Ginkgo biloba Extract Prevents Ethanol Induced Dyslipidemia

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Abstract: Ginkgo biloba extract (EGB) functions as a natural substantial antioxidant and hypolipidemic. Chronic alcohol abuse leads to sustained dyslipidemia characterized by hyperlipidemia and lipid peroxidation. Thus, the present study investigates the effect of EGB on lipid disorders induced by ethanol in rats. Male Sprague-Dawley rats were fed with ethanol (2.4 g/kg), and pretreated with a daily dose of low or high EGB (48 or 96 mg/kg, respectively). During the experiment, serum was collected on day 30, 60, and 90. Serum lipid profile, including lipid peroxidation, was determined by colorimetric methods. Our data showed that ethanol intake resulted in a time-dependent increase in serum levels of triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and malondialdehyde (MDA), and a decrease of the ratio of high-density lipoprotein cholesterol (HDL-C) against TC. EGB prophylactic medication (48 and 96 mg/kg), especially at the high dose, significantly increased HDL-C content, and normalized the abnormal lipid profile and peroxidation in comparison to ethanol-fed only rats. These results suggest that ethanol results in time-dependent hypercholesterolemia, hypertriglyceridemia and promotes serum lipid peroxidation. EGB pretreatment prevents hyperlipidemia and ameliorates lipid peroxidation induced by ethanol.

Keywords: Ginkgo biloba Extract (EGB); Ethanol; Hyperlipidemia; Lipid Peroxidation.

Introduction

Alcohol abuse was considered as an important public health issue due to its high social and economic burden in the world. Habitual binge and alcohol dependence elicit serious

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injury on multi-organs and exhibit extensively deleterious effects on cardio-cerebral vascular, alimentary, nervous, reproductive, and immune systems (Russo et al., 2004; Standridge et al., 2004). Extensive research demonstrated that ethanol, a nutrient with high energy, has multiple implications in lipid metabolism and redox homeostasis, by which ethanol consumption is closely associated with the risk of cardio-cerebral vascular diseases, diabetes and other diseases. The disorders in whole-body fuel utilization, lipolysis, circulating and tissue lipid profile, and lipid peroxidation have all been reported after acute or chronic exposure of ethanol (Siler et al., 1999; Lee, 2004). Moreover, alcoholic hypercholesterolemia and hypertriglyceridemia have been identified as an important pathophysiological mechanism and trigger by which excessive ethanol results in a dramatically increased risk of alcohol-related diseases (Balasubramaniyan and Nalini, 2003). Lipid peroxidation during ethanol metabolism further exacerbates the pathogenetic condition (Wu and Cederbaum, 2003). Hence, it is of clinical importance to prevent ethanol-induced abnormality in lipid metabolism and peroxidation.

Herbal medicine, including its naturally occurring active constituents, has been attracting much attention and widely used for many centuries (Craig, 1999; Heinrich, 2003). Ginkgo biloba is the world’s most ancient extant tree and its medicinal use can be traced back almost 5,000 years in Chinese herbal medicine. The commercial extract from Ginkgo biloba leaves (EGB), standardized with 6% ginkgolides and 24% flavonol heterosides, is recognized as the most commonly used phytomedicine because of its alleged tonic effect and potential curative and restorative properties. The pharmacological mechanism of EGB is attributed primarily to its functions as a neuroprotective agent, an antioxidant, a free-radical scavenger, a membrane stabilizer, and an inhibitor of platelet-activating factor (Cheng et al., 2003; Williams et al., 2004; Koch, 2005). In addition, the flavonoids components of EGB are believed to have a substantial hypolipidemic and anti-sclerotic role (Yao et al., 2004; Auger et al., 2005). However, little is known about the effect of EGB on ethanol-induced dyslipidemia. Therefore, we designed the current study to explore the prophylactic role of EGB on disorders of lipid metabolism provoked by high ethanol administration.

Materials and Methods

Chemicals

Ethyl alcohol absolute (ethanol) was purchased from Zhenxing Chemical Factory (Shanghai, China). EGB, a standard commercial extract containing 24% flavonoid and 6% terpenoid, was obtained from Louian Industry Company Ltd. (Shanghai, China). Thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropane, butylated hydroxy toluene and diethylenetriaminopen taacetic acid were obtained from Sigma-Aldrich Co. (St. Louis, USA). Assay kits for total cholesterol (TC), triglyceride (TG) and HDL-C were provided by Zhongshen Beikong Biotech Co. (China). Other chemicals and organic solvents, including trichloracetic acid (TCA), were of analytical grade and purchased from local reagent retailer.
Animal Treatment

Forty male Sprague-Dawley rats with a body weight of 140–160 g were obtained from Sino-British Sippr/BK Laboratory Animal Ltd. (Shanghai, China). The rats were divided randomly into 5 groups of 8 animals each and treated daily for 90 days as follows: (1) normal control: received physiologic saline (10 ml/kg) twice with 1 hour interval; (2) ethanol group: received ethanol 2.4 g/kg (30%, v/v, 10 ml/kg) 1 hour after ingestion of physiologic saline (10 ml/kg); (3) low EGB plus ethanol: received EGB 48 mg/kg 1 hour prior to administration of ethanol 2.4 g/kg; (4) high EGB plus ethanol: received EGB 96 mg/kg 1 hour prior to administration of ethanol 2.4 g/kg; (3) EGB control: received EGB 96 mg/kg and physiologic saline (10 ml/kg) with 1 hour interval.

All rats were maintained on a 12 hours light/dark cycle in a temperature-regulated room (20–25°C) and cared for according to the Guiding Principles in the Care and Use of Animals. The experiment was approved by Tongji Medical College Council on Animal Care Committee of Huazhong University of Science and Technology (China). Rodent laboratory chow and tap water were available ad libitum during the period. The rats were weighed once a week, and food consumption was monitored daily. Ethanol, EGB and physiologic saline were administrated daily by gastric intubation according to the body weight. Venous blood was collected from the tail 4 hours after intraday final intragastric treatment on day 30, 60, and 90 with an overnight fast. The serum was separated by centrifuge at 3,500 g at 4°C (Eppendorf 5804R, Germany) for 10 min and stored at -80°C (Thermo 8600, USA).

Determination of Serum Lipid

Serum TC and TG were analyzed by enzymatic colorimetric methods (CHOD-PAP for cholesterol and GPO-PAP for triglycerides) with commercially available assay kits according to the instructions of the manufacturer. HDL-C was determined by the same method as TC after precipitation of chylomicrons, very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) using phosphotungstic acid/magnesium chloride reagent. All assays were performed by an automatic biochemical analyzer (Sigma SD 2000, Germany). LDL-C level was calculated in the light of Friedwald formula: LDL-C = TC–HDL–(TG/2.19) (Friedwald et al., 1972).

Determination of Lipid Peroxidation

The extent of lipid peroxidation was evaluated by measuring the production of TBARS (oxidative degradation products react with TBA), including malondialdehyde (MDA), one of the main products (Beuege and Aust, 1978). Briefly, the serum sample was mixed with TCA-TBA-HCl solution, butylated hydroxy toluene and diethylenetriaminopentaacetic acid. The mixture was heated for 60 min in a boiling water bath. After centrifugation at 5,000 g for 10 min, the absorbance of solution was recorded at 532 nm by using 1,1,3,3-tetraethoxypropane as a standard. The lipid peroxidation was expressed as MDA in nmol per ml serum.
Statistical Analysis

Data are expressed as mean ± SD and subjected to one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple range test (SPSS 12.0 software package). A probability value at or less 0.05 was determined to be statistically significant.

Results

Excessive intake of ethanol, a nutrient and toxic drug with high energy, produces a negative impact on lipid metabolism. As shown in Figs. 1 and 2, ethanol consumption led to a time-dependent increase on serum TC and TG. Compared to the ethanol group, low and high EGB prophylactic medication intervention significantly decreased TC and TG levels. Especially the high EGB decreased TC by 16.3% and 15.7%, and TG by 15.4% and 21.6% on day 60 and 90, respectively. These results indicate that EGB exerts a substantially regulating effect on hyperlipidemia induced by ethanol.

In the current study, the ratio of HDL-C against TC gradually decreased after ethanol administration. The alteration of HDL-C level presented the same trend, although no significant difference was obtained between the ethanol group and the control group (Figs. 3 and 4). Of interest is that low EGB preconditioning inhibited the decrease of HDL-C/TC significantly on day 60 and 90 compared to the high EGB group. Moreover, the high EGB supplement further reversed the decline of HDL-C ratio against TC on day 60 and 90, and when compared to the ethanol group on day 90, there was a significant increase in the HDL-C level.

As shown in Fig. 5, the ethanol regime markedly increased LDL-C on day 60 and 90 when compared to the normal control. In comparison with the ethanol group, however, low and high EGB prophylactic medication decreased LDL-C by 20.2% and 33.9% on day 60, and 19.8% and 39.0% on day 90, respectively.

![Figure 1](image.png)

Figure 1. Dynamic response of serum TC level on ethanol administration (2.4 g/kg) and the prophylaxis of EGB (48 or 96 mg/kg) for ethanol-fed rats. Serum was collected 4 hours after intraday final intragastric treatment on day 30, 60 and 90, respectively. TC was determined by CHOD-PAP method and the results were expressed as mean ± SD (n = 8). *p < 0.05, **p < 0.01 versus the normal control; Δp < 0.05, ΔΔp < 0.05 versus the ethanol group.
Figure 2. Time-dependent effect of ethanol on serum TG concentration and the preventive effect of EGB for ethanol-treated rats. Serum was collected 4 hours after intraday final intragastric treatment on day 30, 60 and 90, respectively. TG was assayed by GPO-PAP method and the results were expressed as mean ± SD (n = 8). *p < 0.05, **p < 0.01 versus the normal control; Δp < 0.05, ΔΔp < 0.05 versus the ethanol group.

Figure 3. Dynamic change of serum HDL-C content following ethanol ingestion and the effect of EGB preconditioning for ethanol-fed rats. Serum was collected 4 hours after intraday final intragastric treatment on day 30, 60 and 90, respectively. HDL-C level was measured by CHOD-PAP method after precipitation using phosphotungstic acid/magnesium chloride reagent. The results were expressed as mean ± SD (n = 8). ΔΔp < 0.01 versus the ethanol group.

Figure 4. Dynamic response of serum ratio of HDL-C against TC after ethanol intake and the role of EGB for ethanol-treated rats. Serum was collected 4 hours after intraday final intragastric treatment on day 30, 60 and 90, respectively. HDL-C and TC were examined by enzymatic colorimetric methods and the results were expressed as mean ± SD (n = 8). *p < 0.05, **p < 0.01 versus the normal control; Δp < 0.05, ΔΔp < 0.01 versus the ethanol group.
Lipid peroxidation reflects the damage of cellular membranes from free radical infliction. MDA equivalent, a marker of lipid peroxidation, exhibited time-dependent elevation in response to ethanol administration (Fig. 6). Most notably when compared to the ethanol group, low EGB pretreatment decreased MDA of ethanol-fed rats by 13.5% and 12.3% on day 60 and 90, respectively; high EGB intervention decreased MDA by 16.0%, 17.8% and 17.8% on day 30, 60 and 90, respectively. The inhibitory effect of EGB on ethanol-induced elevation of MDA indicates that EGB possesses a substantially protective effect on alcoholic lipid peroxidation.

Discussion

The present study was performed to assess the prophylactic effect of EGB on ethanol-induced dyslipidemia. The dose selection of ethanol was based on the results of the animal
experiments and epidemiological investigations. For men two drinks daily impart a low risk of cardiovascular and other diseases, whereas the risk dramatically increases when the ethanol dose exceeds 72 or 89 g/day (van Tol and Hendriks, 2001; Standridge et al., 2004). Over 2 g/kg of ethanol to rats may produce an extensive deleterious effect, including elevated lipid peroxidation (Husain et al., 2001).

All rats survived the experiment until sacrifice. Food intake and body weight gain of ethanol-fed rats was slightly lower but there was no statistical significance (data not shown). A compensation of calorific deficit by ethanol ingestion may account for the lack of reduction in body weight gain despite decreased food intake in these animals.

HDL-C is believed to play a crucial role in regulating lipid metabolism by promoting reverse cholesterol transport. Hence, the proportion of HDL-C to TC is an important physiological index reflecting the potential to normalize hyperlipidemia. LDL-C is recognized as one of the key risk factors for cardio-cerebral vascular disorders (Al-Shayji and Akanji, 2004; Balasubramaniyan and Nalini, 2003). In the present study, serum TC, TG and LDL-C were gradually increased, and the ratio of HDL-C/TC was markedly reduced with the extension of unremitting ethanol exposure (2.4 g/kg). This finding is in agreement with other reports (Senthilkumar et al., 2003; Balasubramaniyan and Nalini, 2003; Lee, 2004). Although low to moderate alcohol consumption may restrain TG levels mainly due to peripheral breakdown of VLDL by the actions of lipoprotein lipase in adipose tissue, heavy drinking strongly increases TG serum levels owing to disturbed metabolism of free fatty acids and increased production of VLDL in the liver. This results in a J-shaped relation between TG concentration and alcohol consumption (Kato et al., 2003). During ethanol metabolism, large amounts of reduced nicotinamide-adenine dinucleotid (NADH) is generated, thus, the Krebs cycle and oxidation of fatty acid are inhibited, which favors liver steatosis and serum hyperlipidemia (Lieber, 2004; Rukkumani et al., 2002). Alcoholic hypertriglyceridemia and hypercholesterolemia were gradually exacerbated, and the ratio of HDL-C against TC lowered with ethanol exposure, although no significant reduction on the HDL-C level was found in the current study. Besides the decrease of relative concentration of HDL, Shukla et al. (2001) also reported that chronic alcohol abuse modifies the HDL structure and diminishes the ability of HDL to remove cholesterol and cholesterol efflux.

The beneficial effect of EGB on various diseases has been well-documented both in vivo and in vitro, and the extensively acknowledged mechanism of EGB is mainly based on its antioxidant activity and antagonizing effect on platelet activating factor (Koch, 2005). However, in our study, prophylactic treatment with EGB, especially at a high dose, partially reversed ethanol-induced dyslipidemia. This provides new insight to investigate the underlying mechanism by which EGB prevents ethanol-induced lipid disorders. Similar studies have found that EGB lowers circulating free cholesterol in aging rats, decreases cholesterol influx and increases its efflux (Yao et al., 2004), and inhibits the elevation of serum TG in high cholesterol diet treated rats (Kobayashi et al., 1993). The hypolipidemic action of EGB seems to be principally derived from the flavonoids. Epidemiological data showed that total intake of flavonoids, or a single quercetin component, was inversely correlated with the plasma TC and LDL-C (Arai et al., 2000). Moreover, as a major flavonoid component of EGB, quercetin has been identified as a potent hypolipidemic in
experimental studies (Auger et al., 2005; Kamada et al., 2005). Bok et al. (2002) reported that quercetin promotes an increase in fecal sterols, which in turn leads to a decreased absorption of dietary cholesterol, as well as lower plasma and hepatic cholesterol by inactivating β-hydroxy-methyl-glutaryl CoA (HMG CoA) reductase, a rate-limiting enzyme in cholesterol biosynthesis.

Besides hyperlipidemia, chronic heavy ethanol consumption gave rise to lipid peroxidation as shown with an elevated serum MDA level. Excessive free radicals or reactive oxygen species (ROS) are produced during ethanol metabolism by the alcohol dehydrogenase system, especially the microsomal ethanol oxidation system (MEOS) as cytochrome P450 2E1 (CYP 2E1) is over-induced after long-term ethanol load (Lieber, 2005; Husain et al., 2001). Additionally, an alcohol binge results in an imbalance in iron homeostasis and immunologic response, by which oxidative stress is further induced (Dai et al., 2000; Suzuki et al., 2002). Lipid is particularly sensitive to ROS, and its peroxidation may be an unavoidable consequence when ROS are overproduced (Stadtman, 2002; Liu et al., 2005). The antioxidant properties of EGB provide an alternative protective strategy against ROS-provoked redox abnormity by scavenging ROS, chelating redox-active metals, or blocking chain reaction of ROS (Williams et al., 2004; Cheng et al., 2003). In the present study, alcoholic lipid peroxidation was markedly counteracted by EGB preconditioning, especially at a high dose. Importantly, EGB has been found to defend cells against oxidative damage due to lysophosphatidylcholine or ischemia by stimulating the endogenous antioxidant system, including superoxide dismutase, γ-glutamyl-cysteinyl synthetase (a rate-limiting enzyme for glutathione synthesis) and heme oxygenase-1 (Marfak et al., 2004; Chen et al., 2001; Gohil et al., 2000). Hence, the protective effect of EGB on alcoholic oxidative stress may be implicated in direct and indirect effects on the antioxidant defense system such as trapping free radicals and increasing antioxidative enzymes.

In summary, chronic excessive consumption of ethanol led to gradually aggravated hyperlipidemia and lipid peroxidation. EGB preconditioning, especially at a high dose, favorably counteracted and prevented the disorders induced by ethanol. It is, however, imperative to elucidate the precise pharmacological mechanism of EGB on alcoholic hyperlipidemia and lipid peroxidation.

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References


