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POINT OF VIEW

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THE EFFECT OF CONSUMPTION OF *CHLORELLA VULGARIS* SUSPENSION ON HEMATOLOGIC AND BIOCHEMICAL INDICES OF HUMAN BLOOD

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Studies of the effect of the use of preparations with microalgae C.vulgaris on the human body are important due to its wide distribution and the presence of a large number of biologically active substances. We investigated biochemical and hematologic indices of a group of persons after a course of microalgae suspension reception. There were immunomodulatory effects, expressed as an increase in LYM% and a decrease in ESR, cellular rejuvenation primarily among healthy men, and trends toward increased ALB and TP at younger ages. After 50 years of age, a tendency for GLU levels to decrease after the course was detected. There was an increase in CREA and UREA, which may be related to both improved availability of protein compounds and the composition of the microalgae growth medium, which requires further investigation.

Keywords: suspension, chlorella vulgaris, hematology, biochemistry, age, sex, health status.

Introduction. Interest in the study of C.vulgaris as a promising source of essential and nutritional substances emerged in the 1950s and was linked to the world food crisis [6]. Recently, interest in studying the effects of consuming this microalgae on the human body has only been increasing. This is due to the fact that products with C.vulgaris have a unique composition, which includes a set of all essential amino acids, mineral compounds, dietary fiber, polyunsaturated fatty acids, vitamins [5], including D2 and B12, which are absent in plant foods [1], and other compounds. Consumption of such a quantity of biologically active substances certainly has an effect on the human body, which requires its more extended and in-depth study through various methods of research.

All manufactured forms of the drug can be divided into those in which the chlorella is preserved in its natural state and those in which it is destroyed mechanically. The most common preparations contain destroyed strains of C.vulgaris. This fact is due to the fact that microalgae cells cannot be digested by humans because of the cellulose cell wall, which reduces the digestibility of proteins [1]. Nevertheless, even in its natural state in suspension, C.vulgaris is capable of producing effects on the human body.

The aim of this work is to investigate the effect of C.vulgaris suspension consumption on human blood parameters to identify patterns to its use.

Material and methods of research. The study was conducted on 34 volunteers volunteers. For 30 days (course) they took C.vulgaris suspension of IFR strain №C-111 200 ml in the morning on an empty stomach. The density of the suspension was 60 million microalgae per 1 ml. Every 10 days, volunteers were given 2 liters (2 bottles) of C.vulgaris suspension for the specified period. The study was conducted under the condition that volunteers signed informed consent, in accordance with all provisions of the Declaration of Helsinki and was approved by the local ethical committee at Penza State University. 28 people were able to complete the course. Among the reasons for discontinuing the course, volunteers cited the following: unpleasant organoleptic properties, increased diuresis. At the beginning of the study, before the reception of C.vulgaris suspension, as well as at the end of the course, whole blood sampling was performed. The following indices of general and biochemical blood analysis were investigated: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PDW, MPV, P-LCR, PCT, PLT, LYM%, MXD%, NEUT%, LYM#, MXD#, NEUT#, RDW-

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SD, RDW-CV, RET, ESR 30 min, ESR 1 h, ESR 2 h, ALB, ALP, AMYL, SGPT, SGOT, BILT, D-BIL, ID-BIL, CHOL, CREA, GLU, TP, TG, UREA, LDH.

In addition, the volunteers age, sex, and health status were entered into the database. Some volunteers had the following chronic diseases: polycystic kidney disease, anemia, polynosis, allergies, chronic pancreatitis, 12 peptic ulcer, hypertension, type 2 DM, hepatitis. Volunteers were ranked according to the indicated groups (Table 1). The first age group (FAG) included younger volunteers (36.14±6.4 years) and the second (SAG) older volunteers (54.54±7.3).

Statistical analysis was performed using Microsoft Office 2019 software package. Results were provided as a calculation of the median and the 25% and 75% percentiles. A box diagram was used to visualize the results. The data in the sample were not normally distributed, so the non-parametric Mann-Whitney U-test was used to assess the significance of their difference at a threshold value of $p<0.05$. ROC-AUC analysis of some presented blood parameters was also performed according to age periods: 20-29 years, 30-39 years, 40-49 years, 50-59 years, 60 years and older.

Results and discussion. Most of the hematologic and biochemical blood indices we took did not change significantly after the course we took. Nevertheless, certain changes, both expected and not expected, have been identified. When comparing blood parameters before and after the course, without ranking the sample, the following changes were observed: increase in LYM% (p=0.0384), acceleration of ESR 30 min (p=0.0013), increase in CREA (p=0.0000), increase in UREA (p=0.0198) (Figure 1).

When the volunteer group was ranked by health status, it was found that having a chronic disease decreased MCHC (p=0.0455) and increased CREA to a greater extent (p=0.0000) after the course. Healthy volunteers had increased MPV (p=0.0016), accelerated ESR 30 min (p=0.0016), ESR 1 h (p=0.0065), increased CREA (p=0.0104) and UREA (p=0.0182). Both men (p=0.0257) and women (p=0.0257) had accelerated ESR 30 min. CREA was also significantly increased in both men (p=0.0005) and women (p=0.0000).

The FAG had accelerated ESR 30 min (p=0.0257), increased CREA (p=0.0001) and UREA (p=0.0413). In the SAG, ESR 30 min (p=0.0214) and ESR 1 h (p=0.0307) were accelerated and CREA increased (p=0.0024) (Table 1).

In addition to intra-group data analysis, inter-group data analysis was performed.

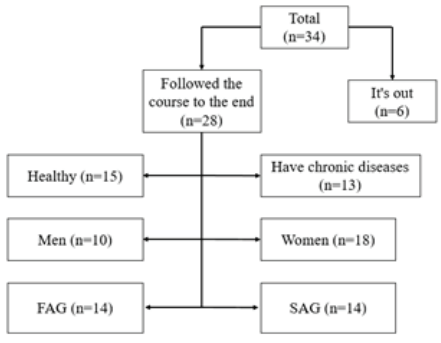

Fig. 1. Design of the study

The disappearance difference (p=0.0477; p=0.8181) between the CREA level of healthy and chronically ill volunteers may indicate its greater elevation in the second group after the course.

The difference in RBC (p=0.0127; p=0.0198), HGB (p=0.0000; p=0.0001), HCT (p=0.0002; p=0.0004) in males and females is explained by the effect of sex hormones.

There was a decrease in MCHC in women, while no change was observed

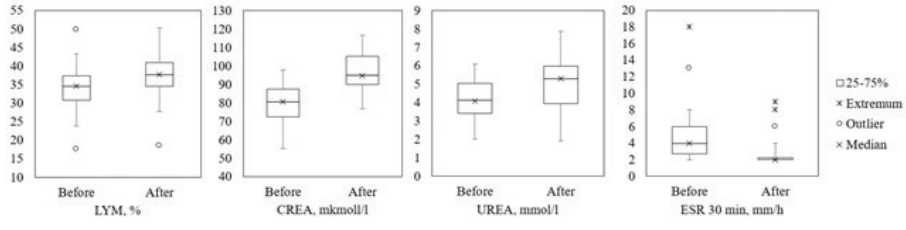

Fig. 2. Significant changes in hematologic and biochemical blood parameters in the total group before and after administration of *C.vulgaris* suspension

Table 1

Intragroup comparison of significant hematologic and biochemical blood parameters before and after administration *of C.vulgaris* suspension

Indicator	Prior to reception	After reception	p	Prior to reception	After reception	p
For health reasons	Healthy (n=15)			Have chronic diseases (n=13)		
MCHC. g/dL	353 [346;356.5]	348 [343;356.5]	0.6965	357 [354.0;363.0]	350 [347.0;358.0]	0.0455
MPV. fL	9.3 [9.2;10.8]	9.9 [9.4;10.9]	0.0016	10.3 [10.1;11.1]	10.4 [10.1;10.8]	0.7948
ESR 30 min. mm/h	5.0 [3.5;7.5]	2.0 [2.0;2.5]	0.0016	4.0 [2.0;5.0]	2.0 [2.0;2.0]	0.1585
ESR 1 h. mm/h	11.0 [6.0;14.0]	5.0 [4.0;8.5]	0.0065	9.0 [4.0;10.0]	5.0 [4.0;8.0]	0.4122
CREA. µmol/l	85.2 [78.1;90.0]	91.1 [89.6;107.2]	0.0104	75.0 [61.5; 85.2]	98.4 [90.1; 102.2]	0.0000
UREA. mmol/l	3.9 [3.5;4.6]	5.3 [4.3;5.7]	0.0182	4.2 [3.4;5.5]	5.37 [3.9;5.9]	0.3575
By sex	Men (n=10)			Women (n=18)		
ESR 30 min. mm/h	4.0 [2.2;6.5]	2.0 [2.0;2.0]	0.0257	4.5 [3.0;5.7]	2.0 [2.0;3.7]	0.0257
CREA. µmol/l	90.0 [85.2;92.3]	105.6 [104.6;110.5]	0.0005	76.1 [63.6; 83.6]	90.1 [89.4; 95.3]	0.0000
По возрасту	ПВГ (n=14)			ВВГ (n=14)		
ESR 30 min. mm/h	3.0 [2.0;4.0]	2.0 [2.0;2.0]	0.0257	5 [4.2;7.7]	2.0 [2.0;4.7]	0.0214
ESR 1 h. mm/h	6.0 [4.2;9.0]	5.0 [4.0;6.7]	0.3472	12.5 [10.0; 14.0]	5.5 [4.2; 12.7]	0.0307
CREA. µmol/l	78.1 [70.8;85.2]	93.3 [90.1;104.5]	0.0001	84.8 [75.5; 87.6]	97.1 [89.4; 107.9]	0.0024
UREA. mmol/l	3.5 [3.2;4.4]	5.0 [3.9;5.5]	0.0413	4.7 [3.8;5.5]	5.3 [4.5;6.1]	0.1556

in men (p=0.0687; p=0.0292). When RET was initially equal after the course, the rate decreased in women and increased in men (p=0.7565; p=0.0110). Also, the initially unobserved difference in ALP after the course increased in men, which was also reflected in the level of significance (p=0.1074; p=0.0018). The difference in CREA levels was present before the course and did not change after the course (p=0.0013; p=0.0004). In the SAG, RDW-SD was higher both before and after the course compared to the FAG, accounting for the difference (p=0.0455; p=0.0348). RDW-SD was higher in the SAG both before and after the course compared to the FAG, accounting for the difference (p=0.0455; p=0.0348). RET levels increased after the course in FAG, while they decreased in SAG (p=0.0366; p=0.5961). ESR 30 min (p=0.0107; p=0.0989), 1 h (p=0.0027; p=0.3125), 2 h (p=0.0038; p=0.3125) before the course was predominant among SAG, while after the course ESR decreased in both groups to equal values.

ALBs increased after the course, more so in FAG (p=0.0703; p=0.0051). ALP levels were relatively increased in SAG both before and after the course (p=0.0307; p=0.0384). CHOL levels were also higher in SAG, however, after the course the difference was worse pronounced due to a non-significant increase in FAG (p=0.0057; p=0.0131). UREA before the course was higher in SAG than FAG, but after the course the difference was worse with a general increase in the two groups (p=0.0292; p=0.3843). LDH both before and after the course was lower in FAG, with an overall decrease (p=0.0146; p=0.0366) (Table 2).

Table 2

Intergroup comparison of significant changes in hematologic and biochemical blood parameters before and after *C.vulgaris* suspension administration

Indicator	Prior to reception		p	After reception		p
For health reasons	Healthy (n=15)	Have chronic diseases (n=13)		Healthy (n=15)	Have chronic diseases (n=13)	
CREA, µmol/l	85.2 [78.1;90.0]	75.0 [61.5;85.2]	0.0477	91.1 [89.6;107.2]	98.0 [90.1;102.2]	0.8181
By sex	Women (n=18)	Men (n=10)		Women (n=18)	Men (n=10)	
RBC, 10^9/l	4.2 [4.3;4.6]	5.1 [4.7;5.4]	0.0127	4.5 [4.2;4.7]	5.0 [4.7;5.3]	0.0198
HGB, g/l	134.0 [130.0;139.0]	156.0 [152.2;165.2]	0.0000	131.0 [126.5;137.7]	152.0 [150.2;157.5]	0.0001
HCT, %	38.0 [36.8;39.1]	43.4 [42.4;45.4]	0.0002	37.7 [36.7;39.0]	42.7 [41.9;45.4]	0.0004
MCHC, g/dL	353.5 [342.7;358.5]	356.5 [353.2;364.5]	0.0687	347.0 [340.0;353.7]	356.5 [351.0;358.0]	0.0292
RET, %	5.0 [3.0;6.0]	5.0 [4.0;8.0]	0.7565	4.5 [4.0;5.0]	7.0 [5.0;10.0]	0.0110
ALP, U/l	50.0 [42.8;55.8]	58.0 [49.6;77.6]	0.1074	50.0 [47.2;62.5]	74.5 [63.2;89.0]	0.0018
CREA, µmol/l	76.1 [63.6;83.6]	90.0 [85.2;92.3]	0.0013	90.1 [89.4;95.3]	105.6 [104.6;110.5]	0.0004
By age	FAG (n=14)	SAG (n=14)		FAG (n=14)	SAG (n=14)	
RDW-SD, %	40.7 [40.1;42.3]	43.2 [40.9;45.2]	0.0455	40.9 [40.1;41.9]	43.8 [41.2;44.6]	0.0348
RET, %	4.0 [3.0;5.0]	6.0 [5.0;8.0]	0.0366	5.0 [4.0;5.7]	5.0 [4.0;9.2]	0.5961
ESR 30 min, mm/h	3.0 [2.0;4.0]	5.0 [4.2;7.7]	0.0107	2.0 [2.0;2.0]	2.0 [2.0;4.7]	0.0989
ESR 1 h, mm/h	6.0 [4.2;9.0]	12.5 [10.0;14.0]	0.0027	5.0 [4.0;6.7]	5.5 [4.2;12.7]	0.3125
ESR 2 h, mm/h	11.0 [8.2;14.5]	20.0 [16.0;25.0]	0.0038	12.5 [9.2;14.7]	13.5 [9.5;25.0]	0.3125
ALB, g/l	45.5 [43.1;48.0]	43.7 [41.2;44.4]	0.0703	46.5 [45.8;47.0]	44.1 [42.0;45.4]	0.0051
ALP, U/l	47.6 [42.1;50.4]	58.0 [53.7;62.2]	0.0307	49.0 [47.2;64.5]	63.5 [58.7;74.5]	0.0384
CHOL, mmol/l	4.5 [4.2;4.9]	5.6 [4.9;6.0]	0.0057	5.0 [4.5;5.3]	5.6 [5.4;6.4]	0.0131
UREA, µmol/l	3.5 [3.2;4.4]	4.7 [3.8;5.5]	0.0292	5.0 [3.9;5.5]	5.3 [4.5;6.1]	0.3843
LDH, U/l	294.0 [266.5;310.7]	320.0 [308.5;339.0]	0.0146	284.0 [258.7;321.7]	316.5 [303.0;360.2]	0.0366

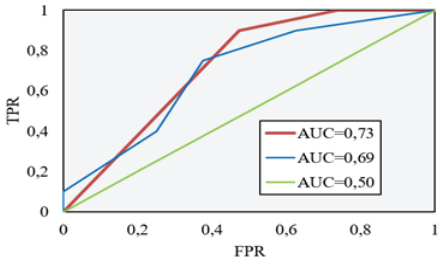

Fig. 3. ROC curves of GLU and TP levels during administration of *C.vulgaris* suspension according to age: red curve – GLU; blue curve – TP; green curve – control

Among the results obtained in the total group as well as in the rest of the groups, an increase in LYM% and ESR at different time intervals of measurement was noticeable, which may indicate the immunomodulatory and anti-inflammatory effects of microalgae suspension on the human body. C.vulgaris is probably characterized by the following mechanism of immunomodulation. The microalgae is rich in arginine, accounting for about 3200 mg per 100 g dry weight, depending on the strain. Arginine promotes the formation of such an important signaling molecule as NO, which in turn is an activator of guanylate cyclase that triggers numerous intracellular chains of reactions, including those leading to immune response in competent cells [1, 7]. In addition, the mechanism of immunomodulation is explained by the presence of a-glucan among the carbohydrates of microalgae, which causes proliferation of splenocytes and restores the level of secretion of cytokines TNF-a and IL-2 [5, 10]. The changes in GLU and TP scores were not significant. However, when considering the trend of these indicators with age, characteristic changes can be found. Thus GLU levels decreased to a greater extent among volunteers over 50 years of age (AUC=0.73). In turn, increased TP was characteristic of volunteers younger than 50 years of age (AUC=0.69) (Figure 2). The antidiabetic effect of C.vulgaris has been described in the literature and may be due to: decreased serum lipid peroxides, [9] increased expression of GLUT4 receptor in skeletal muscle [11], and decreased expression of insulin resistance inducers such as resistrin [3].

The results obtained can be explained by the improved availability of protein compounds, which is also expressed by the tendency to increase ALB and TP, as well as by the joint effect of the composition of the nutrient medium for microalgae cultivation on the human body [1]. It is also worth noting the increase in CREA and UREA, which in itself is not a positive result. Various studies indicate that the preparation property of microalgae depends on the following factors: medium temperature, growth nutrient composition, light availability [8], and the strain itself. Thus, for the synthesis of vitamin B12 and corrinoid compounds [2], Co2+ was added to the growth medium of C.vulgaris, which could also cause an increase in the described parameters. The trend of increased CHOL among young adults is unlikely to indicate the development of a pathologic process, but nevertheless differs from the data presented in the literature. The cell wall of C.vulgaris seems to be supposed to prevent lipid absorption in the intestinal lumen, which accounts for the decrease in CHOL in similar studies [4].

Также непонятно за счет какой фракции липидов (ЛПНП или ЛПВП) происходило повышение показателя. It is also unclear which lipid fraction (LDL or HDL) was responsible for the increase.

Conclusions. Thus, taking C.vulgaris suspension has a pronounced immunomodulatory and anti-inflammatory effect on the human body regardless of sex, age and state of health. Microalgae promotes rejuvenation of erythroid, megakaryocytic and lymphocytic cell lineage, especially in men at a young age. Also at a young age an increase in protein synthesizing function is pronounced. After the age of 50, consumption of microalgae has a hypoglycemic effect. Meanwhile, the detected increase in CREA, UREA, and CHOL warrants further investigation and monitoring of the consumption and production of C. vulgaris.

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