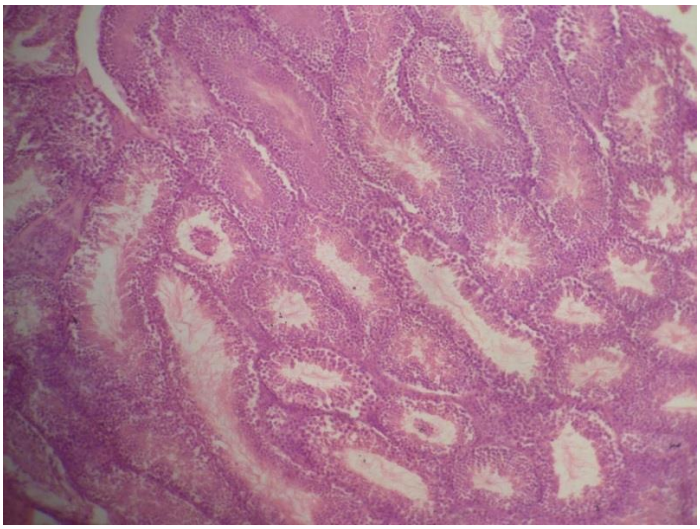


20. Testis-tubules contain several layers of spermatogenic epithelium. (male experimental group IV) Uv. About.10.0. Color: hematoxylin and eosin.

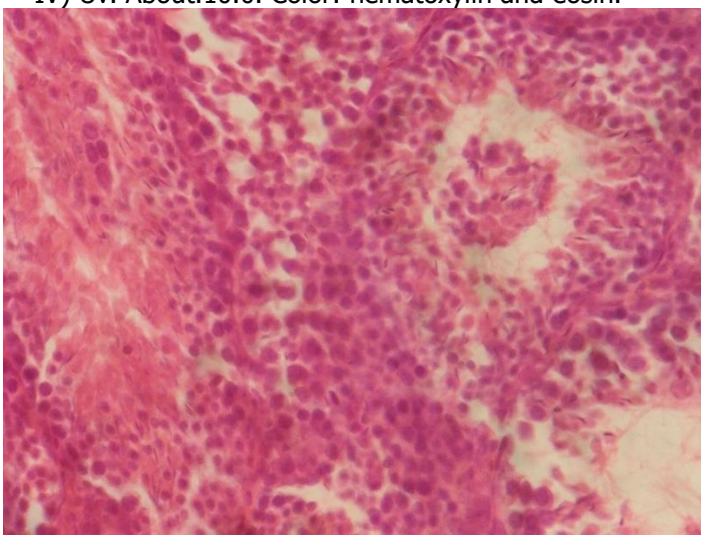
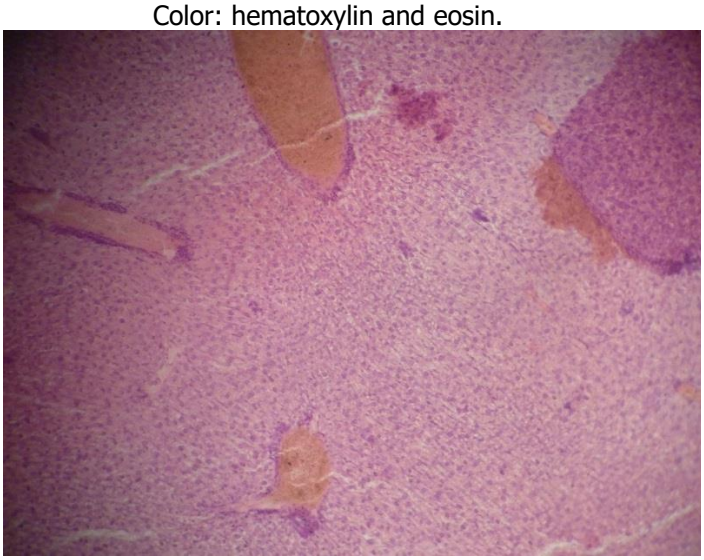
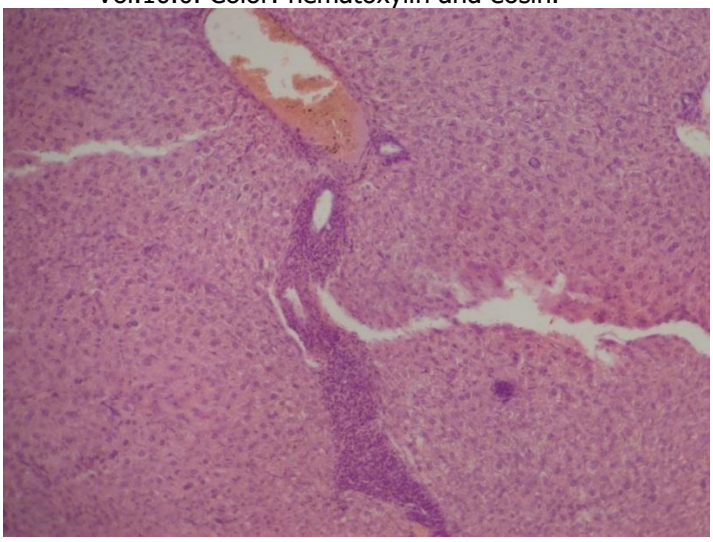


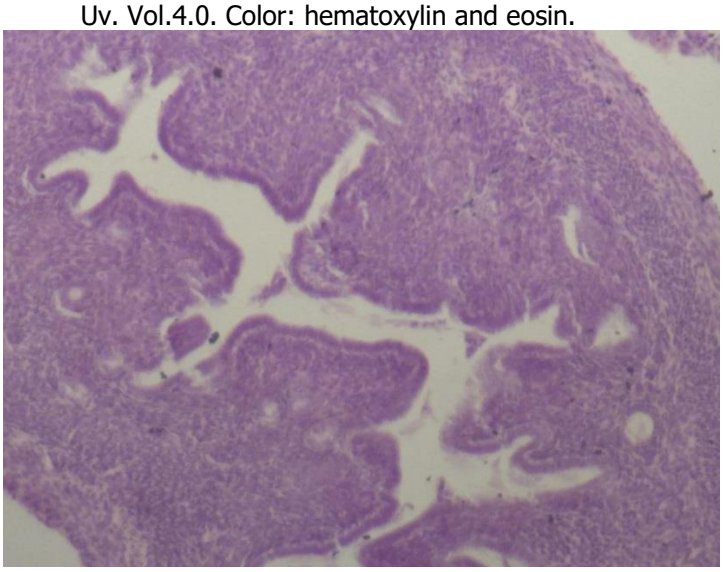
Fig. 21. Testis- spermatogenic epithelium contains spermatocytes of the 1st, 2nd order - the reproduction phase. (male experimental group IV) Uv. About.40.0. Color: hematoxylin and eosin.



22. Liver - blood vessels are dilated and full-blooded - signs of a stagnant process, hepatocytes are unchanged. (female experimental group IV) Uv. Vol.10.0. Color: hematoxylin and eosin.



23. Liver- fullness of the central vein, cell proliferation mesenchymal linear reaction, single hepatocytes with vacuole dystrophy. (female, experimental group IV) Uv. Vol.4.0. Color: hematoxylin and eosin.



24. The uterus is a convoluted lumen, the epithelium covering the endometrium with clear boundaries of the basement membrane, there are glands. Myometrium without signs of focal and nodular lesions. (female experimental group IV) Uv. Vol.4.0. Color: hematoxylin and eosin.

CONCLUSIONS

As a result of studying the chronic toxicity of the new Tribulepil collection, it was found that its long-term (3 months) daily intragastric administration in doses of 25 ml / kg (corresponds to an experimental therapeutic dose), 50 ml / kg (corresponds to an intermediate dose that was 2 times higher than the therapeutic dose) and 250 mg / kg (corresponds to a

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toxic dose that exceeded the therapeutic 10 times) does not cause toxic phenomena and death of animals, does not violate the general condition, appearance, trophism, food and drinking activity, body weight. In addition, the collection of "Tribulepil" does not negatively affect the hematological parameters of blood, does not lead to pathological changes in organs and tissues of experimental animals, i.e. it is safe.

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