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# Garlic oil alleviated ethanol-induced fat accumulation via modulation of SREBP-1, PPAR-α, and CYP2E1

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ABSTRACT

Garlic oil (GO) has been shown to partially attenuate ethanol-induced fatty liver, but the underlying mechanisms remain unclear. The current study was designed to evaluate the protective effects of GO against ethanol-induced steatosis *in vitro* and *in vivo*, and to explore potential mechanisms by investigating the sterol regulatory element binding protein-1c (SREBP-1c), peroxisome proliferators-activated receptor-α (PPAR-α), cytochrome P4502E1 (CYP2E1), and etc. In the *in vitro* study, human normal cell LO2 was exposed to 100 mM ethanol in the presence or absence of GO for 24 h. We found that ethanol increased the protein levels of n-SREBP-1c and CYP2E1, but decreased the protein levels of PPAR-α, which was significantly attenuated by GO co-treatment. In the *in vivo* study, male Kun-Ming mice were pretreated with single dose of GO (50–200 mg/kg body weight) at 2 h before ethanol (4.8 g/kg body weight) exposure. The changes of n-SREBP-1c, PPAR-α and CYP2E1 were paralleled well to those of *in vitro* study. Furthermore, GO significantly reduced the protein levels of fatty acid synthase (FAS), and suppressed ethanol-induced hepatic mitochondrial dysfunction. These results suggested that GO had the potential to ameliorate alcoholic steatosis which might be related to its modulation on SREBP-1c, PPAR-α, and CYP2E1.

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## 1. Introduction

Alcoholic liver disease (ALD) remains to be one of the most common etiologies of liver diseases and is a major cause of morbidity and mortality worldwide (Ramaiah et al., 2004). Alcoholic fatty liver (AFL) is the earliest pathological change of liver when exposed to ethanol. Although AFL is considered benign in the past, recent studies have demonstrated that fat accumulation renders the liver more vulnerable to toxins, and AFL may play crucial roles in the initiation and progression of ALD (Day and James, 1998). Preventing or reversing AFL may block or delay the progression of ALD, and thus it is important to develop effective and safe pharmacological agents to cope with steatosis and to prevent the development of hepatic steatosis to more severe forms such as hepatitis and fibrosis.

AFL may be attributed to many factors, including the changes of the redox condition, transportation impairment of the synthesized lipid, inhibition of fatty acid oxidation, and the enhancement of the lipid synthesis (Zeng and Xie, 2009). Accumulating evidence has demonstrated that sterol regulatory element-binding protein-1c (SREBP-1c) and peroxisome proliferator-activated receptor-alpha (PPAR-α), two nuclear transcription regulators controlling lipid metabolism, are involved in the development of AFL. Ethanol could increase lipogenesis by activating SREBP-1c, but decrease fat catabolism by inhibiting PPAR-α (Crabb et al., 2004; Ji et al., 2006). More recent studies demonstrate that ethanol-inducible cytochrome P4502E1 (CYP2E1) is an important contributor to AFL (Lu et al., 2010, 2008; Wu et al., 2010), although a potential link between CYP2E1 and AFL remains unclear. As the induction of CYP2E1 is a major pathway for ethanol-induced oxidative stress (Albano, 2006; Cederbaum et al., 2009; Dey and Cederbaum, 2006), it is speculated that impairment of the mitochondrial lipid oxidation by oxidative stress and possible link between oxidative stress and hepatic insulin resistance may contribute to the induction of steatosis (Albano, 2008).

Garlic is widely used in folk medicine since ancient times, and is thought to be nature's gift to fight against various physiological threats (Butt et al., 2009). A series of biological benefits (such as antiatherosclerotic, antihypertensive, antimicrobial, anticancer, immunomodulation, and radioprotection) have been reported.

*Abbreviations:* GO, garlic oil; SREBP-1c, sterol regulatory element binding protein-1c; PPAR-α, peroxisome proliferators-activated receptor-α; CYP2E1, cytochrome P4502E1; FAS, fatty acid synthase; AFL, alcoholic fatty liver; ALD, alcoholic liver diseases; DADS, diallyl disulfide; DATS, diallyl trisulfide; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; MDA, malondialdehyde; GSH, glutathione; MPT, mitochondrial permeability transition; $\Delta\Psi m$, mitochondrial membrane potential; CPT-1, carnitine acyl transferase 1.

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Garlic oil (GO), usually prepared by steam distillation, is one of commercial garlic products. Chemical analysis demonstrates that GO contains diallyl (57%), allyl methyl (37%) and dimethyl (6%) mono to hexa sulfides (Amagase, 2006; Banerjee et al., 2003). Previous studies have demonstrated that garlic products including GO possess hypolipidemic effects (Reinhart et al., 2009), and GO could partially attenuate ethanol-induced liver disease (Bobboi et al., 1984; Shoetan et al., 1984; Zeng et al., 2008). However, the underlying mechanisms are still not fully understood.

The current study was designed to evaluate the protective effects of GO against ethanol-induced steatosis in human normal liver cell LO2 and in a widely-used mice model (Enomoto et al., 2000; Wada et al., 2008; Yin et al., 2007), and to investigate the effects of ethanol and GO on SREBP-1c, PPAR-α, CYP2E1, and etc., for the underlying mechanisms exploration.

## 2. Materials and methods

### 2.1. Materials

GO was purchased from Xuchang Yuanhua Biotechnology Co., Ltd. (Xuchang, China). The contents of diallyl disulfide (DADS) and diallyl trisulfide (DATS), two major biological components of garlic, were 31.1% and 29.3% of GO, respectively. Primary antibodies of SREBP-1 (ab-3259), PPAR-α (ab-8934), CYP2E1 (ab-28146), were provided by Abcam (Cambridge, UK). Primary fatty acid synthetase (FAS) antibody (sc-55580), and anti-mouse and anti-rabbit secondary IgG were obtained from Santa Cruz Biotechnologies Corp. (Santa Cruz, CA, USA). Western blotting detecting reagents (enhanced chemiluminescence) was provided by Millipore Corp. (Bedford, MA, USA). The kits of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), malondialdehyde (MDA), glutathione (GSH) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). BCA™ protein assay kits were purchased from Pierce Biotechnology, Inc. (Rockford, IL, USA). All other reagents were purchased from Sigma (St. Louis, MO).

### 2.2. Cell culture and treatments

Human normal liver cell LO2 was obtained from the Chinese Academy of Science, and maintained in RPMI 1640 medium (GIBCO BRL, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified incubator set at 5% $CO_2$ and 37 °C. The cells were exposed to 100 mM ethanol in the absence/presence of GO (2.5, 5.0, 10.0 μg/ml) for 24 h, and were harvested in accordance with the applied technique. The doses of ethanol and GO were chosen by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay.

### 2.3. Animal treatment

Specific pathogen-free male Kun-Ming mice, weighing 18–22 g, were provided by the Laboratory Animal Center of Shandong University. The mice were maintained at approximately 22 °C with a 12-h light: 12-h dark cycle, and had free access to standard chow and tap water. After 3 days acclimation to the laboratory conditions, the mice were randomized into 5 groups, i.e. control group, ethanol group, and three GO groups (50, 100, 200 mg/kg bw). The mice in GO groups were pretreated with different doses of GO at 2 h before intoxication with ethanol (50%, v/v, 12 ml/kg bw), while mice in ethanol group received equal volume of corn oil and then ethanol. The animals in control group were treated with corn oil, and then isocaloric/isovolumetric maltose-dextrin solution. At 4, 8, and 16 h after ethanol exposure, 10 mice in each group were chosen randomly and sacrificed. The liver was dissected quickly, snap-frozen in liquid nitrogen, and stored at −80 °C until analysis. The use of animals was in compliance with the guidelines established by the Animal Care Committee of Shandong University.

### 2.4. Biochemical assay

The activities of ALT and AST were determined using automatic biochemical analyzer (BTS-370, Spanish). The contents of TG, MDA, and GSH, were measured colorimetrically with UV–visible spectrophotometer (UV-2450, SHIMADA, Japan). All procedure were performed strictly according to the instruction of the commercial assay kits.

### 2.5. Liver pathological examination

The specific fat-staining method, Sudan III staining, was used to evaluate the fat accumulation in the liver. Briefly, fresh frozen sections of liver (about 8 μm) were prepared, affixed to microscope slide, and allowed to air-dry at room temperature. The liver sections were fixed in ice cold 10% formalin for 5 min, and rinsed in distilled water for 3 times. After that, the liver sections were stained in Sudan III for 2 min, and then counterstained with hematoxylin for 30 s. After mounting with glycerin jelly, the yellow stained lipid droplets were observed under light microscope (Olympus, Tokyo).

### 2.6. Mitochondria isolation and function assay

Mitochondria isolation and purification was performed by differential centrifugation as previously reported (Song et al., 2003). Mitochondrial permeability transition (*MPT*) and membrane potential ($\Delta\Psi$m) were detected within 2 h after

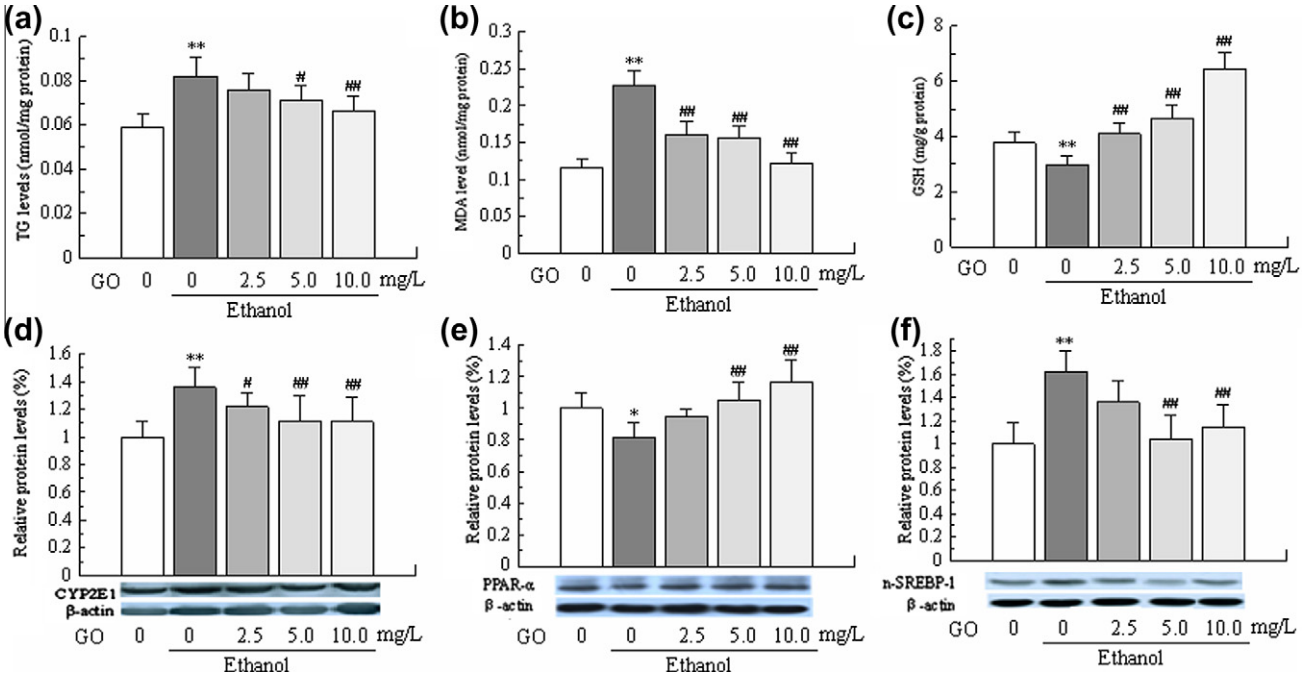


**Fig. 1.** Effects of GO on the contents of TG, MDA, GSH, and protein levels of CYP2E1, PPAR-α and n-SREBP-1c in LO2 cells. LO2 cells were exposed to ethanol (100 mM) in the absence or presence of GO for 24 h. The contents of TG, MDA, and GSH were measured with commercial kits, while the protein levels of CYP2E1, PPAR-α and n-SREBP-1c were determined by western blot. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the ethanol group.

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