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Effects of comprehensive treatment with quercetin administration on biochemical blood parameters and pro-and anti-inflammatory cytokines in nonalcoholic fatty liver disease patients

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Abstract
The effect of additional administration of Quercetin to the basic treatment on the clinical course, biochemical parameters, pro- and anti-inflamatory cytokines plasma levels in nonalcoholic fatty liver disease patients was investigated. Indicators that reflect the processes of cytolysis and metabolic intoxication within two weeks of treatment were more effectively improved in nonalcoholic fatty liver disease patients, who in addition to standard treatment received Quercetin. The decrease in the concentration of total cholesterol by 16,7% (p = 0,03) and triacylglycerols – by 33,3% (p = 0,002) was found in patients of the main group. Patients who additionally to basic treatment were prescribed Quercetin after two weeks of treatment also noted a significant reduction in the contents of tumor necrosis factor-α by 39,8% (p = 0,03) and atrial natriuretic propeptide in 2,07 times (p = 0,04) in the blood, indicating its anti-inflammatory and cardioprotective properties, and substantiates the feasibility of its use as a multifaceted medication in nonalcoholic fatty liver disease patients.

Keywords: nonalcoholic fatty liver disease, interleukin-10, tumor necrosis factor-α, atrial natriuretic propeptide.

Introduction
The common theory of nonalcoholic fatty liver disease (NAFLD) development is the theory of "two strokes", according to which excessive accumulation of lipids occurs in hepatocytes first and liver steatosis develops [14]. Further, the mechanism of liver damage progression includes factors that contribute to the activation of lipid peroxidation and increased proinflammatory cytokine production, such as tumor necrosis factor-α (TNF-α), interleukin 1β, interleukin 6 (IL-6), matrix metalloproteinases that contribute to necrosis of hepatocytes, the development of inflammation in the liver tissue and the occurrence of steatohepatitis [10, 11].
In M. Basaranoglu *et al*. investigation TNF-α was shown to cause destabilization of lysosomes and stimulate cathepsin-B-dependent apoptosis in hepatocytes in NAFLD patients [1]. TNF-α was also found to be an independent predictor of fibrogenesis in NAFLD patients [17]. F. Pinto Lde *et al*. suggest that pharmacological agents that target inhibition of TNF-α and the reduction of obesity-related inflammatory markers may be useful in the treatment of NAFLD patients [15].
IL-10 is an anti-inflammatory cytokine, the inhibition of which stimulates the expression of cytokines involved in inflammation of the liver tissue, worsening of insulin resistance and activation of gluconeogenic and lipidogenic reactions [3, 5]. IL-10 reduces the production of proinflammatory cytokines, TNF-α, IL-1, and IL-6 in particular, and produces hepatoprotective properties against the development of liver fibrosis [12].
An important task of modern hepatology is to improve the treatment of NAFLD patients. For this purpose, the use of flavonoids, compounds of plant origin, which have a high antioxidant activity most pronounced in Quercetin, stimulating the synthesis of proteins, regulating the exchange of phospholipids and having membrane-stabilizing properties is rather promising [2, 18]. In experimental studies the water soluble form of Quercetin has been shown to reduce cholesterol, triacylglycerol, glucose, total bilirubin, alanine aminotransferase (ALT) and alkaline phosphatase (AP) plasma activity, and to reduce the disturbances in the system of prooxidants / antioxidants in the liver [16].
The experimental study of HepG2 cells that modified steatosis and then treated with Quercetin at the dose of 10 micromol/L for 24 hours showed decreased levels of triacylglycerols, insulin

resistance, synthesis of proinflammatory cytokines and increased antioxidants concentrations in them, indicating that Quercetin may be effective in treating NAFLD [21]. H.Z. Ying *et al*. in the experiments of alimentary steatohepatitis modeling in the chickweeds postulated, that the Quercetin administration in the dose of 30 and 60 mg/kg body weight per day reduced the content of triacylglycerols and the size of lipid drops in the liver, the activity of serum transaminases and proinflammatory mediators such as TNF-α and IL-6. In animals receiving Quercetin in the dose of 60 mg/kg, the level of collagen in the liver also decreased [22].

The objective of the study was to find out the effect of additional to the basic treatment Quercetin administration on the clinical course, biochemical parameters, pro- and anti-inflamatory cytokines plasma levels in nonalcoholic fatty liver disease patients.

Material and methods

The main group included 41 NAFLD patients, who in addition to the basic treatment, were prescribed Quertin tablets (Quercetin) (PJSC SIC "Borshchahivskiy CPP", Ukraine) at the dose of 40 mg (1 tablet) three times a day for 30 minutes before meals for 14-16 days. The comparison group were 30 NAFLD patients, who received the standard basic treatment comparable to those of the main group by age and gender. Patients with NAFLD were prescribed basic treatment according to EASL–EASD–EASO Clinical Practice Guidelines for the managementof non-alcoholic fatty liver disease (2016) [13]. The control group consisted of 45 practically healthy individuals, of the correlative age and gender of the patients.

Blood samples were obtained in the morning on empty stomach from the antecubital vein in the first day of hospitalization before the treatment was administered. The investigation was performed in compliance with the Council of Europe Convention on Human Rights and Biomedicine and the Recommendations of the Committee on Bioethics of the Ministry of Public Health of Ukraine, and was approved by the Biomedical Ethics Commission of the Higher State Educational Establishment of Ukraine "Bukovinian State Medical University" (Chernivtsi, Ukraine). Written consents were obtained from all the participants.

All of the observed patients and healthy individuals underwent comprehensive clinical, laboratory and instrumental diagnostic investigations. In order to exclude a viral etiology of the liver disease all of the patients were tested on possible hepatitis B and C infections with the help of polymerase chain reaction method. Biochemical studies were performed on the blood biochemical analyzer "Accent-200" ("Cormay SA", Poland). The range of indicators of biochemical blood analysis included: total bilirubin and its fractions, cholesterol, triacylglycerols, total protein and albumin, urea, creatinine, plasma enzyme activity (aspartate aminotransferase (AST), ALT, lactate dehydrogenase (LDG), gamma-glutamyl transferase (GGT), AP).

Investigation of cytokine profile was performed on immunoenzyme analyzer "Statfax 303/Plus" ("Awareness Technology Inc.", USA). The plasma levels of TNF-α ("Bender MedSystems GmbH", Austria), IL-10 ("Bender MedSystems GmbH", Austria), atrial natriuretic propeptide (1-98) (proANP) ("Biomedica", Austria) were studied in the examined patients and healthy individuals.

Results and discussion

Faster for one to three days improvement of patient well-being, reduce of discomfort in the right hypochondrium and unpleasant sensations in the heart area, and increase tolerance to physical activity were observed in NAFLD patients, who additionally to the basic treatment received Quercetin in the indicated dosage.

The dynamics of the studied biochemical parameters in patients of both groups are mentioned in Table 1. AST activity significantly decreased by 37,2% (p = 0,03) and ALT by 50,4% (p = 0,01) (Table 1) during treatment of the patients of the main group. The abovementioned results are consistent with L.M. Sheremet data, who find out similar properties of Quercetin in the experimental modeling of hepatitis [19]. For the patients of comparison group only a tendency to decrease of these enzymes activity was detected. This indicates the effective properties of Quercetin on the reduction of cytolysis activity in patients of the main group as compared to those who received only basic therapy. The dynamics of the studied results was associated with regression of clinical symptoms, which reflected the general-somatic status and conditions of the liver and the cardiovascular system. GGT activity reduced after two-week treatment in patients of both groups: in the patients of the main group – by 89,9% (p = 0,007), and in the comparison group – only by 27,7% (p = 0,03) (Table 1). This reflects a decrease in the manifestations of endogenous intoxication and cholestatic syndromes in the observed individuals. The revealed biochemical changes corresponded to the clinical symptoms of the patients of the main group, who noted faster improvement of overall condition and decrease of dyspeptic disorders. Patients of young age demonstrated more prominent decrease in GGT activity and better recurrence of clinical manifestations of intoxication and cholestatic syndromes in comparison with patients of older age.

The investigation of the effect of Quercetin on the lipid blood spectrum, as the biochemical basis of numerous pathological processes, primarily of atherosclerotic genesis was performed. The decrease in the concentration of total cholesterol by 16,7% (p = 0,03) and triacylglycerols – by 33,3% (p = 0,002) (Table 1) was mentioned for patients of the main group. The content of these factors did not decrease during the two-week treatment period in patients of the comparison group. This decrease in the content of cholesterol and triacylglycerols in the blood of patients who were treated with the addition of Quercetin confirms its hypocholesterolemic and antiatherogenic properties [4, 9]. In particular, in S. Egert *et al*. study in patients with overweight and obesity with metabolic syndrome, administration of Quercetin in the daily dose of 150 mg/day for six weeks contributed to a decrease in the concentration of atherogenic low density lipoproteins in the blood [4].

Table 1: Biochemical blood indicators in nonalcoholic fatty liver disease patients in the dynamics of treatment (M±m, n, p)

Plasma level	Healthy volunteers, n = 45	Comparison group, n = 30		Main group, n = 41	
		Before the treatment	After the treatment	Before the treatment	After the treatment
Total bilirubin, mkmol/L	11,1 ± 0,79	15,9 ± 1,32 $p_1 < 0,001$	13,7 ± 1,20 $p_1 = 0,03$	12,8 ± 2,97	9,5 ± 1,63
Direct bilirubin, mkmol/L	3,1 ± 0,31	4,5 ± 0,72 $p_1 = 0,02$	3,4 ± 0,44	3,1 ± 0,38	2,8 ± 0,31
Cholesterol, mmol/L	4,6 ± 0,15	5,5 ± 0,24 $p_1 = 0,001$	5,1 ± 0,23 $p_1 = 0,04$	5,6 ± 0,48 $p_1 = 0,02$	4,8 ± 0,45 $p_2 = 0,03$
Triacylglycerols, mmol/l	1,0 ± 0,07	2,0 ± 0,18 $p_1 < 0,001$	1,8 ± 0,14 $p_1 < 0,001$	2,0 ± 0,23 $p_1 < 0,001$	1,5 ± 0,12 $p_2 = 0,002$
Albumin, g/L	45,0 ± 0,41	43,8 ± 1,10	45,1 ± 0,88	44,4 ± 1,67	43,4 ± 1,32
Total protein, g/L	69,3 ± 0,62	73,8 ± 1,47 $p_1 = 0,01$	71,2 ± 1,65	74,1 ± 2,58	71,1 ± 1,94
Urea, mmol/L	4,2 ± 0,23	5,2 ± 0,30 $p_1 = 0,01$	5,5 ± 0,41 $p_1 = 0,01$	6,4 ± 1,56 $p_1 = 0,03$	5,4 ± 1,19
Creatinine, mkmol/L	82,6 ± 1,80	90,0 ± 3,59 $p_1 = 0,04$	85,7 ± 3,69	89,0 ± 4,35 $p_1 = 0,04$	85,3 ± 5,81
Aspartate aminotransferase, units of action/L	22,6 ± 1,37	32,8 ± 3,36 $p_1 = 0,001$	29,5 ± 2,33 $p_1 = 0,006$	41,3 ± 5,07 $p_1 < 0,001$	30,1 ± 4,21 $p_2 = 0,03$
Alanine aminotransferase, units of action/L	18,5 ± 1,46	34,7 ± 4,15 $p_1 < 0,001$	31,8 ± 3,32 $p_1 < 0,001$	41,8 ± 3,67 $p_1 < 0,001$	27,8 ± 3,30 $p_2 = 0,01$
Lactate dehydrogenase, units of action/L	387,0 ± 13,59	465,7 ± 27,70 $p_1 = 0,02$	442,9 ± 21,09 $p_1 = 0,04$	454,0 ± 29,89 $p_1 = 0,04$	425,2 ± 34,92 $p_1 = 0,04$
Alkaline phosphatase, units of action/L	80,3 ± 3,20	89,6 ± 6,69	88,5 ± 4,49	88,7 ± 8,15	78,3 ± 6,28
Gamma-glutamyl transferase, units of action/L	21,9 ± 1,62	53,0 ± 7,51 $p_1 < 0,001$	41,5 ± 3,86 $p_1 < 0,001$, $p_2 = 0,03$	58,3 ± 4,97 $p_1 < 0,001$	30,7 ± 4,56 $p_2 = 0,007$

p_1 – significance of differences as compared to the indicators in the healthy individuals; p_2 – significance of differences as compared to the indicators before the treatment.

The results of the investigation of cytokine profile of the blood in NAFLD patients in the dynamics of treatment are mentioned in table 2. TNF-α concentration in the blood of patients of the main group significantly decreased by 39,8% (p = 0,03) (Table 2) as compared to proper indicator before the treatment. Patients of the comparison group showed no significant changes in the activity of this proinflammatory cytokine during two-week treatment. Similar results concerning reduction of TNF-α plasma concentration under the influence of Quercetin administration were found in the experimental studies performed by H.Z. Ying *et al*. [22]. Meanwhile, the content of
IL-10 in the blood did not significantly change during the treatment, both in patients of the main and comparison groups.

It was considered expedient to study the dynamics of proANP level as an accurate marker of cardiovascular insufficiency, since the major comorbid diseases in NAFLD patients are cardiovascular diseases [6, 20, 23]. There was a significant decrease in the concentration of proANP in the blood in 2,07 times (p = 0,04) during two weeks of treatment with Quercetin (Table 2). Significant decrease in the concentration of proANP in the blood that was observed in patients of the main group, indicates its positive cardiotropic properties, which was also noted in the studies of other scientists [7, 8]. Clinically the abovementioned was accompanied by faster improvement of patient's clinical condition, reduction of general weakness and feeling of heaviness in the right hypochondrium, decrease of unpleasant sensations in the heart area and shortness of breath, increase of tolerance to physical activity. Meanwhile, no significant reduction in proANP content in the blood was detected in patients of the comparison group.

Table 2: Indicators of cytokine profile in the blood of nonalcoholic fatty liver disease patients in the dynamics of treatment (M±m, n, p)

Plasma level	Healthy volunteers, n = 20	Comparison group, n = 15		Main group, n = 15	
		Before the treatment	After the treatment	Before the treatment	After the treatment
Interleukin-10, pg/ml	3,9 ± 0,34	4,5 ± 1,04	3,9 ± 0,79	4,8 ± 1,43	4,2 ± 0,38
Tumor necrosis factor-α, pg/ml	15,3 ± 0,95	30,6 ± 8,14 $p_1 = 0,02$	24,8 ± 3,94 $p_1 < 0,001$	33,7 ± 6,94 $p_1 = 0,02$	24,1 ± 4,32 $p_1 = 0,003$ $p_2 = 0,03$
Atrial natriuretic propeptide, nmol/l	1,2 ± 0,23	3,0 ± 0,99 $p_1 = 0,03$	2,2 ± 0,70 $p_1 = 0,04$	2,9 ± 0,86 $p_1 = 0,03$	1,4 ± 0,33 $p_2 = 0,04$

p_1 – significance of differences as compared to the indicators in the healthy people; p_2 – significance of differences as compared to the indicators before the treatment.

Conclusions

Additional to standard treatment Quercetin prescription to nonalcoholic fatty liver disease patients caused more effective improvement of indicators that reflect the processes of cytolysis and metabolic intoxication within two weeks of treatment. Patients of this group after two weeks of treatment also noted a significant reduction in the tumor necrosis factor-α and atrial natriuretic propeptide blood levels, indicating

Quercetin's anti-inflammatory and cardioprotective properties, and substantiates the feasibility of its use as a multifaceted medication in nonalcoholic fatty liver disease patients. However, an incomplete correction of the studied parameters during the two-week treatment indicates the need to continue Quercetin administration at the outpatient period.

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