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PATHOMORPHOLOGICAL STATE OF LIVER TISSUES IN RATS WITH EXPERIMENTAL METABOLIC SYNDROME WITH QUERCETIN CORRECTION

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Using an experimental model, we studied the features of the state of the rat's liver tissues under the conditions of experimental metabolic syndrome with quercetin correction. Features of the state of tissues and their morphometric characteristics were microscopically determined in semi-thin histological sections. The majority of hepatocytes visually did not differ from those in the control group and were characterized by a polygonal shape with distinct contours. Hepatic cells with necrotic changes were not detected. Hepatocytes with signs of fatty dystrophy, in which osmiophilic inclusions were present in the cytoplasm, were periodically encountered. In general, sinusoidal capillaries contained some blood corpuscles, some moderately dilated. Although the cross-sectional area of hepatocytes decreased compared to the previous experimental group without correction, it remained larger compared to intact animals. The area of the cytoplasm of liver cells also decreased significantly. The cross-sectional area of the hepatocyte nucleus did not differ much from the previous experimental group. Accordingly, the nuclear-cytoplasmic ratio significantly increased in hepatocytes after quercetin correction, but was lower compared to intact animals. An increase in the number of mononuclear hepatocytes, which practically did not differ from the corresponding indicator in intact animals, should also be considered a morphological manifestation of the protective action of the polyphenol Quercetin in metabolic syndrome.

Keywords: metabolic syndrome, quercetin, liver, hepatocytes, nuclear-cytoplasmic ratio.

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ПАТОМОРФОЛОГІЧНИЙ СТАН ТКАНИН ПЕЧІНКИ ЩУРІВ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ МЕТАБОЛІЧНОМУ СИНДРОМІ З КОРЕКЦІЄЮ КВЕРЦЕТИНОМ

На експериментальній моделі ми дослідили особливості стану тканин печінки щурів за умов експериментального метаболічного синдрому з корекцією кверцетином. В напівтонких гістологічних зрізах мікроскопічно визначали особливості стану тканин та їх морфометричні характеристики. Більшість гепатоцитів не відрізнялися від таких у тварин контрольної групи та характеризувалися полігональною формою з виразними контурами. Печінкових клітин з некротичними змінами не виявлялось. Періодично зустрічались гепатоцити з ознаками жирової дистрофії в яких були наявні осміофільні включення в цитоплазмі. Синусоїдні капіляри містили помірну кількість формених елементів крові, деякі з них були помірно розширеними. Площа поперечного перерізу гепатоцитів хоча і зменшилася порівняно з попередньою експериментальною групою без корекції, але залишалася більшою порівняно з інтактними тваринами. Площа цитоплазми печінкових клітин також помітно зменшилася. Площа перерізу ядра гепатоцитів мало відрізнялася від попередньої експериментальної групи. Відповідно ядерно-цитоплазматичне співвідношення значно збільшилось у гепатоцитах після корекції кверцетином, але було меншим порівняно з інтактними тваринами. Морфологічним проявом протекторної дії поліфенолу «Кверцетин» при метаболічному синдромі також слід вважати збільшення кількості одноядерних гепатоцитів, що практично не відрізнялося від відповідного показника у інтактних тварин.

Ключові слова: метаболічний синдром, кверцетин, печінка, гепатоцити, ядерно-цитоплазматичне співвідношення.

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In modern clinical practice, metabolic syndrome (MetS) is considered a cluster of metabolic dysregulations, which combines carbohydrate and lipid metabolism disorders, oxidative stress, increased body weight, arterial hypertension, and chronic inflammation of liver tissues [1, 4, 6, 9].

MetS is significantly associated with an increased risk of diabetes and cardiovascular disease, significant causes of morbidity and mortality worldwide [11, 12].

In a recent European publication, the authors compared the results of a study of the prevalence of clinical criteria for MetS in the population aged 20–74 years, where a particularly significant increase (from 43 % to 57 %) was observed in men aged 60–74 years and the leading causes were considered to be an increased prevalence of carbohydrate metabolism disorders, abdominal obesity and dyslipidemia. Arterial hypertension was the only criterion for MetS that became slightly less common during the study period [11].

According to the Centers for Disease Control and Prevention in the United States, there was a 35 % increase in the prevalence of MetS from the time the term was coined in the 1980s to 2012 [12]. The prevalence of MetS has been catastrophic, capturing one-third of the American adult population. Although recently published data from the National Health and Nutrition Examination study show a decrease in the

number of patients by 24 % in men and 22 % in women, however, prominent prevalence figures worry specialists [13, 14]. According to WHO data in Ukraine in 2019 statistical reports, 59.1 % of Ukrainians are overweight, and 24.8 % are obese [6].

Metabolic-associated fatty liver disease, an inflammatory disease involving the accumulation of lipid molecules in hepatocytes, is present in 25 % of the world population and 15–49 % of the European population [11]. The same factors moderate its pathogenesis, and progression leads to nonalcoholic steatohepatitis, from which fibrosis develops in 50 % of patients within 8–13 years, leading to cirrhosis in 5–25 % of cases [13].

Dangerous in the epidemiology of the syndrome is the emphasis of many authors on the formation of MetS in childhood when the frequency of liver lesions is observed from 17.5 % to 50 % of the children's population [1, 7].

In the pathogenesis of MetS, the liver is the central organ of pathomorphological transformations with a further chain of pathophysiological processes that lead to the above-mentioned clinical symptoms [2, 10]. Therefore, in our previous studies, we discussed the latest current knowledge of histopathology and pathophysiology MetS. They also presented the main pathomorphological characteristics of the structural components of the liver in the experimental study MetS with an indication of the leading state of the chronic inflammatory process, both in parenchymal and stromal components [1, 4, 6].

Polyphenols are special and interesting for use in the treatment process. The ability of bioflavonoids to limit oxidative and nitrosative stress in liver tissues under experimental conditions substantiates the feasibility of their further research as potential safe means of treatment and prevention of liver pathology under the action of pathogenic factors of the "western" lifestyle (appropriate diet, violation of circadian rhythms) [4, 6].

Administration under experimental conditions of bioflavonoids – epigallocatechin-3-gallate and quercetin – effectively limits the formation of reactive forms of oxygen and nitrogen in liver tissues, namely the rate of superoxide anion-radical production by various sources (by NADPH-dependent microsomal monooxygenases and constitutive NO-synthases in the unconjugated state, the respiratory chain of mitochondria and NADPH-oxidase of leukocytes), suppresses the activity of NO-synthase due to its inducible isoform, reduces the concentration of peroxynitrite [4, 6].

In our presented study, we summarized the latest updates of scientific results regarding the potential treatment and prevention of this clinical syndrome based on the practical use of one of the representatives of the polyphenol group – quercetin, which, according to our data, significantly counteracts the aggressive influence of the main etiological factors on liver structures [1, 4, 6].

The purpose of the study was to determine the pathomorphological features of the state of rats' liver tissues under the conditions of experimental metabolic syndrome and correction with quercetin.

Material and methods. The experimental model was reproduced on sexually mature male Wistar rats, taking into account the prevalence of MetS in men [1, 5, 10]. The animals involved were divided into 3 groups. The first group consisted of intact animals. The second group consisted of animals modulated by MetS using the "Method for modelling metabolic syndrome", for which a utility model Patent No. 122249 dated 26.12.2017 was obtained. We presented the results of this experimental group in previously published materials [4, 5]. The third experimental group was formed by rats, which were given a 200 mg/kg quercetin solution to their food at the same time as a carbohydrate-lipid diet, dissolving it in a 20 % warm aqueous solution of fructose, which increased its solubility and bioavailability [4].

Animals were kept and used in the standard conditions of the university vivarium by the developed resolutions "General ethical principles of animal experiments", adopted by the VII National Congress on Bioethics 2019 and the law of Ukraine of 13.02.2020 [3].

After fulfilling all the conditions for experimenting, the rats were sacrificed by dousing with thiopental anesthesia and subsequent decapitation. The rat's body was dissected with a ventral median incision to determine pathomorphological signs and obtain histological material comprehensively. Liver tissues were carefully removed, cut into 1×1×2 mm pieces with a razor blade, and immersed in a 4 % glutaraldehyde solution for fixation and subsequent manufacture of epoxy blocks. From the latter, semi-thin sections (up to 2 μm) were made, stained with toluidine blue and enclosed in polystyrene for permanent storage according to generally accepted methods.

After receiving, the histological material was examined at the microscopic level using a trinocular microscope Optica B510, to which a digital camera Sigeta M3 CMOC 8500 with software for processing digital images. The obtained images of liver structures were subjected to a comprehensive study according to a self-developed scheme. After obtaining digital images, the morphometric part of the study was performed using the Toup Viwe program of the ToupTek Corporation version 3.7.13127.2018.1016.

Among the studied parameters were the relative indicators of the structural components of the liver tissue, such as the number of mono- and binucleated hepatocytes and their ratio, S cytoplasm of hepatocytes, S nuclei of hepatocytes, nuclear-cytoplasmic proportions, number and area of sinusoidal cells. Processing of the results of morphometric data was provided using the methods of variational statistics. The Student's t-test estimated the probability of the difference between the control and experimental groups.

Results of the study and their discussion. After correcting the metabolic syndrome with quercetin, signs of moderate parenchymal edema were microscopically determined in the liver of animals. In contrast, the structure of the liver cords was mainly preserved. The majority of hepatocytes visually did not differ from those in the control group and were characterized by a polygonal shape with distinct contours (Fig. 1).

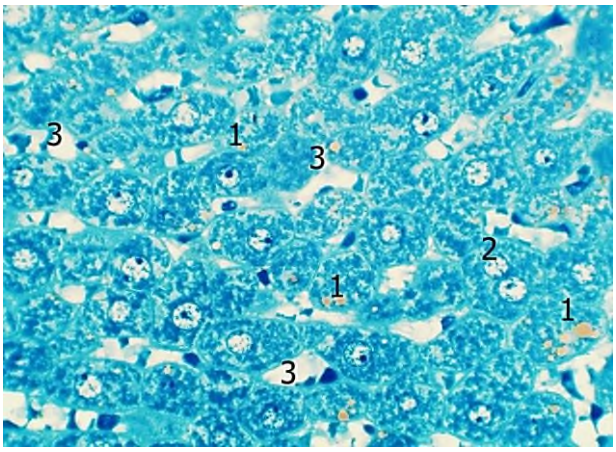

Fig. 1. Liver parenchyma of rats with experimental metabolic syndrome after correction with quercetin. 1 – hepatocytes with signs of fatty dystrophy; 2 – binucleated hepatocytes; 3 – sinusoidal capillaries. Semi-thin section. Toluidine blue staining. Magn. 40×.

The cytoplasm of these cells was characterized by amphiphilicity and, in some cases, moderately pronounced granularity. Nuclei had a regular rounded, somewhat less elliptical shape, were located in the centre of cells and contained from one to four nucleoli. Hepatocytes mostly contained one nucleus, and binucleated cells were much less common.

Compared to the previous experimental group, hepatocytes with hydrophilic dystrophy phenomena were much less common. As before, they were located mainly in the centre of the liver lobules. As a rule, small focal agglomerations formed only in isolated observations. We periodically encountered hepatocytes with signs of fatty dystrophy, the characteristic feature of which was the presence of osmiophilic inclusions of medium and small sizes in the cytoplasm (Fig. 1). These cells were located both in the liver lobes' central parts and on the latter's periphery, near portal tracts. We did not find liver cells with a morphological pattern characteristic of irreversible necrotic changes.

The central veins of the liver lobes were characterised by varying degrees of hematoma, which in some cases spread to the terminal sections of the sinusoidal capillaries. In general, sinusoidal capillaries contained some blood corpuscles, some moderately dilated (fig.2).

The latter showed swelling of the sinusoidal spaces and a significant number of Kupffer cells with swollen nuclei. The portal tracts were not significantly different from the animals of the control group. No necrotic changes were detected in them. In most observations, blood vessels of the portal tracts were characterized by moderate hyperemia. Endothelial swelling occurred in some arterial vessels. The bile ducts were characterized by a typical structure, usually they did not contain bile. Fibroblasts, lymphocytes, plasmocytes, macrophage-monocytic cells, and a few mast cells located in the perivascular zones were constantly found in the perivascular zones (fig.3).

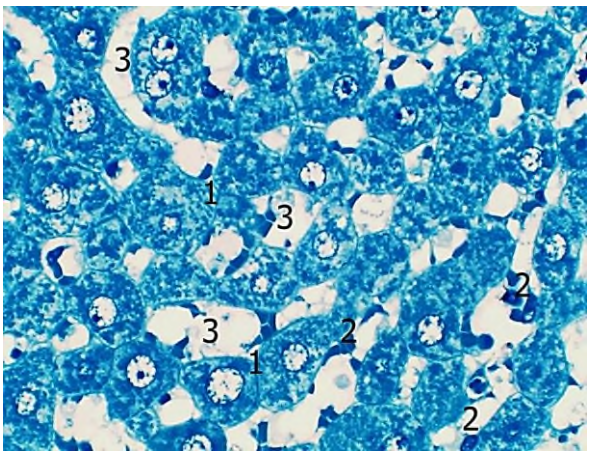

Fig. 2. Liver parenchyma of rats with experimental metabolic syndrome after correction with quercetin. 1 – hepatocytes forming liver cords; 2 – Kupffer cells with swollen nuclei; 3 – sinusoidal capillaries. Semi-thin section. Toluidine blue staining. Magn. 40×.

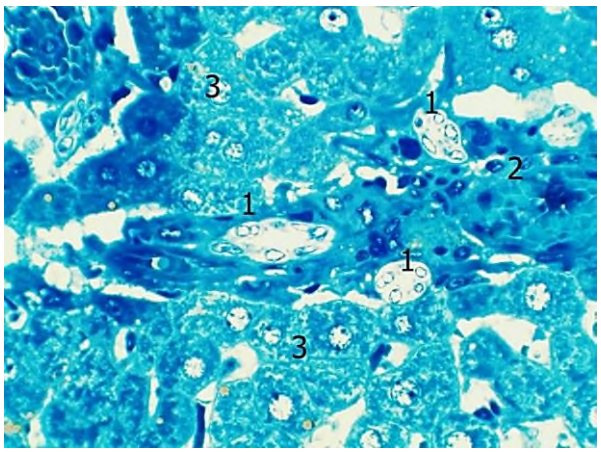

Fig. 3. Liver parenchyma of rats with experimental metabolic syndrome after correction with quercetin. 1 – unchanged intrahepatic bile ducts; 2 – periportal connective tissue; 3 – hepatocytes. Semi-thin section. Toluidine blue staining. Magn. 40×.

According to the morphometric studies, the cross-sectional area of hepatocytes significantly decreased compared to the previous experimental group without correction. It was also more minor compared to intact animals by 306.38±3.67 μm. The area of the cytoplasm of the liver cells was also significantly reduced compared to the MetS-modelled animals, 250.52±26.71 μm and was also more minor compared to the control. The cross-sectional area of the nucleus of hepatocytes also had a decrease in indicators from the previous experimental group and the control group and was 55.86±8.49. Accordingly, the nuclear-cytoplasmic ratio significantly increased in hepatocytes after quercetin correction by 0.23±0.04 and was also more significant compared to intact animals.

An increase in the number of mononuclear hepatocytes to 27.21±5.24 should also be considered a morphological manifestation of the polyphenol Quercetin protective action in MetS. Especially in comparison with the group of animals without correction, and practically did not differ from the corresponding indicator in intact animals 26.21±3.56. The same changes are observed with binucleated hepatocytes, which become significantly more compared to the experimental and intact groups.

Sinusoidal cell area values decreased markedly after MetS correction and amounted to 15.47±4.46. While the number of the latter approached the indicators of intact animals. It should also be noted that the ratio of sinusoidal cells to the number of mononuclear hepatocytes increased, which in this group of animals was 1.24±0.27.

Studies of morphometric indicators of tissue structures of the liver during the correction of MetS with quercetin showed the following results (Table 1).

Table 1

Morphometric parameters of liver cells by experimental groups

Parameters		Intact liver	MetS	MetS + quercetin
Cross-sectional area in microns	Hepatocyte	400.25±49.68	488.08 ±44.51	306.38 ±36.70
	Nucleus	59.88±10.59	59.69 ±8.48	55.86 ±8.49
	Cytoplasm	340.37±45.77	428.39±40.97	250.52 ±32.91
Nuclear-cytoplasmic ratio		0.18±0.03	0.14±0.02	0.23 ±0.04
Number of hepatocytes in the field of view of the microscope at 400 × magnification	Single-core	26.21±3.56	20.36±2.46	27.21 ±5.24
	Binucleated	4.57±1.14	4.29±1.08	6.43 ±2.35
Ratio of the number of binuclear hepatocytes to the number of mononuclear hepatocytes		0.18±0.04	0.22±0.07	0.25 ±0.11
Area of sinusoidal cells		20.19±4.58	21.36±5.12	15.47 ±4.46
Number of sinusoid cells		39.93±4.36	19.36±3.46	32.79 ±5.42
Ratio of the number of sinusoidal cells to the number of mononuclear hepatocytes		1.54±0.15	0.99±0.26	1.24 ±0.27

Inflammatory and purinergic liver signaling modulates several physiopathological processes, such as proliferation, differentiation, migration, and necrosis in response to damage, which we observed in the second experimental group [6, 8, 9]. The morphological analysis of the obtained results shows that the correction of MetS with quercetin leads to the normalisation of most of the studied structural parameters of the liver, bringing the latter's values closer to those of intact animals. However, first, it should be noted that the animals of the third experimental group did not have liver cells with necrosis and a noticeable decrease in hepatocytes with dystrophic changes and severe edema. At the same time, we observed the approximation of metric parameters of hepatocytes to intact animals.

Similar results were obtained by the team of authors of a multi-vector study on the therapeutic effect of polyphenols, which showed the connection between apoptosis and liver diseases and the anti-apoptotic role of polyphenols. Positive nuclear immunoreactivity was found when studying differences in proliferation in liver tissues in response to damage. However, it was limited to Kupffer cells, and the administration of polyphenols reduced proliferation levels [7].

Our results are fully correlated with the experimental and clinical data of other modern studies, which indicate that polyphenols reduce inflammation and fibrosis of the liver. Most likely, the restoration of the typical structural organisation of hepatocytes occurred due to hypoxia reduction in the liver parenchyma. Evidence of this will be considered morphological signs of partial normalisation of blood flow both in the blood vessels of the portal tracts and in the hepatic sinusoids.

Thus, we hypothesise, like previous authors, that the mechanism of the therapeutic role of polyphenols in liver diseases is reversing apoptosis and fibrosis, reducing inflammation, without excluding their other pleiotropic effects. Also, using quercetin leads to the normalisation of the structural organisation of the biliary system of the liver and a significant increase in the quantitative composition of sinusoidal cells.

Conclusions

1. Correction of the metabolic syndrome with quercetin morphologically in the liver tissues reduces the signs of inflammatory processes both in the parenchyma and in the stroma of the organ, which preserves structural and functional integrity.

2. The use of quercetin contributes to the normalisation of blood flow in the blood vessels of the portal tracts and the hepatic sinusoids.

3. The polyphenol "Quercetin" significantly reduces the negative impact of the carbohydrate-lipid diet on the portal tracts of the liver, which did not differ substantially from the control group's animals.

Prospects for further research in this direction will include morphological and biochemical studies of experimental models under the conditions of a carbohydrate-lipid diet and the determination of cytological and histological features of liver structures during correction with various polyphenols and their combinations

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