EFFECTS OF NUTRITIONAL FORMULA SUPPLEMENTATION IN PEOPLE WITH ELEVATED LIVER ENZYMES

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ABSTRACT

The controlled randomized intervention study to evaluate the effects of nutritional formula supplementation in people having elevated liver enzymes was implemented in Ninh Binh province from March to September 2023. 120 participated subjects were randomly divided into 2 groups, each group had 60 subjects: the intervention group supplemented with 45 g of formula twice daily and the control group with regular diet. Results showed that indicators of liver enzymes, nutrition, and health in the intervention group were better than the control group with statistical significance: average aspartate aminotransferase (AST) level decreased by 45.32 UI/L (-4.29 ± 8.36 vs. 1.03 ± 0.45); average alanine aminotransferase (ALT) level decreased by 53.96 UI/L (-56.98 ± 6.53 vs. -3.02 ± 1.35) (p<0.001); Reducing amount of food decreased by 20% (p<0.01); Loss of appetite decreased by 16.7% (p<0.05); Constipation reduced by 15% (p<0.05); Diarrhoea decreased by 11.7% (p<0.05); No-deep sleep decreased by 40% (p<0.05); Fitful sleep decreased by 18.4% (p<0.05); Some indicators tended to be better but not statistically significant (p>0.05): Difficulty sleeping decreased by 10.0%; Itching decreased by 13.4%; Urticaria decreased by 6.6%; Respiratory infections decreased by 1.6%; Fatigue decreased by 10%; Anxiety and emotional changes decreased by 8.4%; and average weight increased by 0.1 kg with p>0.05. Product acceptability of users was 100% of which there were 73.3% very satisfied; 21.7% satisfied; and 5.0% accepted./.

Key word: *Nutrition, elevated liver enzymes, AST, ALT*

I. INTRODUCTION

Elevated liver enzymes are indicators showing that the liver cells are destroyed in people having liver disorder who account for a significant proportion of population. Energy-rich nutrition and appropriate multi-micronutrients, vitamins and minerals are very important roles in supporting to protect, regenerate and restore cells and improve liver function as well as the function of the body's organs and immune system. To meet nutritional needs through the daily diet faces many difficulties, meanwhile people with poor liver function often get difficulty digesting food and anorexia, creating a cycle of malnutrition that affects liver function and vice versa. Research into convenient, appropriate, and feasible nutritional supplements for people with liver dysfunction is necessary; especially nutrition formula seems one of affordable and feasible solutions. However, dosage and ingredients are still issues needing more research [1]. This also following The National Nutrition Strategy for the period 2021-2030 with the goal of " *Implementing relevant nutrition to improve nutritional status suitable for each subject, locality, region, region and group of population"* [2]. This study was conducted to evaluate the effectiveness of an optimal

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supplementing formula for people having elevated liver enzymes.

II. RESEARCH SUBJECTS AND METHODS

Research subjects: People with elevated liver enzymes (AST, ALT >40 UI/L); volunteer to participate in research; not under treatment or suffering from serious illness; not use nutritional supplements or participate in other research studies.

Study design: Randomized controlled clinical intervention study.

Time: from February 2023 to September 2023.

Sample size, sample selection: 120 eligible subjects were randomly divided 1:1 into 2 intervention group and control group.

Intervention materials: Leanmax Ligos, a nutritional product produced by NutriCare Nutrition Joint Stock Company, was selected as intervention material. 100 g of formula includes 443kcal of energy; protein (18.8 g); arginine (894 mg); methionine (260 mg); BCAA (3320 mg); fat (15.1g); MCT (4.33 g); MUFA (5463 mg); PUFA (1486 mg); carbohydrates (60.1 g); FOS/Inulin (3.27g); colostrum (147 mg); IgG (26.7 mg); choline (500 mg); and 13 vitamins: A (1373 IU), D3 (412 IU), E (13.7 IU), K1 (31.4 mcg), C (103 mg), B1 (1169 mcg), B2 (753 mcg), B3 (13979 mcg), B5 (4996 mcg), B6 (1711 mcg), B9 (122 mcg), B12 (5.15 mcg), Biotin (28.1 mcg); and 14 minerals: Sodium (207 mg), Potassium (169 mg), Chlorine (362 mg), Calcium (352 mg), Phosphorus (99.9 mg), Magnesium (70.9 mg), Iron (3.09 mg), Zinc (8.09 mg), Manganese (927 mcg), Copper (206 mcg), Iodine (63 mcg), Selenium (18.1 mcg), Chromium (20.3 mcg), Molybdenum (20.2 mcg).

Intervention and assessment methods*:* 60 subjects in the intervention group used formula 45 g twice daily for 6 weeks and 60 subjects the control group used regular diet; Results were compared before and after intervention in 2 groups regarding liver enzyme level, nutritional status, health, quality of life index.

Data analysis and processing: Data were entered using MS.Excel 2016 and Epidata 3.1 software and analysed using IBM SPSS 20.0 software.

Research ethics: The study was approved by the IRB of the Institute of Health Sciences & Technology and adhered to good clinical practice.

III. RESULTS

Table 1. ***Characteristics of study participants***

Index	Intervention group (n = 60)	Control group (n = 60)	All
Age (min, max)	56.5 (25.69)	57.8 (30.69)	57.3(25.69)
Male	31 (51.7%)	30 (50%)	61 (50.8%)
Weight (kg)	55.5 ± 4.7	55.8 ± 4.9	55.7 ± 4.8
Height	158.1 ± 5.8	158.2 ± 6.0	158.2 ± 5.9
BMI (kg/m^2)	22.6 ± 1.5	22.7 ± 1.6	22.7 ± 1.6
AST (UI/L)	73.45 ± 25.35	69.83 ± 22.55	72.6 ± 28.68
ALT (UI/L)	86.59 ± 11.45	90.02 ± 15.72	87.9 ± 25.65

There were similarities between the intervention group and the control group in terms of age, gender, average weight, and body mass index (BMI) (Table 1). Average liver enzyme levels in the intervention group and the control group were small differences and not statistically significant (p>0.05): AST (73.45 ± 25.35 and 69.83 ± 22.55 UI/L), and ALT (86.59 ± 11.45 and 90.02 ± 15.72 UI/L).

Table 2. ***Effectiveness in improving elevated liver enzyme***

Index	Time	Intervention group	Control group	p [a]
AST (UI/L)	T0	73.45 ± 25.35	69.83 ± 22.55	>0.05
	T1	29.16 ± 12.15	70.87 ± 14.34	<0.001
	T1-T0	- 44.29 ± 8.36	1.03 ± 0.45	<0.001
ALT (UI/L)	T0	86.59 ± 11.45	90.02 ± 15.72	>0.05
	T1	29.60 ± 7.88	87.00 ± 22.16	<0.001
	T1-T0	-56.98 ± 6.53	2.92 ± 1.35	<0.001

(a): T-test for the average of 2 independent groups.

The results showed that liver enzyme levels before (T0) and after 6 weeks of intervention (T1) were better (Table 2): Average AST before and after intervention decreased by 44.29 UI/L (29.16 ± 12.15 vs. 73.45 ± 25.35 UI/L); the intervention effect comparing intervention group and control group was decreased by 45.32 UI/L (-44.29 ± 8.36 vs. 1.03 ± 0.45 UI/L) having a statistically significant difference (p<0.001). Average ALT level before and after intervention decreased by 56.98 UI/L (29.60 ± 7.88 vs. 86.59 ± 11.45 UI/L) and the intervention effect comparing intervention group and control group was decreased by 59.90 UI/L (-56.98 ± 6.53 vs. 2.92 ± 1.35 UI/L) and statistically significant (p<0.001).

Table 3. ***Effectiveness in improving skin problems***

Index	Time	Intervention group	Control group	p
Itchy skin	T0	14 (23.3%)	11 (18.3%)	>0.05 [b]
	T1	4 (6.7%)	9 (15.0%)	<0.05 [c]
	T1-T0	-10 (-16.7%)	- 2 (3.3%)	<0.05 [c]
Rash on the skin	T0	9 (15.0%)	7 (11.7%)	>0.05 [b]
	T1	4 (6.7%)	6 (10.0%)	>0.05 [c]
	T1-T0	- 5 (- 8.3%)	- 1 (-1.7%)	>0.05 [c]

(b): Chi-square test; (c): *Fisher's exact - test, Bootstrap 1000 samples.*

Study results showed the change rate of skin itching before and after intervention (Table 3): Skin itching decreased by 16.7%, the intervention effect comparing intervention group and control group was decreased by 13.4% (-16.7% vs. -3.3%) with statistical significance (p<0.05); The rate of skin rashes after intervention decreased by 8.3%; the intervention effect comparing intervention group and control group was decreased by 6.6% (-8.3% vs. -1.7%) with p>0.05.

Table 4. ***Effectiveness in improving nutritional and digestive status***

Index	Time	Intervention group	Control group	p
Weight (kg)	T0	55.5 ± 4.7	55.8 ± 4.8	>0.05 [a]
	T1	55.7 ± 4.8	55.9 ± 4.9	>0.05 [a]
	T1-T0	0.2 ± 0.1	0.1 ± 0.1	>0.05 [a]
Reducing amount of food	T0	15 (25.0%)	13 (21.7%)	>0.05 [b]
	T1	5 (8.3%)	15 (25.0%)	<0.01 [b]
	T1-T0	-10 (-16.7%)	2 (3.3%)	<0.01 [c]
Loss of appetite	T0	21 (35.0%)	19 (31.7%)	>0.05 [b]
	T1	8 (13.3%)	1 7 (28.3%)	<0.05 [b]
	T1-T0	-13 (-21.7%)	-2 (-3.3%)	<0.0 5 [c]
Indigestion	T0	23 (38.3%)	25 (41.7%)	>0.05 [b]
	T1	12 (20.0%)	24 (40.0%)	<0.05 [b]
	T1-T0	-11 (-18.3%)	-1 (1.7%)	<0.05 [c]

(a): T-test for the average of 2 independent groups; (b): Chi-square test; (c): Fisher's exact - test, Bootstrap 1000 samples.

Table 4 shows the results of improving nutritional status after intervention (T1): weight increased 0.2 ± 0.02 kg, the intervention effect comparing intervention group and control group was decreased by 0.1 kg (0.2 ± 0.02 kg vs. 0.1 ± 0.01 kg) (p>0.05); The rate of reducing amount of food decreased by 16.7% and the intervention effect comparing intervention group and control group was decreased by 20% (p<0.01); Loss of appetite decreased by 21.7% and the intervention effect decreased by 18.4% (p<0.05); Bloating /indigestion decreased by 18.3% and the intervention effect decreased by 16.6% (p<0.05).

Table 5. ***Effects on improving sleep and mental health***

Index	Time	Intervention group	Control group	p
No deep sleep	T0	42 (70.0%)	38 (63.3%)	>0.05 [b]
	T1	16 (26.7%)	36 (60.0%)	<0.05 [b]
	T1-T0	-26 (-43.3%)	-2 (-3.3%)	<0.05 [c]
Fitful sleep	T0	13 (21.7%)	11 (18.3%)	>0.05 [b]
	T1	8 (13.3%)	12 (20.0%)	>0.05 [b]
	T1-T0	-5 (-8.3%)	1 (1.6%)	---
Difficulty falling asleep	T0	13 (21.7%)	12 (20.0%)	>0.05 [b]
	T1	9 (15.0%)	14 (23.3%)	>0.05 [b]
	T1-T0	-4 (-6.7%)	2 (3.3%)	>0.05 [c]

(b): Chi-square test.; (c): *Fisher's exact - test, Bootstrap 1000 samples.*

The results of the table above show improvement in sleep in the intervention group compared to the control group: No deep sleep decreased by 43.3%, the intervention effect was reduced by 40% compared to the control group (-43.3% vs. -3.3%) (p<0.05); Fitful sleep decreased by 18.4%, the intervention effect decreased by 9.9% (-8.3% vs. 1.6%) (p>0.05); Difficulty sleeping decreased by 6.6%, the intervention effect decreased by 10.0% (- 6.6% vs. 3.6%) (p>0.05).

Table 6. ***Effects in immune system and other indicators***

Index	Time	Intervention group	Control group	p
Respiratory infections	T1-T0	-2 (-3.3%)	-1 (-1.7%)	---
Diarrhoea	T1-T0	-8 (-13.3%)	-1 (-1.7%)	---
Constipation	T1-T0	-8 (-13.3%)	1 (1.7%)	<0.05 [c]
Fatigue	T1-T0	-5 (8,3%)	1 (1,7%)	---
Anxiety	T1-T0	-7 (11,7%)	-2 (3,3%)	>0,05[c]

(b): Chi-square test.; (c): *Fisher's exact - test, Bootstrap 1000 samples.*

Analysis results in Table 6 show improvement in infection after intervention: Respiratory infections decreased by 3.3%, intervention effect decreased compared to the control group by 1.6% (-3.3% vs. -1.7%) (p>0.05); Diarrhoea decreased by 13.3%, the intervention effect was reduced by 11.7% compared to the control group (-13.3% vs. -1.7%) (p<0.05); Constipation decreased by 13.3%, the intervention effect was reduced by 5.0% compared to control group 1 (-13.3% vs. 1.7%) (p<0.05); Fatigue decreased by 10% (-8,3% vs. 1,7%); Anxiety and emotional changes decreased by 8.4% (-11,7%) vs - 3,3%).

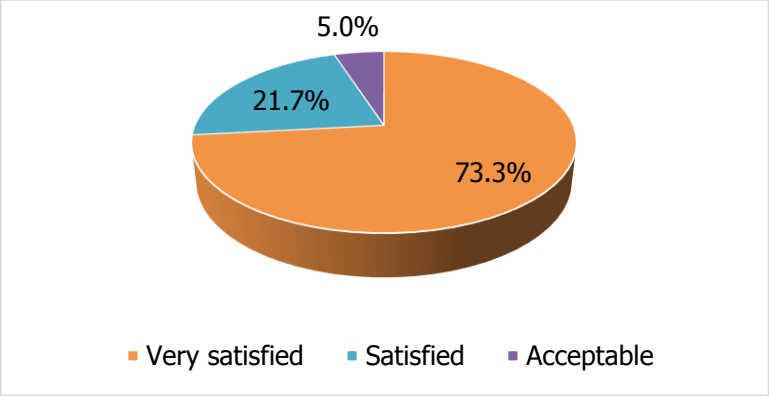

Figure 1. ***Rate of product acceptability***

The results showed that 100% of users accepted the use of the product, of which 73.3% of users were very satisfied with the product, 21.7% were satisfied and 5.0% were acceptable (Figure 1).

IV. DISCUSSION

The intervention had positive effects on reducing liver enzyme, protecting liver cells, detoxifying and supporting liver functions: Liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are intracellular enzymes those levels increase when having destroyed liver cells due to inflammation or toxicity [1]. Normal value allows AST<25 UI/L in men and <21 UI/L in women, ALT<22 UI/L in men and <17 UI/L in women [1]. Meeting the body's optimal nutritional needs can be a solution to help improve health and improve liver function. The formula provides energy, essential amino acids and 27 vitamins and minerals for 6 weeks showed that the average AST level decreased by 45.32 UI/L (-4.29 ± 8.36 vs. 1.03 ± 0.45); average ALT level decreased by 53.96 UI/L (-56.98 ± 6.53 vs. -3.02 ± 1.35) (p<0.001); This result reflects the role of ingredients in nutritional milk in contributing to reducing inflammation and cytotoxicity: arginine (894 mg), methionine (260 mg) and an amino acid chain including leucine, isoleucine, valine with a content of 3320 mg. Those helped to prevent protein catabolism, improved cell metabolism and enhanced the liver's detoxification function. Choline (500 mg/100 g formula) also contributed for catalysing metabolism and enhancing liver function. Research results of Vali Musazadeh and colleagues in Iran on the effects of supplementing camel milk with ingredients rich in energy and nutrients for people with increased liver enzymes also showed that average ALT decreased by 10.54 IU/L and average AST decreased by 10.19 UI/L while improving the immune system and reducing inflammation at a statistically significant level (p<0.05) [3]. Vitamins of groups D, E, and K have been proven to have a role in supporting the treatment of people with liver disorder. Providing optimal dietary supplements is a solution to support liver protection from the effects of free radicals, which are harmful chemical intermediates produced during incomplete metabolism (p<0.05) [4]. The results also showed that in the intervention group, skin itching improved by 16.7% and the intervention effect decreased by 13.4% compared to the control group (16.7% vs.

3.3%) with statistical significance (p<0.05); The rate of skin rashes tended to decrease (8.3% vs. 1.7%) but the difference was not statistically significant (p>0.05). This result can be explained that supplementing dairy products with a diverse composition of micronutrients could have positive effects on the immune system. Some micronutrients, having antioxidant effects and neutralize free radicals due to homeostasis, had important effects to improve, maintain and stimulate immune responses [5].

Supplementing nutrients contributed to enhancing immune system: The colostrum ingredients in the product provided contribution in strengthen the body's antibodies such as colostrum (147 mg), IgG (26.7 mg) and choline (500 mg) in 100 g of formula supporting to enhance the immune system [5]. A review of 29 randomized controlled clinical trials found that dietary nucleotide supplementation could increase immunoglobulin concentrations [6]. Research results showed a trend of reducing infections in the intervention group with a reduced rate of diarrhoea by 13.3%, the intervention effect decreased by more than 11.7% compared to the control group (13.3% vs. to 1.6%) with statistically significant (p<0.05); Constipation rate decreased by 13.3%, intervention effect decreased by more than 15.0% compared to the control group (-13.3% vs. 1.7%) with statistically significant (p<0.05) and the rate of respiratory infections also tended to decrease by 3.3% (p>0.05). It can be explained that the nutritional ingredients in formula helped to improve immune status, enhance resistance and digestive system function, and reduce the incidence of acute infections. This is relevant with current knowledge on micronutrients with antioxidant properties play an important role in enhancing and improving the immune system to support the body against infectious diseases, in which, in addition to amino acids, vitamins and minerals also play important roles [7].

The intervention improved digestion, sleep, nutritional status, and quality of life: The ingredients in the nutritional formula supplement energy and micronutrients that help neutralize harmful free radicals in the blood, reduce inflammation and restore liver cells, strengthen body resistance and increase beneficial intestinal bacteria. Weight and body mass index (BMI) improved but did not cause overweight or obesity: after intervention, average weight increased 0.2 kg in the intervention group compared to 0.1 kg the control group. The role of unsaturated and polyunsaturated fatty acids provides energy provided up to 5463 mg in 100 g of nutrition formula, contributing to supplementing energy to meet Vietnam's recommended nutritional threshold [7]. The provided energy around 700 kcal, met nearly 40% of the day's energy needs, improving thinness and not causing weight gain; the milk ingredients do not contain saturation fatty acids causes excess energy and bad effects [8]. 100g of researched milk contains 3.27 g of FOS and inulin, which are fibres that help stimulate the growth of beneficial intestinal bacteria, limit the growth of harmful bacteria, increase resistance, and increase immunity, enhance absorption of nutrients and prevent constipation [7]. The results of this study also improved the condition of poor appetite and the use of food volume with the rate of poor appetite reduced by 16.7% and the intervention effect reached 20% (p<0.01) compared to the control group; The rate of poor appetite decreased by 21.7%, the intervention effect was achieved

18.4% (-21.7% vs. -3.3%) compared to the control group ($p<0.05$); The rate of bloating/indigestion decreased by 18.3%, the intervention effect was achieved 16.6% (-21.7% vs. -3.3%) with $p<0.05$. A study of 124 overweight and obese people on a diet rich in MUFAs (about 20% of total calories) or a high-carbohydrate diet for one year resulted in weight loss equivalent to about 4 kg ($p<0.05$). Formula ingredients with vitamin B also supported to improve the digestive system and food utilization, especially with the optimal content of 100 g of formula providing B1 (1169 mcg), B2 (573 mcg), B6 (1711 mcg), B12 (5.15 mcg). Results showed that intervention had a good influence on sleep [7] and the results of this study showed that sleep status improved significantly, the rate of poor sleep decreased by 43.3% ($p<0.05$), the rate of lack of sleep decreased by 8.3% with intervention effectiveness reduced by 9.9%; the rate of difficulty sleeping decreased by 6.6% with the intervention effect decreasing by 10.0% ($p>0.05$) and this result may explain the role of fatty acids and vitamins in milk ingredients in improving immune function, improving nutritional status, digestion and sleep, mental health, especially in people with existing liver disorders.

Product acceptability: In our research, we received good comments from users and researchers on the product's ingredients, nutritional content, tastes and smells. The product acceptability of users rate reached 100%, of which 73.3% of users were very satisfied, 21.7% were satisfied and 5.0% were acceptable.

Research results showed that the product provides energy, protein, fat, carbohydrate, soluble fibre (FOS/Inulin) and 27 vitamins and minerals had positive effects on reducing liver enzymes, improving liver function, enhancing immunity, digestive function and sleep contribute to promote the patient's health, physical condition, mental health and quality of life. The product can be applied as a simple, safe and effective community intervention to improve liver function and support treatment for people with elevated liver enzymes.

V. CONCLUSION

Research results on supplementing the nutritional product Leanmax Ligos on people having elevated liver enzymes showed positive effects and the product acceptability was high. Comparing the results of intervention group versus the control group showed:

- Reduced liver enzyme levels with statistical significance: average AST level decreased by 45.32 UI/L (-4.29 ± 8.36 vs. 1.03 ± 0.45); average ALT level decreased by 53.96 UI/L (-56.98 ± 6.53 vs. -3.02 ± 1.35) ($p<0.001$).

- Improved health status with statistical significance: Reducing amount of food decreased by 20% ($p<0.01$); Loss of appetite decreased by 16.7% ($p<0.05$); Constipation reduced by 15% ($p<0.05$); Diarrhoea decreased by 11.7% ($p<0.05$); No-deep sleep decreased by 40% ($p<0.05$); Fitful sleep decreased by 18.4% ($p<0.05$); Some indicators tended to be better but not statistically significant ($p>0.05$): Difficulty sleeping decreased by 10.0%; Itching decreased by 13.4%; Urticaria decreased by 6.6%; Respiratory infections decreased by 1.6%; Fatigue decreased by 10%; Anxiety and emotional changes decreased by 8.4%; and average weight increased by 0.1 kg ($p>0.05$).

- Product acceptability of users was 100% of which there were 73.3% very satisfied; 21.7% satisfied; and 5.0% accepted./.

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