

## The Influence of Eleutheroside on Blood Glucose and Blood Lipid of D-Galactose-Induce Rats through Inhibiting Blood Superoxide Dismutase Activities

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**Abstract:** The present study aims at evaluation mechanisms of natural plant eleutheroside extracts for ameliorating the blood lipid and blood glucose. The eleutherosides derived from the roots of *eleutherococcus senticosus* and is purported to behave as an “adaptogen”, we assessed effects of eleutheroside at doses of 3.6, 7.2 and 14.4 g/(kg day) on SD rats injected daily with D-gal (50 mg/(kg day)). Eleutheroside-fed rats showed higher the level HDL-C, decrease the level of HCT, TG, TC, LDL-C compared with D-gal-treated rats. We further examined the mechanisms involved in effects of 3.6, 7.2 and 14.4 g/(kg day) on rat blood. In summary, eleutheroside significantly increased Superoxide Dismutase (SOD) activity and decreased the Malondialdehyde (MDA) level.

**Keywords:** Blood glucose, blood lipid, Eleutheroside, superoxide dismutase

### INTRODUCTION

Plant extracts provide a potential source through which novel anti-inflammatory compounds may be identified (Clardy and Walsh, 2004). *Acanthopanax* is a widely used herbal product in China, other Asian countries and the United States. For thousands of years, people in China have used *acanthopanax* as a tonic and, in emergency medicine, to rescue dying patients. The eleutherosides derived from the roots of *eleutherococcus senticosus* and is purported to behave as an “adaptogen”, a class of herbal medications that confer resistance to the effects of stress (Gaffney *et al.*, 2001). It have been given the most attention and are believed responsible for the proposed adaptogenic activity. But appearing no report about eleutheroside to impacts of blood glucose and blood lipid.

In the present study, we explored whether Eleutheroside has a protective effect against D-gal-induced response in rat blood glucose and blood lipid. We further investigated the potential mechanism underlying its action.

### MATERIALS AND METHODS

**Material:** Eleutheroside (eleutheroside B content of 8%) is produced by Hunan nine department of modern Chinese medicine Co. Ltd. D-galactose (D-gal) is produced by sigma company.

Superoxide Dismutase (SOD) Detection Kit and Malonaldehyde (MDA) Detection Kit are produced by Nanjing Jiancheng Bioengineering Institute.

**Measuring method animals and diets:** Sprague-Dawley pathogen-free rats weighing 300-400 g were

purchased from Shanghai Slac Laboratory Animal Ltd (Shanghai, China) and fed a commercial rat chow (M01-F, Shanghai Slac Laboratory Animal Ltd) for three days before the study started. The rats were then randomly assigned to five groups (each group n = 8):

- **Normal group:** Using normal saline to inject and perfuse gastric
- **Model group (MG):** Inject D-gal (100 mg/(kg day)) and using normal saline to perfuse gastric
- **Low-concentration group:** Inject D-gal and use eleutheroside 3.6g/(kg day) perfuse gastric
- **Medium-concentration group:** Inject D-gal and use Establishment 7.2 g/(kg day) perfuse gastric.
- **High-concentration group:** Inject D-gal and use establishment 14.4g/(kg day) perfuse gastric for 8 weeks. All rats during dosing as well as rats destined for the studies, were singly housed in polycarbonate cages in the animal facility with a 12:12 h, light:dark cycle and maintained at 20±1°C and 50±10% humidity. Rats were given diets and water ad libitum. Body weight was recorded once a week and food intake was recorded twice a week. At the end of the study, rats were fasted overnight and anesthetized by intraperitoneal sodium pentobarbital (60 mg/kg body weight). The rats were killed and blood samples were collected into heparinized syringes from the abdominal vena cava. Plasma was prepared by centrifugation at 800 g for 20 min at 4°C and frozen in liquid nitrogen for further examination (Table 1).

**Determination of blood glucose and blood lipid:** Exteriorize blood from abdominal aorta and don't

Table 1: The content of rats blood lipid (n = 8,  $\bar{X} \pm S$ ) (nmol/L)

Groups	GLU	CHO	TG	LDL-C	HDL-C
Control group	6.87±1.30	1.86±0.07**	1.17±0.15**	0.78±0.04**	1.59±0.03**
Model group	6.41±0.69	2.20±0.1	2.45±0.12	1.18±0.04	1.13±0.31
Low-concentration group	6.10±0.35	2.11±0.10	2.28±0.10*	1.14±0.04	1.32±0.09*
Medium-concentration group	6.51±0.88	2.06±0.12*	2.23±0.08**	1.13±0.04*	1.31±0.04*
High-concentration group	6.30±1.15	1.98±0.14**	1.91±0.22**	1.07±0.03**	1.34±0.05**

Compared with model group\*\*p<0.01 \*p<0.05

Table 2: The content of rats blood lipid (n = 8,  $\bar{X} \pm S$ ) (nmol/L)

Traits	GLU	CHO	TG	LDL-C	HDL-C
Control group	6.87±1.30	1.86±0.07**	1.17±0.15**	0.78±0.04**	1.59±0.03**
Model group	6.41±0.69	2.20±0.1	2.45±0.12	1.18±0.04	1.13±0.31
Low-concentration group	6.10±0.35	2.11±0.10	2.28±0.10*	1.14±0.04	1.32±0.09*
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anticoagulation, centrifugal at 3000 r for 10 min, then separate serum, determined by fully automatic biochemical analyzer in 24 h. Project including total cholesterol (CHO), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), glucose (GLU).

**SOD assay:** SOD activity was measured according to the method described by Beyer and Fridovich (1987). Solution A was prepared by mixing 100 mL of 50 mM PBS (pH 7.4) containing 0.1 mM EDTA and 2  $\mu$ mol of cytochrome c with 10 mL of 0.001N NaOH solution containing 5  $\mu$ mol of xanthine. Solution B included 0.2U xanthine oxidase/mL and 0.1 mM EDTA. 50 microlitres of a tissue supernatant were mixed with 2.9 mL of solution A and the reaction was started by adding 50  $\mu$ L of solution B. Change in absorbance at 550 nm was monitored. A blank was run by substituting 50  $\mu$ L ultra pure water for the supernatant. SOD levels were expressed as U/mg protein with reference to the activity of a standard curve of bovine copper-zinc SOD under the same conditions.

**MDA assay:** The level of MDA in blood tissue was determined using the method (Vchiyama and Mihara, 1987). Half a milliliter of homogenate was mixed with 3 mL of H3PO4 solution (1%, v/v) followed by addition of 1 mL of thiobarbituric acid solution (0.67%, w/v). Then the mixture was heated in a water bath for 45 min. The colored complex was extracted into n-butanol and the absorption at 532 nm was measured using tetramethoxypropane as standard. MDA levels were expressed as nmol per mg of protein.

**Statistical treatment:** Each result shown in figures was the mean of at least three replicated treatments. Data were subjected to analysis of variance (one-way ANOVA) using SPSS software and expressed as means $\pm$ SD( $\bar{x} \pm S$ ). p-values<0.05 were considered significant.

## RESULTS

**Blood lipid and blood glucose:** From Table 2, we can know that concentration of CHO, TG, LDL, HDL in model group is very outstanding significant; compared with model group, concentration of CHO, TG, LDL, HDL in High-concentration group, TG and HDL in Low-concentration group, CHO, LDL, HD in Medium-concentration group is also significant, concentration of TG is significant rather than GLU; compared with different concentration groups, concentration of CHO in Low-concentration group and High-concentration group is very outstanding significant, concentration of TG in low, high concentration groups and medium, high concentration groups is significant, concentration of LDL in low, huge concentration groups and medium, high concentration groups is also significant, besides, there is no differences in others.

**SOD activity and MDA levels:** D-gal increased the erythrocyte MDA level compared with the control group. This D-gal induced increase was prevented in all eleutheroside groups (Fig. 1). D-gal-treated rat decreased the activity of erythrocyte SOD compared with the control group (Fig. 2). This D-gal induced decrease in activity was prevented by High-concentration group, but not low and Medium concentration Group (Fig. 1).

The D-gal treatment resulted in significant decreases in Cu, Zn-SOD activity in the blood (p<0.01) (Fig. 1). The D-gal-treated rat received eleutheroside showed a significant attenuated in the Cu, Zn-SOD activity; high dose of eleutheroside had greater effect than low dose (p<0.05).

Figure 2 described the levels of MDA in the blood of five groups. Compared with the control group, D-gal-treated group showed a significant increase in MDA level (p<0.01). This increase in MDA was also attenuated in the blood of eleutheroside fed rat. As compared with the D-gal-treated group, a higher dosage of eleutheroside (14.4 and 7.2g/(kg day)) exerted more significant difference on MDA level than the

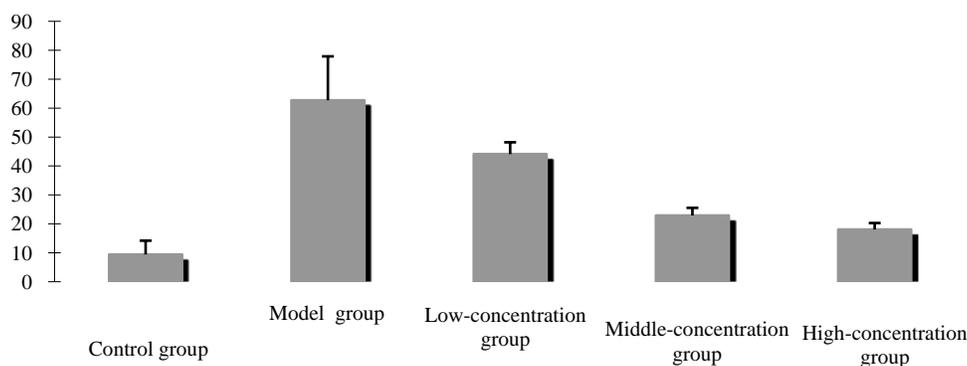


Fig. 1: Blood superoxide dismutase (SOD) Activities

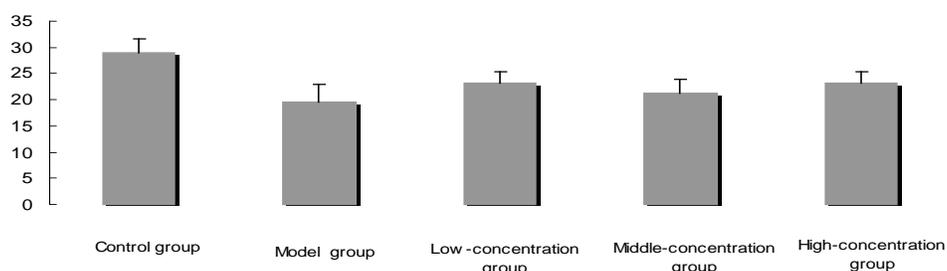


Fig. 2: Blood malondialdehyde (MDA) levels

eleutheroside at the dose of 3.6 g/(kg day) weight ( $p < 0.05$ ) in the blood of rat.

### DISCUSSION

In recent years, some researchers suggest that D-gal can interfere with normal sugar and lipid metabolism. But also for protein non-enzymatic glycosylation affection, saccharification proinsulin, insulin and its receptor, leading to the reduction of insulin sensitivity, inducing hyperinsulinemia and weakening the physiological function of islet turbulent. Our experimental results show that there is no significant difference on blood glucose between D-gal rats and model group. Blood lipid increased significantly which will due to a higher risk of hyperlipidemia, promoting aging and many other geriatric diseases. Studies have thought that the content of TC, TG increases and HDL-C declines will thicken the artery wall which resulting in increased risk of cardiovascular disease. Our study found that treated-rats serum TC and TG content increased than normal rats significantly while HDL-c content decreased obviously. CG can reduce rat blood TG, TC, LDL-C and improve the rat HDL-C. Besides, it can reduce rats blood lipid, blood thickness levels and dose effect.

Toxicity by oxygen radicals has been suggested as a major cause of cancer, aging, heart disease and cellular injury in hepatic and extrahepatic organs (Hartman, 1981; Troll and Weisner, 1985; Gram *et al.*,

1986). There is compelling evidence that SOD are essential for biological defence against the superoxide anion (Fridovich, 1983). MDA is a major biomarker of lipid peroxides (Rogina and Helfand, 2000). Long term administration of D-gal induced changes of these redox-related biomarkers in rat, including decrease in SOD increase of MDA level. Oxygen species, which have been implicated in several physiological and pathophysiological processes. Antioxidants also ensure defenses against ROS-induced damage to lipids, proteins and DNA.

In the present study, we found that Eleutheroside could significantly increase the activities of anti-oxidative enzymes such as SOD and decrease the production of MDA level in the D-gal-treated rat blood. This indicated that eleutheroside, to some extent, could attenuate the oxidative injury induced by D-galactose. In other words, the protection of eleutheroside against oxidative stress to blood may be involved in the mechanism of its action to ameliorate the blood lipid and blood glucose.

In summary, our results showed that eleutheroside could reverse the chronic D-gal-induced toxicity. Eleutheroside fed mice showed an improved performance in blood lipid and blood glucose. Based on our data, we concluded that the mechanism of the protective effects of eleutheroside on D-gal-induced toxicity may be increasing the antioxidant enzyme activities, for example, SOD and increasing the erythrocyte MDA level.

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