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Kh.I. Kurylo, I.M. Klishch, Z.M. Nebesna, M.Ya. Furdela, A.S. Volska, S.O. Lytvynyuk
SHEI "I. Horbachevsky Ternopil State Medical University", Ternopil

HISTOLOGICAL CHANGES IN LIVER AND KIDNEYS IN EXPERIMENTAL TYPE 2 DIABETES MELLITUS AND ITS CORRECTION BY ADMINISTRATION OF PHYTOCOMPOSITIONS COMPRISING *GALEGA OFFICINALIS L.*

e-mail: kurylokh@tdmu.edu.ua

The experimental studies of the morphological state of the white rats' kidney and liver in conditions of the simulated Type 2 diabetes mellitus and the use of pharmaceutical preparation comprising *Galega officinalis L.* and *Vaccinium myrtillus*, as well as administration of "Galevit" liposomal formulation have been carried out. In the group of animals without correction the drastic destructive-degenerative damage to all structural components of the studied organs, as well as significant vascular disorders has been found. Application of the remedial pharmaceutical preparation comprising *Galega officinalis L.* and *Vaccinium myrtillus* has a positive effect on morphofunctional state of the liver and kidneys of laboratory animals, especially the administration of the "Galevit" liposomal formulation. The degree of the reparatory processes in the studied organs in experimental type 2 diabetes mellitus shows that the new "Galevit" composition has more apparent positive effect as compared to the pharmaceutical preparation comprising *Galega officinalis L.* and *Vaccinium myrtillus.*

Keywords: liver, kidneys, Type 2 diabetes mellitus, *Galega officinalis L.*, *Vaccinium myrtillus*, liposomal formulation.

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Diabetes mellitus (DM) is one of the major medical and social problems, ranking the third place in the world after cardiovascular and oncological diseases. The International Diabetes Federation (IDF) reports about 120 to 180 million patients with diabetes worldwide, accounting for 2-3% of the total population of the planet [1, 3, 4, 6]. This causes the relevance of the study of the novel effective medications to prevent and treat DM sequelae.

The purpose of the paper was to determine the histological changes in the liver and kidneys in streptozotocin-induced type 2 diabetes mellitus and the effect of pharmaceutical preparation comprising *Galega officinalis L., Vaccinium myrtillus* and taurine, as well as its liposomal formulation with conventional name "Galevit".

Materials and Methods. The object of the pharmacological studies was the pharmaceutical combination preparation comprising *Galega officinalis L.* and *Vaccinium myrtillus* and its liposomal formulation «Galevit». The composition is comprised of dry extracts of 50 mg *Galega officinalis L.* and *Vaccinium myrtillus* and 1.4.mg taurine. Liposomal formulation of the composition was obtained by the conventional technique. The study was carried out on 50 outbreed male white rats with body weight of 260-280 g. Type 2 DM was induced by streptozotocin (STZ, "Sigma", United States). STZ was dissolved *extempore* and injected on the citrate buffer (pH 4.5), since in alkaline and neutral medium it quickly degrades to inactive metabolites and loses its diabetogenic activity. To simulate the type 2 DM, rats were injected intraperitoneally with a single dose of (65 mg/kg body weight) STZ solution according to the Islam S., Choi H. (2007) technique [7]. To reduce the diabetogenic activity of STZ prior (15 minutes) to its administration nicotinamide (N) was injected intraperitoneally with a dose of 230 mg/kg. The rats were fed a high-calorie diet for 12 weeks before administration of STZ [8]. The investigated formulations were administered endogastrically once a day for 21 days with treatment-and-prophylactic purpose. The first injection of the drugs started within 24 hours after induction of diabetes. A group of animals of controlled pathology (CP) were administered with distilled water in a similar way. The animals were randomized into 4 groups. Group 1 (the control group; intact animals (IC)); Group 2 (animals of control pathology); Group 3 (STZ + N-induced diabetic animals administered with pharmaceutical preparation comprising *Galega officinalis L.* and *Vaccinium myrtillus* with a dose of 50 mg/kg; *peros* taurine with a dose of 1.4 mg/kg); Group 4 group (STZ + N-induced diabetic animals administered with "Galevit" liposomal formulation. The experiments were performed in compliance with the requirements of international principals of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg,1986) and " General Ethical Principles for Scientific Experiments on Animals", approved by the I National Congress

of Bioethics (Kyiv, 2001). No violations of ethical standards in the conduct of scientific research have been revealed by the commission on bioethics at SHEI «I. Horbachevsky Ternopil State Medical University» (Minutes No. 41 as of 15.03.2017). For histological studies the slices of the kidneys and liver were fixed in 10% neutral formalin solution. The subsequent processing of the material with the follow up embedment into paraffin blocks was conducted using the conventional methods [2]. The sections, obtained on the sliding microtome, were stained with hematoxylin-eosine. Histological specimens have been studied using the light microscope SEO SCAN and the images were made with Vision CCD Camera with image output system of histological preparations.

Results and Discussion. The Group 1 animals (IC) were used for control, involving white rats, kept in the standard conditions of the vivarium and not exposed to pathogenic factors. The histological study of the liver and kidneys detected no inconsistencies with the descriptions of the microstructure of these organs found in the publications. The resulting microscopic studies of the liver in animals of Group 2 (control pathology) have revealed the significant destructive-degenerative changes in all structural components of the organ on day 22 after the confirmed STZ + N -induced type 2 diabetes mellitus. The significant vascular lesions have been detected; lumens of the central and sublobular veins were plethoric, dilated, their walls were thinned and with unclear contours. Wide veins, filled with blood corpuscles, were found in the triads. Arteries had smaller diameters and thickened walls. The destruction of the majority of the lobuli and impaired beam-type arrangement of the cells was detected in the parenchyma. In the central parts of the lobuli the alternative changes in hepatocytes were noted often in the form of atomized fat dystrophy, sometimes with the development of the centrolobular necrosis. The cytoplasm of cells was weakly oxyphilic, partially or completely cleared, their nuclei were pyknotic. Generally, sinusoids were observed only on the periphery of the liver lobuli and were dilated (Fig. 1).

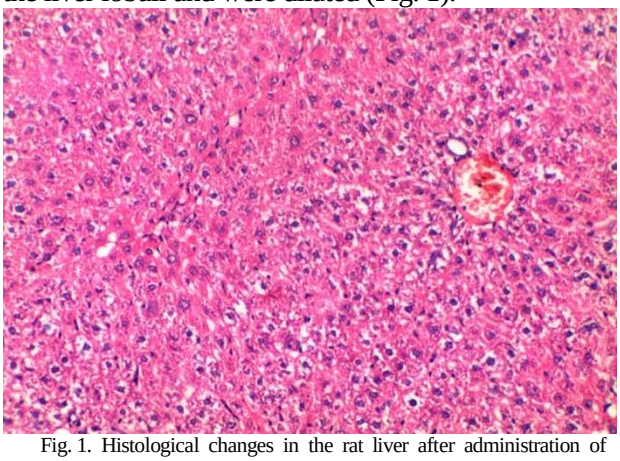

Fig. 1. Histological changes in the rat liver after administration of STZ+N. Impaired beam organization of the organ and dystrophic changes in hepatocytes. H&E stain. Magnification × 200.

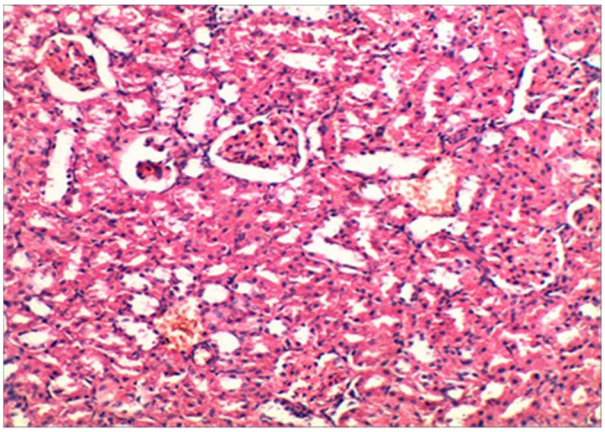

Fig. 2. Histological changes in the rat kidney after administration of STZ+N. Dilated, plethotic lumens of the vessels; atrophy of glomeruli. H&E stain. Magnification ×200.

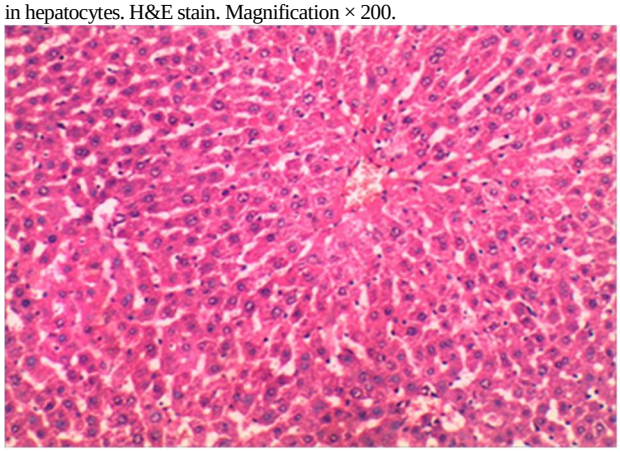

Fig. 3. Histological hepatic changes in STZ + N-induced diabetic animals, administered with pharmaceutical preparation comprising *Galega officinalis L.* and *Vaccinium myrtillus*. Altered lobular-beam structure, destruction of hepatocytes, dilated lumens of the sinusoids. H&E stain. Magnification × 200.

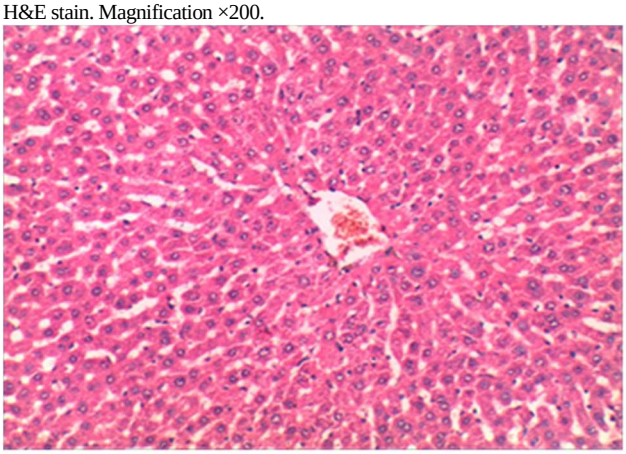

Fig. 4. Microscopic state of the liver of the STZ + N-induced diabetic animals, administered with "Galevit" liposomal formulation. Central veins; area of the triad. Preserved lobular-beam structure of the organ. Moderately dilated lumens of the sinusoids. H&E stain. Magnification ×200.

Histological studies of the kidneys of diabetic animals have established the destructive changes of all components of the organ. Inhomogeneous blood filling with a predominance of moderate plethora of the renal cortex has been noted. The lumens of vessels, especially the venous ones, were markedly dilated, plethoric, their walls were thinned; hemorrhages, sludge of erythrocytes and stasis were detected. Drastic contraction of

the vascular glomeruli was observed in the renal corpuscles, as well as their poor blood filling and dilatation of the lumens of Shumlyanskiy - Bowman capsules. Degenerative changes in the epithelial cells of the vast majority of the sinuous canaliculi have been found. Optically empty vacuoles have been found in their edematic cytoplasm, indicating about the focal development of hydropic degeneration. The lumen of many tubules was dilated or stenosed, sometimes was filled with homogenous eosinophilic masses (Fig. 2).

The findings of histological study of the kidney and liver of STZ + N-induced diabetic white rats of Group 3, who were administered with pharmaceutical preparation comprising *Galega officinalis L.* and *Vaccinium myrtillus* have established changes, specific to experimental DM model; however, in comparison with the previous group their intensity was less manifested. The microscopic study of the liver in the animals of Group 3 has established that lobular-beam structure of the organ was partially broken and the events of disintegration have been noted in some areas.

The lumens of many vessels were dilated comprising the blood corpuscles. Portal tracts were not principally dilated, and their blood vessels were plethoric. Sinusoids were with clear contours, mostly in the central parts of the lobuli, and dilated. At the same time the focal destructive changes in hepatocytes with clarification of their cytoplasm have been noted. Moreover, the enlargement of the area of hepatocytes' nuclei and their intense basophilia has been detected. Such changes prevailed in the central parts of the lobuli and were less pronounced in the periportal areas. The increase in binuclear hepatocytes, indicating the enhancement of the regenerative processes has been also noted (Fig. 3). Histological study of the kidneys of animals of Group 3 has found that the circulatory disorders were the hallmark of the specific changes of the renal cortex and medulla of kidney. They were manifested by the dilatation of numerous blood vessels, capillaries of the peritubular reticulum and part of the blood capillaries of the glomeruli. Perivascular connective tissue was edematic.

The architectonics of the majority of renal glomeruli was preserved. Part of the renal corpuscles and their vascular glomeruli were hypertrophied and sporadic degenerated glomeruli in diminished renal corpuscles in which moderately dilated lumens of the Shumlyanskiy - Bowman capsules were noted. Microscopic changes have been also found in the proximal and distal parts of the nephron tubules. Tubules with dilated lumens and collapsible tubes were noted. In other tubules with narrowed lumens the epithelial cells were edematic, sometimes the areas of desquamation of their apical sections were found. The correction of hyperglycemia in experimental STZ + N-induced diabetic animals of Group 4, administered with "Galevit" liposomal formulation minimized the hepatic and renal lesions. The architectonics of the lobuli and renal beam organization was preserved with, practically, no events of disintegration. Disorders of blood circulation were not found, and sporadic dilated lumens of the central veins were detected; the plethora was insignificant. Scarce red blood cells and macrophages were detected in the lumens of moderately dilated and clearly circumscribed sinusoids. Hepatocytes were clearly structured with no signs of edema and destruction, intercellular contacts were not disturbed. At the same time moderately pronounced focal destructive changes in the hepatic cells, mainly on the periphery of the lobuli, were detected.

The structure of cells in all areas of the lobulus was homogeneous. The cytoplasm was oxyphilic, round nuclei were clearly circumscribed and found in all lobular hepatocytes. The increased amount of the binuclear hepatocytes indicated about the active progress of regenerative processes. Portal tracts were not dilated; their blood vessels were plethoric (Fig. 4). Histological study of the kidney of animals of Group 4 showed that the structural changes of the components of the organ were not significant as compared to the group of animals, not administered with corrective "Galevit" preparation. In the renal cortex and medulla of kidney insignificant disorders of blood circulation were detected, manifested by inhomogeneous vascular plethora. In the renal cortex the hypertrophied renal corpuscles with minor lumens of the capsules and moderately plethoric vascular glomeruli were noted.

Hypertrophied renal corpuscles were not detected. The proximal and distal parts of the nephron were changed insignificantly. The lumens of the tubules were partially dilated, histostructure of the epithelial cells were without apparent signs of edema and destruction. Hyperglycemia is the key metabolic factor that triggers a cascade of pathological changes in the cells of the renal glomeruli and tubules in diabetes mellitus. It induces unfermentable glycosylation of the membrane and other proteins, activates the sorbitol bypass of the glucose metabolism, oxidative stress, action of growth factors, cytokines, which causes kidney damage at the cellular level.

Glycosylation of the proteins of the basal membrane of the renal glomeruli leads to changes in its structure and properties, thickening of the basal membrane of the vessels of glomerulus, dilatation of the mesangial matrix, resulting in the reduced level of the glomerular filtration. Our studies confirm the described processes of the progress of diabetes mellitus [5, 9].

Conclusions

1. The simulated diabetes mellitus (experimental Group 2) is accompanied by the marked degenerative and focal necrobiotic changes in parenchymatous structures of the liver and kidneys and their blood flow.
2. Correction of the abovementioned pathological condition contributes to the decrease in the intensity of these changes with the simultaneous activation of the compensatory mechanisms, and more positive dynamics of the recovery of structural-functional state of the affected organs occurred in the STZ + N-induced diabetic animals of Group 4, administered with "Galevit" liposomal formulation, as compared with STZ + N-induced diabetic animals of Group 3, administered with pharmaceutical preparation comprising *Galega officinalis L.* and *Vaccinium myrtillus*.
3. The degree of the reparatory processes in the studied organs in experimental type 2 diabetes mellitus shows that the new "Galevit" composition has more apparent positive effect as compared to the pharmaceutical preparation comprising *Galega officinalis L.* and *Vaccinium myrtillus*.

The perspectives of further research. *New scientific data obtained during the experiment can be subsequently used for study of the effect of phytocomposition on the liver and kidneys in type 2 diabetes mellitus.*

References

1. Antopolskaya EV, Shveynov IA. Morfometricheskie pokazateli sostoyaniya tkani pecheni pri poliorgannoy nedostatochnosti na fone ostroy patologii organov bryushnoy polosti. Kurskiy nauchno-prakticheskiy vestnik. Chelovek i ego zdorove. 2009(4):78-81.
2. Horalskyi LP, Khomych VT, Kononskyi OI. Osnovy histolohichnoi tekhniky i morfofunktsiohalni metody doslidzhen u normi ta pry patolohii. Zhytomyr: Polissia; 2011. 288 s.
3. Dedov II. Saharnyiy diabet: ostryie i hronicheskie oslozhneniya. DIabet. GostrI ta hronIchnI uskladnennya, 2011: 480 s.
4. Fira L, Volkov K, Nikolaiev T, Klishch I, Oleshchuk O, Lykhaitskyi P. Vyvchennia vplyvu preparatu enteroshel na strukturni komponenty nyrky pry tryvalomu vvedenni i yoho efektyvnosti za eksperymentalnoi nyrkovoi nedostatnosti. Ukrainskyi zhurnal nefrolohii ta dializu. 2009;2(22):41-6.
5. Chilelli NC, Burlina S, Lapolla A. AGEs, rather than hyperglycemia, are responsible for microvascular complications in diabetes: a "glycoxidation-centric" point of view. Nutrition, Metabolism and Cardiovascular Diseases. 2013 Oct 31;23(10):913-9.
6. Federation ID. IDF diabetes atlas. Brussels: International Diabetes Federation. 2013. 155 p.
7. Islam MS, Choi H. Nongenetic model of type 2 diabetes: a comparative study. Pharmacology. 2007;79(4):243-9.
8. Islam MS, Choi H. Comparative effects of dietary ginger (Zingiber officinale) and garlic (Allium sativum) investigated in a type 2 diabetes model of rats. Journal of medicinal food. 2008 Mar 1;11(1):152-9.
9. Vallon V, Thomson SC. Targeting renal glucose reabsorption to treat hyperglycaemia: the pleiotropic effects of SGLT2 inhibition. Diabetologia. 2017 Feb; 1:1-1.

Реферати

ГИСТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ ПЕЧЕНИ И ПОЧЕК ПРИ ЭКСПЕРИМЕНТАЛЬНОМ САХАРНОМ ДИАБЕТЕ 2 ТИПА И ИХ КОРРЕКЦИЯ ВВЕДЕНИЕМ ФИТОКОМПОЗИЦИЙ НА ОСНОВЕ КОЗЛЯТНИКА ЛЕКАРСТВЕННОГО

Курило К. И., Клищ И.Н., Небесная З. М., Фурдела М.Я., Вольская А. С., Литвинюк С. А.

В эксперименте на белых крысах проведены исследования морфологического состояния почек и печени в условиях экспериментального СД II типа и применения лекарственного средства на основе козлятника лекарственного, черники обыкновенной, введения липосомальной формы Галевит. В группе животных без коррекции, установленны глубокие деструктивно-дегенеративные повреждения всех структурных компонентов исследуемых органов и значительные сосудистые расстройства. Применение корректирующего лекарственного средства на основе козлятника лекарственного и черники обыкновенной оказывает положительное влияние на морфофункциональное состояние печени и почек лабораторных животных и особенно введение липосомальной формы Галевит. По степени репаративных процессов в исследуемых органах при экспериментальном сахарном диабете 2 типа новая композиция Галевит имеет более выраженное положительное влияние по сравнению с фармацевтическим средством на основе козлятника лекарственного и черники обыкновенной.

Ключевые слова: печень, почки, сахарный диабет 2 типа, козлятник лекарственный, черника обыкновенная, липосомальная форма.

HISTOLOGICAL CHANGES IN LIVER AND KIDNEYS IN EXPERIMENTAL TYPE 2 DIABETES MELLITUS AND ITS CORRECTION BY ADMINISTRATION OF *GALEGA OFFICINALIS L.* PHYTOCOMPOSITIONS

Kurylo Kh.I., Klishch I.M., Nebesna Z.M., Furdela M.Ya., Volska A.S., Lytvynyuk S.O.

The experimental studies of the morphological state of the white rats' kidney and liver in conditions of the simulated Type 2 diabetes mellitus and the use of pharmaceutical preparation comprising Galega officinalis L. and Vaccinium myrtillus , as well as administration of «Galevit» liposomal formulation have been carried out. In the group of animals without correction the drastic destructive-degenerative damage to all structural components of the studied bodies and significant vascular disorders has been found. Application of the remedial pharmaceutical preparation comprising Galega officinalis L. and Vaccinium myrtillus has a positive effect on morphofunctional state of the liver and kidneys of laboratory animals, especially the administration of the «Galevit» liposomal formulation. The degree of the reparatory processes in the studied organs in experimental type 2 diabetes mellitus shows that the new «Galevit» composition has more apparent positive effect as compared to the pharmaceutical preparation comprising Galega officinalis L. and Vaccinium myrtillus.

Keywords: liver, kidneys, Type 2 diabetes mellitus, Galega officinalis L., Vaccinium myrtillus, liposomal form.

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