Role of Synbiotic in Preventing the Progression of Alcoholic Liver Disease Targeting Gut-Liver-Adipose Tissue Axis

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ABSTRACT

Consumption of ethanol has been carried out since ages and it has been an important cause of death worldwide. Ethanol mediated liver injury is also known as Alcoholic liver disease (ALD) is caused due to surplus intake of alcohol. Several studies have proposed molecular pathways that may be leading to ALD. One of the factors that may affect the pathway is gut dysbiosis. The gut microbiota produces a various compound that play a crucial role in regulating distal organs such as adipose tissue and the liver. Dysbiosis causes bacteremia, hepatic encephalopathy, small intestinal bacteremia, increased intestinal permeability. Recent research has better understanding of the gut and liver axis and how it controls systemic metabolic modifications in liver cirrhosis. Reports of clinical and experimental studies demonstrates the association with gut-microbiota and fatty acid metabolism. Another factor that may affect the ALD pathway is dysfunction of adipose tissue metabolism. Excessive alcohol consumption disturbs lipid metabolism, by increasing lipolysis and decreasing or unchanged lipogenesis, impaired glucose tolerance of adipose tissue which results in ectopic fat deposition inside the liver. Adipokines are adversely modified upon chronic alcohol consumption including adiponectin, leptin, and resistin. When these two factors are combined develops a pro-inflammatory state within WAT. The futuristic therapeutic approach for treatments and prevention for liver cirrhosis patients must be focused on evaluation of the gut-liver-adipose tissue metabolic network and the modification of these interactions using probiotics, synbiotics, and prebiotics. This work focuses on the progression and metabolism of ALD, the effect of ethanol on gut and adipose tissue, the effect of ethanol on adipokine secretion on 3T3-L1 cells.

INTRODUCTION

According to world health organization, there are 3.3 million deaths every year from the overuse of alcohol that is 5.9% of all deaths.[1] The pathogenesis of alcoholic liver disease (ALD) is a complex biological process. Ethanol is metabolized to acetaldehyde and reactive oxygen species (ROS) that plays a prominent role in the clinical and pathological spectrum of ALD. Ethanol oxidative mechanism stimulates intracellular signaling pathways leading to fat deposition, changing adipokines expression in adipose tissue and stimulation of innate and adaptive immunity leading to progression of alcoholic liver disease. Acetaldehyde and ROS are lethal to the liver that modifies lipid homeostasis via AMP-activates protein kinase (AMPK)- dependent pathway that modulates autophagy that is essential for the removal of lipid droplets. Furthermore, acetaldehyde disrupts the tight junctions of intestinal barrier and promotes lipopolysaccharide (LPS) translocation. Combination of acetaldehyde and LPS induces Kupffer cell activation to release ROS and proinflammatory cytokines and chemokine, which contribute to the progression and development of ALD. Adipose tissue comprises of large volume of biologically functioning fat globule. Adipocytes are a critical component of metabolic pathways and functioning of endocrine organs. Adipokines play an active role in autocrine, paracrine or endocrine metabolic functions. Thus, the treatment/ prevention of ALD involves the prevention of translocation of LPS and modulates immune gut system focusing on the potential role of synbiotic as a new therapeutic approach for alcoholic liver disease. **Synbiotic is defined as a mixture of prebiotic and probiotic** that result into syngerstic effect on gastrointestinal function of the host by improving the survival and implantation of live microbial dietary supplements in gastrointestinal tract.[2] As a result promoting the growth of healthy bacteria.[4] Thus, improving the host welfare. Here we report anti-inflammatory effect of synbiotic administration (**aged garlic extract and *Lactobacillus rhamnosus***) as a new molecular target for progression of alcoholic liver disease.

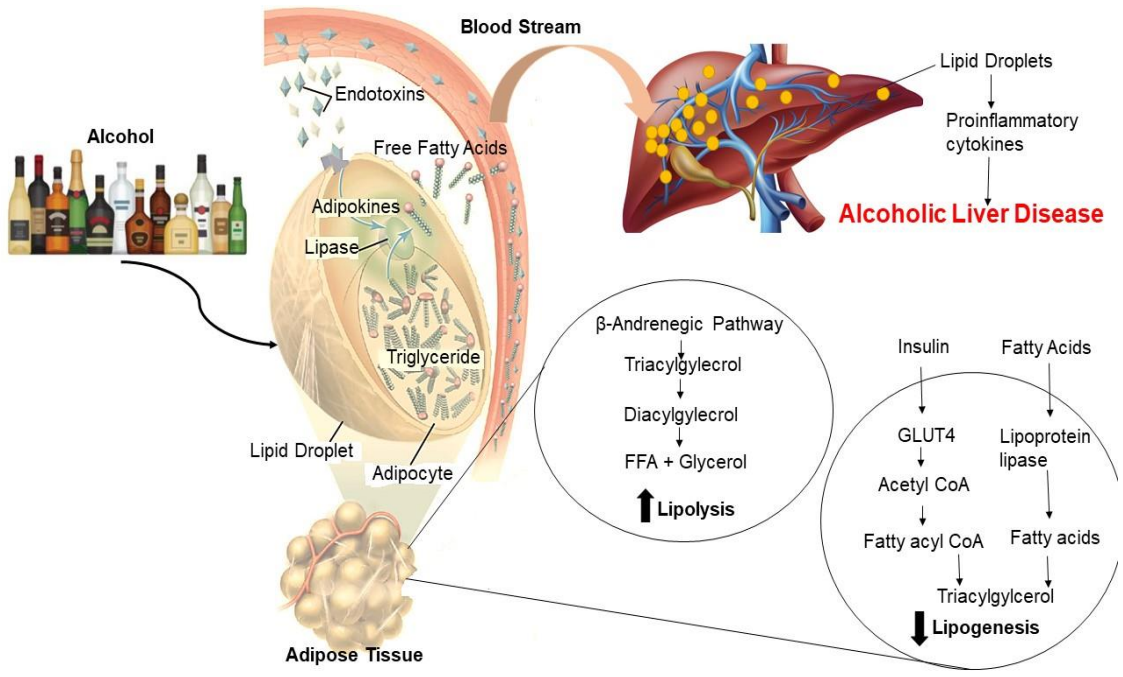


Figure 1: Alcohol effect on pro inflammatory cytokines of adipose tissue and liver.

RESULTS

Bacterial Growth Curve

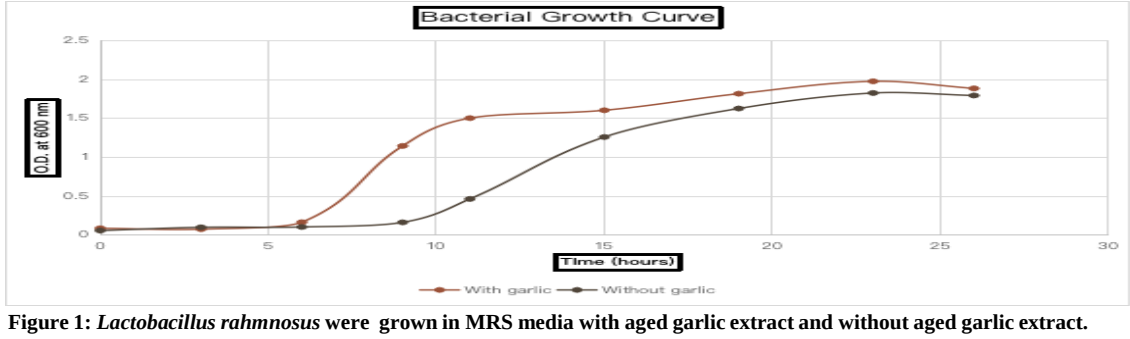
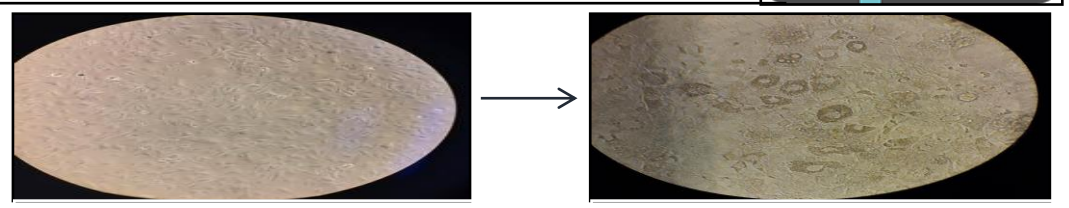


Figure 1: *Lactobacillus rahmnosus* were grown in MRS media with aged garlic extract and without aged garlic extract. O.D was taken at 600 nm in UV-Visble spectrophotometer.

In Vitro Studies on 3T3-L1 Cells.: Culturing of mature 3T3-L1 cells.

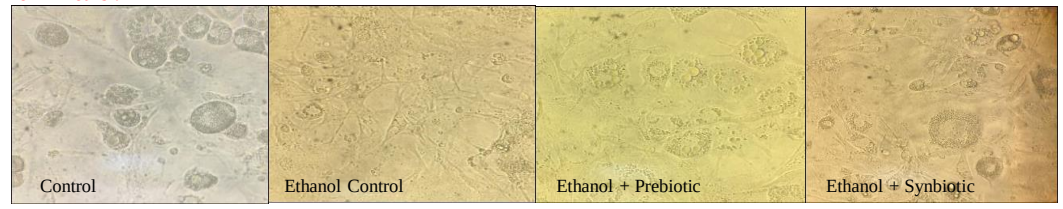
3T3-L1 cells were acquired from National Center of Cell Sciences (NCCS), Pune, India was grown until confluent in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS and 1% antibiotic-antimycotic solution at 37°C in a 5% CO_2 environment. **Post confluence (Day 0):** Cells were differentiated with DMEM medium containing 10%FBS, 0.5 µM isobutylmethylxanthine, 1 µg/ml insulin and 1M dexamethasone for 2 days. **Day 3:** Cells were cultured in DMEM medium containing 10%FBS and 1ug/ml insulin for next 2 days. **Day 6:** Cells were cultured only in DMEM medium supplemented with 10% FBS for next 4 days. **Day 12:** 90% of cells differentiated into mature adipocytes characterized by the formation of lipid droplets.[3] **Note:** Medium was replaced every 48 hr. This mature adipocytes are then used for further experiments with ethanol and synbiotic dose.



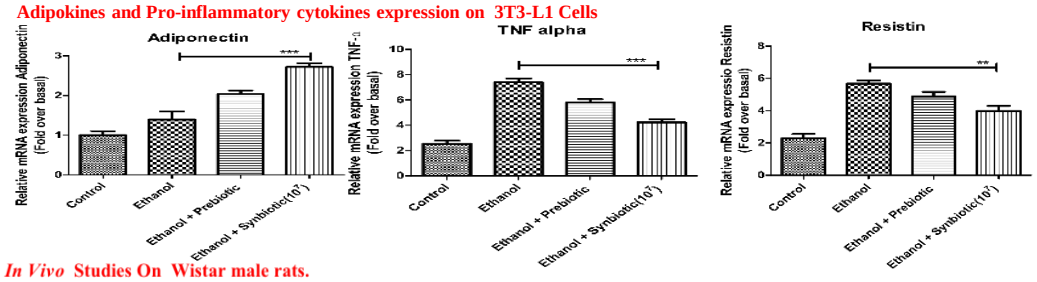
3T3-L1 : Pre-adipocytes (10X)
Day 0: Seeding (10^5 cells/ml)
Day 1: 24 hr after seeding

3T3-L1 : Mature adipocytes (10X)
Day 12 : After differentiation; before treatment

Cells were treated with 100 mM ethanol and exposed to 10 µg of aged garlic extract and 10^{8-10} cfu/ml cell free supernatant (Probiotic) for 24 hours .



Adipokines and Pro-inflammatory cytokines expression on 3T3-L1 Cells



In Vivo Studies On Wistar male rats.

Initiation	Animals (n 30) will be euthanized as per CPCSEA guidelines and organ were dissected and washed in sterile phosphate buffered saline and incubated in -80°C			
Group	I	II	III	IV
Treatment	Control	Pairfed	Ethanol	Synbiotic Treatment per dose
Dose	Pure drinking water + chow fed diet (*ad libitum*)	Maltosedextrine	95% Ethanol	95% Ethanol + (AGE + *L.rhamnosus*)
Number of animals	5	5	5	5
Duration of Treatment	24 hr	24 hr	24 hr	24 hr
Route of administration	Oral gavage administration			
Termination	After 5 weeks, blood was collected from Retro orbital plexus, and the animals were sacrificed and stored in -80°C			

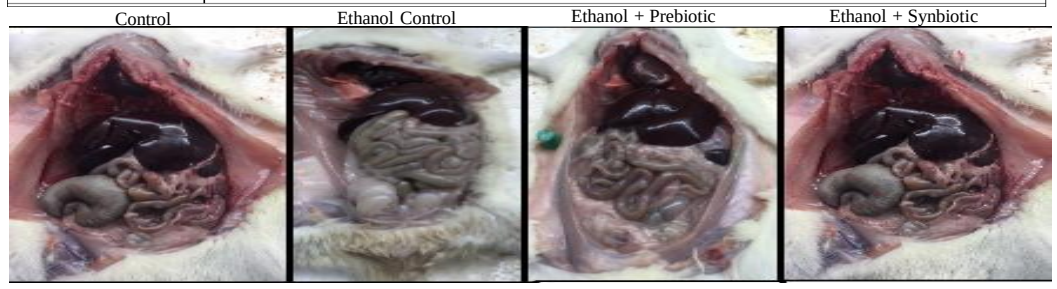
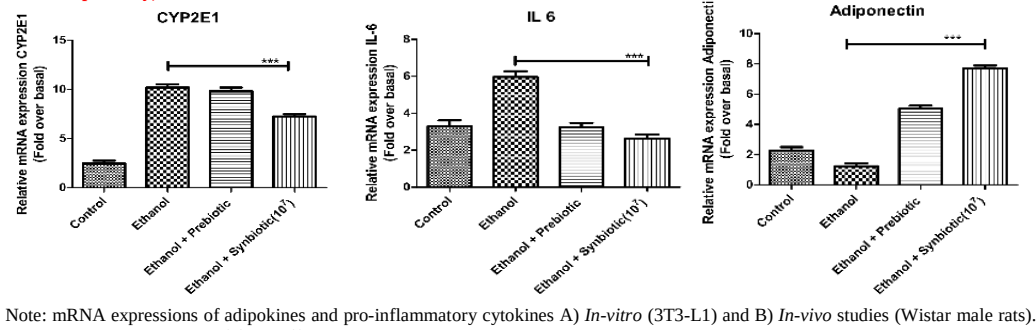


Figure 3. Ethanol and Synbiotic effect on Wistar male rats for five weeks of Liber di Carlie diet.

Adipokines and Pro-inflammatory cytokines expression on Wistar Rats (Ethanol: 95%, Synbiotic dose: SYN 200 mg/kg,+ 10^{8-10} cfu/ml respectively)



Note: mRNA expressions of adipokines and pro-inflammatory cytokines A) *In-vitro* (3T3-L1) and B) *In-vivo* studies (Wistar male rats). Results are means ± SEM of four different experiments. p<0.05 when compared to ethanol control. *p < 0.05, **< 0.01, ***<0.001, ****<0.0001.

CONCLUSION

The present study identifies that new synbiotic combination that is capable of reducing inflammation caused by ethanol in adipose tissue. Thus, our synbiotic combination may prove as one of the effective preventive strategy for alcoholic induced liver disease.

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