

The effect of synbiotics supplement on alcohol use disorders identification test and biochemical parameters, gamma glutamyl transferase, lipopolysaccharide and immunoglobulin a levels, in high risk alcoholics



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ABSTRACT

Background: Alcohol consumption does not result in dependence or abuse among most people. Nevertheless, a significant group of the population as a whole unavoidably is troubled by chronic alcoholism. Alcohol is involved in a number of diseases, disorders, and injuries, and several social problems. **Aims and Objective:** To investigate the possible effects of synbiotics supplement affecting to gut-brain axis in high risk alcohol drinkers through alterations between improving of gut related parameters and changes of alcohol use disorders identification test (AUDIT). **Materials and Methods:** Single group, pre- and post-test study. Participants: 24 male participants, alcohol use disorders identification test at 8 or above. Exclusions included clinical diagnosis of cirrhosis, immunodeficiency, autoimmune disorder, use of drugs other than alcohol, pregnancy and lactation, use of antibiotics and herbs during the course of study. Intervention: Synbiotics containing probiotics 7 species and prebiotic 3 types once a day before bedtime for 8 weeks. Main outcome measures: Primary outcome- the efficacy of synbiotics supplement improving subjective AUDIT score. Secondary outcome- changes on gut related biochemical parameters (gamma glutamyl transferase, lipopolysaccharide and immunoglobulin A levels). **Results:** Twenty high risk alcoholic participants (with an average age of 42.50 ± 11.66 years) were supplemented with synbiotics contained 6.25 billion cells of probiotics per day for 8 weeks. After the end of intervention, there was significantly improved total AUDIT score ($p=0.001$). The changes in gamma glutamyl transferase (GGT), lipopolysaccharide and immunoglobulin A level was calculated. GGT (from 90.62 ± 56.65 U/l to 67.67 ± 57.00 U/l), lipopolysaccharide (from 23.19 ± 9.57 to 16.67 ± 4.52 mg/ml) and immunoglobulin A (from 377.13 ± 229.88 to 484.16 ± 290.98 ng/ml) levels were significantly changed when compared to the baseline value ($p < 0.05$). **Conclusion:** The results of the current study suggested that the consumption of synbiotics significantly improved subjective and objective parameters involving gut-brain axis in high risk alcoholic patients, and further studies are mandatory to reveal the effects of synbiotics on gut health link to central neurological system.

Key words: Synbiotics; Gut-Brain; Alcoholics; AUDIT

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INTRODUCTION

Among most people, alcohol consumption does not result in dependence or abuse. Nevertheless, a significant group of the population as a whole unavoidably is troubled by chronic alcoholism. Alcohol consumption is the third world ranking of risk factor for morbidity and represents 5.9% of all deaths worldwide.¹ Alcohol is involved in a number of diseases, disorders, and injuries, and several social problems.²⁻⁴

Dysbiosis takes place when lifestyle factors distort the gut ecology of bacteria.⁵ Disturbance to the normal gut flora can also happen when there is an overall overgrowth of bacteria. The 2016 study reported that both acute and chronic alcohol consumption altered specific qualifications of the microbiome composition, bacterial overgrowth, and breakdown of the mucosal barrier.⁶ Numerous studies reported that an imbalance of the intestinal microbiota, gut dysbiosis, can lead to many diseases including allergy,⁷ diabetes mellitus,⁸ inflammatory bowel diseases,⁹⁻¹⁰ and obesity as well as results in an elevation in the release of endotoxins, presented by gram-negative and gram-positive bacteria. Endotoxins trigger proteins and immune cells enhancing inflammation.¹¹ Although bacterial overgrowth is able to be triggered directly by alcohol, a number of studies report that it may be an indirect byproduct of poor digestive and intestinal function resulted from alcohol consumption.

Collective data demonstrate that the gut microbiome significantly produces the bidirectional communication between the gastrointestinal tract and the brain, which has been known as the microbiota-gut-brain axis. Two vast sources of alcohol related inflammation inducers are alcohol damaged cells and gut microflora, particularly, lipopolysaccharide. Ethanol and their toxic metabolites directly cause the production of reactive oxygen species, known for their mechanism to induce triggering of a main inflammatory transcription factor nuclear factor- κ B (NF- κ B).¹² Ethanol induces lipopolysaccharide, a key outer membrane of all Gram-negative bacteria, translocation across the gut through several mechanisms, supported by Fukui et al.'s study showing alcoholic individuals with liver diseases had significantly increased circulating lipopolysaccharide.¹³ Lipopolysaccharide is able to imitate bacterial infection leading to an acute inflammatory response.

After translocating via the gut epithelium, lipopolysaccharide in the interstitial fluid is able to go into the systemic blood circulation by two pathways: the portal vein and the gastrointestinal tract lymphatic vessels.¹⁴ Then lipopolysaccharide in systemic circulation is reachable

to several organs and is involved in multi-organ damage, especially when detoxification process in liver is impaired. Lipopolysaccharide and pro-inflammatory cytokines are able to trigger the neuroendocrine response in the central nervous system resulting in the activation of the hypothalamo-pituitary-adrenal axis and inflammation in central nervous system.

The current study was conducted to evaluate the effect of synbiotics supplement on gut-liver-brain axis in high risk alcoholic patients.

MATERIALS AND METHODS

Participants

Twenty-four male participants 20–65 years old with high risk alcohol drinker classified by score of the alcohol use disorders identification test (AUDIT) at 8 or above were participated in this pre- and post-test clinical trial. Exclusion criteria included major abnormal cirrhotic signs, symptoms and laboratory investigations such as jaundice, ascites, asterixis, vomiting blood, hypoalbuminemia, and coagulopathy, history of central nervous system and psychiatric disorders such as epilepsy and brain trauma, human immunodeficiency virus or other immunodeficiency and autoimmune disorder, regular use of drugs other than alcohol, pregnancy and lactation, use of antibiotics during the course of this study, consumption of others dietary supplements and herbs during the course of this study, history of side effects towards pro- or prebiotic supplements. The study was approved by and performed under the guidelines of the Research Ethics Committee of Mae Fah Luang University, Thailand, and a written consent was obtained from all participants. The participants were screened and included into the study according to the inclusion and exclusion criteria. Informed consent was obtained from all study participants before initiating study procedures. Information on demographics and medical history were recorded.

Preparation of synbiotics intervention

Synbiotics were manufactured by Lactomason Korea Co., Ltd. and contained *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus reuteri*, *Lactobacillus salivarius*, *Bifidobacterium lactis*, *Bifidobacterium breve*, *Bifidobacterium longum*. Total of probiotics contained 6.25×10^9 colony forming unit as well as included Inulin 4 grams, Fructooligosaccharide 2 grams and Galactooligosaccharide 2 grams.

The intervention

The aluminum foil sachets contained synbiotics were given to the subjects. The subjects consumed one sachet per day for 8 weeks. They were reminded to take it daily by phone

and allowed to drink normally alcohol but not to ingest the others including others dietary supplements and herbs during the course of this study.

Sample collection

Blood samples for biochemical tests were collected in a sterile blood collecting tube at baseline and after 8 weeks of intervention and stored on ice or 4°C until analysis.

Biochemical analysis

After the sample collection, biochemical tests included blood Gamma Glutamyl transferase (GGT) (Kinetic photometric method), blood lipopolysaccharide (Mybiosource Human Lipopolysaccharide ELISA kit) and Immunoglobulin A (Elabscience Human IgA ELISA kit) were taken at baseline and end of treatment, 8th week of treatment.

Data analysis

Descriptive analyses were performed for demographics utilizing characteristic measures such as percent, mean and standard deviation. After using Kolmogorov-Smirnov (KS) test for determining parametric or nonparametric data, analytic analyses used statistical paired sample t-test or Wilcoxon signed rank test for evaluating mean differences

in AUDIT scores and biochemical parameters between baseline and end of treatment (8th week). Computer statistical program was used for statistical analyses, and *p*-values of < 0.05 were considered statistically significant.

RESULTS

A. Demographic information of the subjects

Twenty male participants were registered and completed the study. Four participants were dropped out due to loss of follow up. The mean age of the participants was 42.50 ± 11.66 years. According to Western Pacific regional office of the WHO's Asian BMI cut-off recommendation, about a half of participants are abnormal BMI persons (underweight 10 %, Overweight 10 %, and Obesity 30 %) (Table 1). The mean compliance scores calculated from the remaining supplements returned were 90 ± 6 %. Relied on completion of compliance forms, all participants reported to have consumed about 81% of assigned synbiotics between trials. Analysis of food frequency questionnaire data showed there were no differences in participants consumption habits throughout the intervention period (*p* > 0.05).

B. Changes in alcohol use identification test (AUDIT) score

There was significantly improved total AUDIT score (*p*=0.001) as well as the data showed significant decrease in scores of frequency of consuming and blackouts problems from alcohol drinking (*p*=0.011 and 0.014 respectively). No other differences were observed between trials (*p* > 0.05) (Table 2).

C. Changes in biochemical levels

The serum GGT, plasma lipopolysaccharide and immunoglobulin A levels were measured at baseline and after 8 weeks of synbiotic intervention. The level

Table 1: Baseline Characteristics of the Participants Participated in the Study

Characteristic	Number (Percentage)	Mean±SD
Age (years)		42.50±11.66
Less than 30	4 (20.0)	
30–49	10 (50.0)	
50 or more	6 (30.0)	
Weight (kg)		62.55±11.51
BMI (kg/m ²)		22.50±3.36
Less than 18.5	2 (10.0)	
18.5–22.9	10(50.0)	
23.0–24.9	2 (10.0)	
25.0–29.9	6 (30.0)	
Body fat (%)		16.69±4.43

Table 2: Pre-and Post-Alcohol Use Disorders Identification Test (AUDIT) Scores

Domains	Question content	Pre-test		Post-test		Mean delta ^a	p value ^b
		n	Mean (SD)	n	Mean (SD)		
Hazardous alcohol use	Frequency of drinking	20	3.35 (0.75)	20	2.90 (0.91)	-0.45	0.011*
	Typical quantity	20	2.40 (0.75)	20	2.45 (0.61)	+0.05	0.564
	Frequency of heavy drinking	20	2.95 (0.69)	20	2.65 (0.75)	-0.30	0.096
Dependence symptoms	Impaired control over drinking	20	3.05 (0.76)	20	2.90 (0.79)	-0.15	0.180
	Increased salience of drinking	20	2.95 (0.83)	20	2.80 (0.70)	-0.15	0.317
	Morning drinking	20	2.90 (0.72)	20	2.60 (0.75)	-0.30	0.058
Harmful alcohol use	Guilt after drinking	20	2.60 (0.68)	20	2.65 (0.67)	+0.05	0.317
	Blackouts	20	3.05 (0.51)	20	2.60 (0.60)	-0.45	0.014*
	Alcohol-related injuries	20	2.20 (0.62)	20	2.20 (0.52)	0	1.00
	Others concerned about Drinking	20	2.20 (0.62)	20	2.10 (0.55)	-0.10	0.157
Total		20	27.50 (4.07)	20	25.65 (3.98)	-1.85	0.001*

SD=standard deviation. ^aCalculated using Wilcoxon signed rank test for paired pre-test and post-test data. ^bRepresents mean of pre-test to post-test score delta for all individual paired data. *indicates the significant difference between samples (*P*< 0.05).

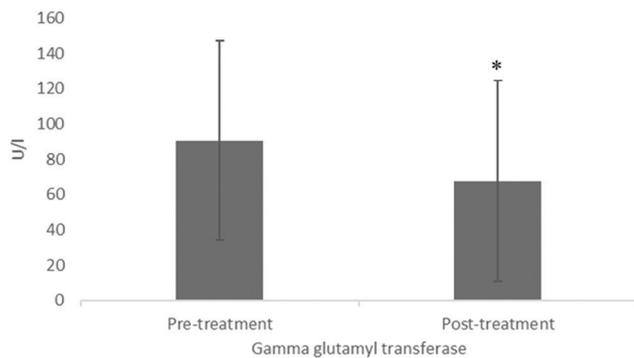


Figure 1: Changes in the level of gamma glutamyl transferase after 8 weeks of synbiotics intervention in high risk alcoholic participants. *indicates the significant difference between samples ($p < 0.05$).

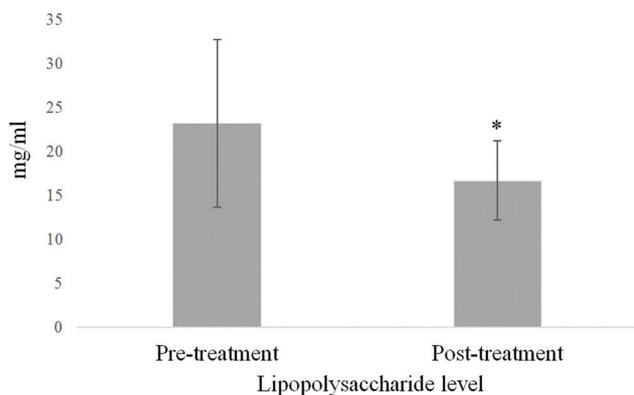


Figure 2: Changes in the level of lipopolysaccharide after 8 weeks of synbiotics intervention in high risk alcoholic participants. *indicates the significant difference between samples ($p < 0.05$).

of GGT, lipopolysaccharide and immunoglobulin A at baseline (Pre-treatment) were 90.62 ± 56.65 U/l, 23.19 ± 9.57 mg/ml and 377.13 ± 229.88 ng/ml, respectively. Whilst after 8 weeks of synbiotics supplementation, the level of GGT, lipopolysaccharide and immunoglobulin A were 67.67 ± 57.00 U/l, 16.67 ± 4.52 mg/ml and 484.16 ± 290.98 ng/ml, respectively (Figure 1, 2 and 3). After the synbiotics intervention, GGT and lipopolysaccharide level were significantly decreased ($p < 0.05$), whereas immunoglobulin A level was significantly increased ($p < 0.05$).

D. Adverse events

Four participants reported experiencing gastrointestinal (GI) symptoms during the study. The most commonly reported GI symptoms were bloating ($n = 2$), and diarrhea ($n = 2$). No serious adverse events were registered during trials.

DISCUSSION

Alcohol consumption is able to directly affect several aspects of gut health, such as increased intestinal permeability and

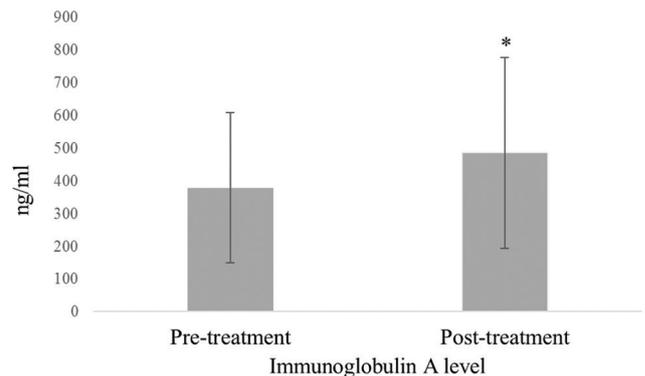


Figure 3: Changes in the level of immunoglobulin A after 8 weeks of synbiotics intervention in high risk alcoholic participants. *indicates the significant difference between samples ($p < 0.05$).

gut dysbiosis, as well as brain functions, including cognitive impairment and increased inhibitory error. Meanwhile, gut and brain provide a communication each other. Key mediators of bidirectional signaling comprise of serotonin (5-HT), opioid, and endocannabinoid, gut hormones, cytokines, and growth factors.¹⁵⁻¹⁶ Hence, some effects of the one organ can indirectly impact to another one. Several clinical studies show depression and psychological stress are related with exacerbations of inflammatory bowel disease and with the pathogenesis of irritable bowel syndrome.¹⁷ It may be described by serotonergic dysregulation and decline of gut barrier function through mast cell-dependent and mast cell-independent mechanisms.¹⁸⁻¹⁹

The synbiotics administration in alcoholics providing intestinal microbial balance and improving gut health gained much attention in the recent years. According to gut-brain connection, the synbiotics may play a role in amelioration brain functions among chronic alcohol drinkers. Michels et al. showed the effects of probiotics likely associated cognitive control processes further on modulating affective processes and affected prefrontal cortex.²⁰ Moreover, several studies supported the positive effects of probiotic supplementation on improving anxiety and depressive symptoms that is considered to stimulate an inclination toward drinking in alcoholic patients.²¹⁻²² The results of the current study also demonstrated that the synbiotics supplementation decreased subjective alcohol use identification test (AUDIT) score and biochemical parameters (gamma glutamyl transferase and lipopolysaccharide) as well as increased immunoglobulin A levels in high risk alcoholic participants.

CONCLUSION

The results supported that the consumption of synbiotics contained 6.25 billion cells of probiotics per day for 8 weeks

significantly improved subjective and objective parameters involving gut-brain axis in high risk alcoholic participants. However, the current study has some downsides including limited sample size, short duration of the study, no-placebo control, vast age differences among the participants (\pm 11.66 years), and limited studied parameters. Thus, a further extended study is required to confirm the results of the present study.

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