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Original Research Article

Hepatogomax Improves Serum Albumin and Transaminase Enzymes Activity Levels in Sprague Dawley Rats Liver Cirrhosis

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Abstract

Background: Patients with liver cirrhosis had liver cell damage and malnutrition risk. Hepatogomax enteral formula consists of soybean flour and goat's milk flour which could reduce serum albumin, AST, and ALT levels because it contains BCAA amino acids (valine, leucine, and isoleucine) and MCT.

Objective: To examine the effect of Hepatogomax enteral formula based on Soybean flour and goat's milk flour on serum albumin, AST, and ALT levels in Sprague Dawley rats liver cirrhosis.

Methods: The true experimental study – pre-post control group used 24 adult Sprague Dawley rats in 4 groups. Groups K(+), P1, and P2 were induced by Thioacetamide 400 mg/kgBW. The groups of P1 and P2 were given the Hepatogomax enteral formula made from soybean flour and goat's milk flour at a dose of 4.87 g/200gBW and 14.6 g/200gBW for 28 days. Serum albumin levels were determined using the Bromocresol Green (BCG) method while serum AST and ALT were determined using spectrophotometry. Statistical analysis had used Paired T-Test and Kruskal Wallis test with Mann Whitney follow-up test.

Results: Serum albumin, AST, and ALT levels in the P1 and P2 groups had significant differences ($p<0.05$) against the K(-) and K(+) groups. Enteral formula based on soybean flour and goat's milk flour could increase serum albumin levels and reduce serum AST and ALT levels of P1 and P2 groups. The most significant improvement in serum albumin, AST, and ALT levels was at dose 14.6 g/200gBW.

Conclusion: Hepatogomax enteral formula based on Soybean flour and goat's milk flour could increase serum albumin and reduce AST, and ALT levels Sprague Dawley rats liver cirrhosis.

Keywords: *Liver Cirrhosis, Hepatogomax, Albumin, Transaminase Enzyme Activity*
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INTRODUCTION

Liver cirrhosis occurs when normal cells are replaced with scar tissue so that the performance of essential functions in the human body is disturbed.[1] The prevalence of liver cirrhosis in 2017 increased to 10.6 million cases and caused the death of 1.32 million worldwide populations.[2] Patients with liver cirrhosis were at risk of malnutrition by 20% and increased by 60% with the complication of other diseases.[3] Protein Calorie Malnutrition (PCM) often occurs in patients experiencing liver disease with a prevalence of 50-90% in patients with cirrhosis.[4,5] PCM occurs due to energy and protein intake deficit. PCM in liver cirrhosis is caused by factors related to changes in nutritional status in patients, such as decreased energy intake by 13-34%, hypermetabolic with resting energy expenditure over 120%, metabolic disorders, increased β-adrenergic activity, and malabsorption in fat.[5,6]

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These can occur because the liver cannot produce sufficient amounts of bile, decrease in micellar formation, and fat malabsorption can reduce the number of calories in the body.[5] Malnutrition in liver cirrhosis patients can lead to loss of muscle mass and adipose tissue. In different studies, research on lean body mass showed liver cirrhosis in patients who experienced a 20-60% decrease in body mass.[7]

Liver cirrhosis patients who cannot meet needs of nutrient intake needs through oral intake may be considered for enteral therapy. The administration of enteral therapy aims to sufficient nutritional needs, prevent malnutrition, improve and maintain nutritional status, stimulate the improvement of liver function, and reduce mortality rates.[8,9] The enteral formula used in this study is the Hepatogomax enteral formula based on soybean flour and goat's milk flour for the liver disease developed by Rahmadanti, TS in 2020.[10] The development of Hepatogomax as a hospital enteral formula can reduce the costs of spending on enteral formulas because it is made from local food ingredients at a lower price compared to commercial enteral formulas.[11]

Soybean flour is a source of protein containing branched-chain amino acids (BCAA), namely valine 1.59 g, leucine 2.39 g, and isoleucine 1.57 g in 100 g.[12,13] Besides soybean flour, BCAA contents are also found in goat's milk flour, which is valine 9% and isoleucine 4%.[14] BCAA can improve nutritional status, increase albumin levels, prevent progressive liver damage, and stimulate protein synthesis.[15,16] In addition to containing BCAA, Hepatogomax enteral formula contains medium-chain triglyceride (MCT) fat derived from goat's milk flour. Goat's milk flour contains 4.5 fat with a high MCT of about 36%.[14,17] The administration of fat in the form of MCT is used to improve the absorption of long-chain fatty acids to prevent the occurrence of fat malabsorption.[18] The present study aims to examine the Hepatogomax enteral formula effect on serum albumin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels in Sprague Dawley rats liver cirrhosis.

MATERIALS AND METHODS

Experimental Animals

This study was a True Experimental Pre-Post Test with Control Group Design using adult Sprague Dawley rats with the body weight of 180-250 g and ages 8-12 weeks; as many as 24 rats adapted for one week at the Experimental Animal Laboratory of the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta. This study was conducted from February to April 2022. All rats were divided into four groups of 6 rats each ($n = 6$) : (1) Healthy Rats Group / K(-); (2) Liver Cirrhosis Rats Group / K(+); and Liver Cirrhosis Rats Group with Treatments / P1 and 2. All groups were given AD-II Comfeed as feed standard during the study period. The group of K(+), P1, and P2 were induced with Thioacetamide (TAA) at 400 g/kgBW for two weeks.[19] After TAA induction, P1 and P2 groups were given Hepatogomax enteral formula at doses of 4.87 g/200gBW and 14.6 g/200gBW for 28 days.

Data collection on serum albumin, AST, and ALT levels was carried out 2 times during the study, namely pre and post intervention. Rats were fasted for 12 hours before taking blood. Blood was taken through the retro-orbital plexus of rats, as much as ±1% of the body weight of rats.[20] Measurement of serum albumin levels was using the bromocresol green (BCG) method, and serum levels of AST and ALT were used the spectrophotometric method.

Ethical Clearance

This study was reviewed and approved by the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University, with the issuance of Ethical Clearance No. 13/EC/H/FK-UNDIP/II/2022.

Preparation of Hepatogomax Enteral Formula

The enteral formula of Hepatogomax is made with basic ingredients of 63 g of soybean flour, 77 g of goat's milk flour, 7 g of virgin coconut oil (VCO), 45 g of sugar, and 70 g of maltodextrin. Dry ingredients such as soybean flour, goat's milk flour, sugar, and maltodextrin were mixed and stirred manually for 3 minutes. After that, VCO was added to the dry ingredients mixture and stirred for 2 minutes. All the ingredients that have been stirred manually will be stirred again using a mixer for 8 minutes to be homogeneously mixed. The enteral formula that has been homogeneously mixed is sifted to produce a smoother formula.[10]

Analysis of BCAA and MCT Content

BCAA content of Hepatogomax enteral formula was carried out using High-Performance Liquid Chromatography (HPLC) method (HPLC Series 1100, Agilent Technologies, Germany®) at the Testing, Calibration, and Certification Service Laboratory, Institut Pertanian Bogor (IPB) University. While the MCT content was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) method (GC-2010 Plus, Shimadzu Europe®) at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada.

Data Analysis

Statistical analysis was performed using SPSS software 21 (IBM/SPSS Inc). The research data were analyzed using the Shapiro-Wilk normality test. All data pre and post intervention were normally distributed so that the Paired T-Test was used to determine the differences in serum albumin, AST, and ALT levels pre and post intervention. Differences in influence between groups were analyzed using Kruskal Wallis as a non-parametric statistic with Mann Withney as a follow-up test because it was not normally distributed. All results are expressed as mean±SD. Value $p < 0.05$ was considered to be significant.

RESULTS

BCAA and MCT content in Hepatogomax Enteral Formula

The results of BCAA and MCT content in 262 g of Hepatogomax enteral formula can be seen in **Table 1** shows that Hepatogomax contains more MCT compared to BCAA. The total BCAA content in Hepatogomax was 4.54 g or 2.09%, while the MCT content was 76.66 g or 29.26%.

Table 1. BCAA and MCT content in Hepatogomax

Hepatogomax	BCAA						MCT	
	Valine		Leucine		Isoleucine			
	%	g	%	g	%	g	%	g
Sample 1	0.48	1.26	0.74	1.94	0.45	1.18	29.36	76.92
Sample 2	0.50	1.21	0.81	2.12	0.48	1.26	29.16	76.40
Average	0.49	1.29	0.78	2.03	0.82	1.22	29.26	76.66

Table 2. Albumin Serum Levels Pre and Post Intervention

Albumin Levels (g/dL)	Pre- Intervention	Post-Intervention	p^1	ΔChanges	p^2
	Means±SD	Means±SD		Means±SD	
K(-)	4.46±.0.42	4.45±0.27	0.032*	-0.19±0.16[a,b]	0.000*
K(+)	1.28±0.10	1.24±0.08	0.015*	-0.04±0.03[a,b]	
P1	1.14±0.04	2.40±0.08	0.000*	1.26±0.11 [a,b]	
P2	1.16±0.05	4.11±0.07	0.000*	2.95±0.05 [a,b]	

[1]*paired t-test*, [2]*kruskal wallis* test, *p= p value*,* significant difference (p<0.05), [a]significant difference against control group (*Mann Whitney* test), [b]significant difference against intervention group (*Mann Whitney* test)

Table 3. AST and ALT Serum Levels Pre and Post Intervention

Variable	Pre- Intervention	Post-Intervention	p^1	ΔChanges	p^2
	Means±SD	Means±SD		Means±SD	
AST (U/L)					
K(-)	25.98±0.51	26.7±0.75	0.007*	0.73±0.40[b]	
K(+)	45.48±0.59	47.01±1.12	0.003*	1.54±0.72[b]	0.000*
P1	45.40±0.67	37.63±1.29	0.000*	-7.77±1.23[a,b]	
P2	45.64±1.27	28.24±0.36	0.000*	-17.4±1.35[a,b]	
ALT (U/L)					
K(-)	18.04±0.37	18.53±0.36	0.000*	0.48±0.01[b]	
K(+)	33.91±0.72	35.60±0.79	0.009*	1.70±1.01[b]	0.000*
P1	34.39±1.08	29.62±0.81	0.001*	-4.78±1.70[a,b]	
P2	34.15±0.50	20.88±0.43	0.000*	-13.27±0.73[a,b]	

[1]*paired t-test*, [2]*kruskal wallis*test, *p= p value*,* significant difference (p<0.05), [a]significant difference against control group (*Mann Whitney* test), [b]significant difference against intervention group (*Mann Whitney* test)

Hepatogomax Improves Liver Function Changes of TAA-induced Cirrhosis

Serum Albumin

The result of the mean changes in serum albumin levels is shown in Table 2, which shows an increase in serum albumin levels in the intervention groups. In the pre-intervention condition, TAA-induced groups had lower serum albumin levels than the K(-) group. After the intervention, the P2 group had albumin levels almost the same as the K(-) group. The Paired T-Test showed significant differences in serum albumin levels pre and post intervention in all groups (p<0.05).

The results of the Kruskal Wallis test showed a significant difference in changes in serum albumin levels between groups (p<0.001). The highest increase in serum albumin levels was found in the P2 group. The Mann-Whitney test showed that the increase in serum albumin levels in the P1 and P2 groups had a significant difference against the K(-) and K(+) groups (p<0.05).

Transaminase Enzymes Activity

Table 3 showed decreased serum AST and ALT levels in the P1 and P2 groups after the interventions. Serum levels of AST and ALT pre-intervention in the TAA-induced groups were higher than in the K(-) group. In the post-intervention, serum AST and ALT levels of the P2 group showed almost the same decrease approaching the K(-) group.

There was a significant difference in the mean changes of serum AST and ALT levels pre and post intervention between all groups using the Kruskal Wallis test (p<0.001). The most decrease in serum AST and ALT levels was found in the P2 group. The Mann-Whitney test results showed that the control group K(-) and K(+) had significant differences against the intervention groups P1 and P2 (p<0.05).

DISCUSSION

This study demonstrates that Hepatogomax enteral formula increases serum albumin levels and decreases transaminase enzymes (ALT and AST) in rats induced cirrhosis. Furthermore, the effect of Hepatogomax on serum Albumin and transaminase enzymes were dose dependent manner. The enteral formula of Hepatogomax contains 4.54 g of BCAA, which is lower than the commercial formula (9.36 g). However, the MCT content of 76.66 g in Hepatogomax is higher than the commercial formula (45 g). The BCAA content in Hepatogomax comes from soybean flour and goat's milk flour, while the MCT content comes from goat's milk and VCO oil. Based on research by Rahmadanti, TS, in 2020, it was shown that macronutrient content in the Hepatogomax was 1175 kcal, 71.0 g of fat, 17.55 g of protein, and 172.85 g of carbohydrates. Overall, the nutritional content of Hepatogomax enteral formula has met the requirements of the enteral liver cirrhosis diet according

to the European Society for Clinical Nutrition and Metabolism (ESPEN) by containing low fat, moderate protein digestibility, and containing BCAA and fat with MCT form.[10,21]

Serum albumin levels were used as a biomarker of nutritional status in liver cirrhosis patients to see the occurrence of malnutrition and disease severity.[22] Liver cirrhosis patients experienced a decrease in serum albumin levels due to a decrease in protein synthesis caused by impaired liver function.[23–25] In this study, there was a decrease in serum albumin levels in the TAA-induced groups. The previous study used TAA at lower doses than this study, as much as 200 mg/kgBW 2 times per week for six weeks showing lower serum albumin levels (3.39 g/dL) compared to the control group (4.19 g/dL).[26] Induction of TAA at higher doses (>300 mg/kgBW) would lead to higher mortality and more severe liver cell damage, allowing for a higher serum albumin decrease.[27]

TAA induces toxicity in the liver, causes impaired liver function and forms fibrosis that will develop into cirrhosis. In addition, TAA also attacks deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis, which alters intrahepatic metabolic processes.[19] Decreased albumin levels are related to impaired liver function caused by TAA induction and abnormal distribution of portal blood flow resulting in decreased albumin synthesis. Damage to RNA causes disturbances in the albumin synthesis process which it is requires mRNA for translation, amino acid supply, and transcription. This damage can decrease the formation of polysomes synthesizing pre-albumin, reducing albumin production.[28–30]

Besides, the decrease in albumin synthesis is caused by chronic acidosis and pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α).[23] In liver cirrhosis, increasing TNF-α indicates inflammation, proliferation, and apoptosis and can cause fibrosis in the liver.[25] In the albumin synthesis process, increasing TNF-α will decrease the transcription of albumin genes so that mRNA albumin in the hepatic lobule decreases. It causes albumin production to decrease so that reducing serum albumin levels.[31]

The administration of Hepatogomax enteral formula with protein intake containing BCAA increased serum albumin levels associated with albumin synthesis. Patients with liver cirrhosis experience protein and energy deficiencies that cause PCM. Serum BCAA levels tend to decrease compared to serum Aromatic Amino Acid (AAA) levels, causing a low Fisher ratio (BCAA/AAA) in liver cirrhosis. [5,6,32] Cells in the liver can secrete albumin normally if protein intake is sufficient. Increased protein intake can accelerate the synthesis of albumin mRNA so that albumin levels can increase. Protein containing BCAA is needed in the albumin synthesis process because it can activate hepatic mTOR signaling, which will increase the production of eukaryotic initiation factor 4E-binding protein-1 and ribosomal protein-6 kinase so that albumin synthesis will increase. BCAA leucine will stimulate nuclear protein binding polypyrimidine tract that will bind to albumin mRNA so that it can increase translation in the albumin synthesis process.[33,34] The increase in serum albumin levels due to the administration of Hepatogomax enteral formula containing BCAA was the same as in the previous study. It showed that the liver cirrhosis rats group given BCAA supplementation of 10 mg/kgBW for 16 weeks had higher serum albumin levels of 2.39 g/dL than the cirrhosis control group.[35]

AST and ALT are transaminase enzymes available in the liver and are associated with impaired liver function and damage to liver cells.[36] Liver cirrhosis patients tended to have high serum AST and ALT.[37] TAA induction in this study led to higher serum AST and ALT levels than in the healthy rats group. TAA can damage the cells tissue in the liver and form fibrosis. A damaged state in the liver causes the release of AST and ALT into the blood, so serum levels of AST and ALT increase during the examination. Besides that, serum AST levels will increase if the liver or muscles are bruising, trauma, necrosis, infection, and neoplasm.[38,39] High serum ALT levels are characterized by hepatocyte damage associated with increased mortality in liver disease.[39,40] A sustained increase in circulating ALT levels may reflect increased ALT production in regenerative liver tissue or continued release of hepatocytes.[41] The results of elevated serum levels of AST and ALT in rats after being induced TAA 400 mg/kgBW for two weeks in this study were to the previous study, which showed serum AST levels high of 653 U/L and ALT of 767 U/L after TAA-induced at the same doses.[19]

The decrease in AST and ALT serum levels occurred in the groups given Hepatogomax enteral formula containing MCT fat. MCT has rapid absorption and high solubility, preventing hydroxylation into MCFA and being transported directly to the liver through the hepatic portal vein.[42,43] MCT increases thermogenesis and is stored in adipose tissue in small amounts. The administration of MCT fat can improve the absorption of long-chain fatty acids to prevent the occurrence of fat malabsorption and have a protective effect on the liver.[42,44,45] Besides that, MCT can increase the regulation of fatty acid oxidation in the liver to reduce fat accumulation, which can cause steatosis.[46] The decrease in the development of steatosis can reduce inflammation and damage to cell tissues in the liver so that release of AST and ALT that comes out into serum can be reduced.[42] Therefore, the administration of Hepatogomax enteral formula containing MCT reduced serum levels of AST and ALT in rats with liver cirrhosis. The results in this study were from a previous study where goat milk feeding of 50 mg/kgBW for 30 days in hepatotoxicity rats showed a decrease in serum AST levels to 121.2 U/L and ALT to 38 U/L.[47] Then, other studies by giving VCO oil of 10 ml/kgBW also showed serum AST levels of 7.90 U/L and ALT of 6.97 U/L were lower than those of the hepatotoxicity rats control group.[45]

This study has limitations because researchers did not examine BCAA levels in serum. Serum BCAA levels tend to be decreased in patients with liver cirrhosis. Therefore, it is necessary to examine BCAA levels in serum to determine the biological mechanism of improvement in serum albumin, AST, and ALT levels, whether based on BCAA content in the Hepatogomax formula or on the increase in BCAA serum levels.

CONCLUSION

Based on this study, it can be concluded that the administration of Hepatogomax enteral formula at doses of 4.87 g/200gBW and 14.6g/200gBW can increase serum albumin levels and reduce the activity of transaminase enzymes. Hepatogomax enteral formula at a dose of 14.6g/200gBW improved serum albumin, AST, and ALT levels which were almost the same close to the condition of the healthy rats.

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