

# Research on antioxidant activity of EOB-F and its function in the diagnosis of heart and cerebral vessels

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**Abstract:** This paper introduces the research progress related to the antioxidant activity of bamboo leaf flavonoid (EOB-f) and its pharmacological activity of heart and cerebral vessels. The paper studied what role EOB-f played in the diagnosis of heart and cerebral vessels, based on the *in vitro*, *in vivo* and animal model as well as the pharmacological research experiment. 1) The *in vitro* and *in vivo* experiments indicated that EOB-f has the function of anti-reactive-oxide species, anti-aging and anti-fatigue; 2) The research of animal model indicated that EOB-f can significantly decrease the triglyceride (TG) content in serum, significantly increase high density lipoprotein cholesterol (HDL-C) content in serum, regulate blood lipids and reduce the risk of atherosclerosis; 3) The pharmacological study showed that EOB-f has the effect to resist the whole animal anoxia, can effectively dilate coronary vessels, increase coronary flow, increase myocardial contractility, obviously improve myocardial ischemia and diminish the myocardial infarction scope, inhibit the coagulation process and reduce platelet aggregation, and has certain protective effect on cerebral ischemia. EOB-f has the potential to develop as the natural drug and functional foods for prevention of cardiovascular and cerebrovascular diseases.

**Keywords:** Bamboo leaf flavonoid; antioxidant activity; heart and cerebral vessels.

## INTRODUCTION

Bioflavonoids or vitamin P, which belongs to plant secondary metabolites, and often coexists with vitamin C, is widely distributed in nature. Licorice, kudzu vine root and ginkgo leaves which are rich in flavonoids, according to the study, are found with the functions of antioxidant, scavenging free radicals, eliminating fatigue, preventing arteriosclerosis, protecting blood vessels, dilating telangiectasia, activate cells of brain and other organ and anti-aging. At present, the plant flavonoids which are being developed in large scale, are mainly ginkgo flavonoids, pueraria isoflavones, tea polyphenol, etc., which have been widely used in cardiovascular disease prevention (Fu *et al.*, 2001).

Research and development of effective components of bamboo is very common in China. Bamboo leaves flavonoid (EOB-f) is a kind of plant flavonoid preparations developed by Zhang Ying *et al.* in 1990's with the mountain wild lophatherum graciles as the raw material (Wenwen *et al.*, 2011; Yuhong *et al.*, 2009). Its functional factor is flavone glycoside, with the carbon glycoside flavone as majority. The flavone glycoside is the compound formed by the connection of flavone mother nucleus with glycosyl in some positions. It is divided into carbon glycoside flavone and oxygen glycoside flavone. The so-called carbon glycoside flavone refers to that the glycosyl is connected with the mother nucleus with-O-C- and the oxygen glycoside flavone with-O-C-.

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Flavonoids compounds, a kind of natural antioxidant, can inhibit the oxidation and rot of food and protect other components in food; in addition, it can eliminate free radical, which can prevent aging, cardiovascular disease, cancer, etc (Tao *et al.*, 2009; Hong and Chunshui, 2004). Therefore, the research on flavonoids compounds' elimination function on free radical is of great significance.

## MATERIALS AND METHODS

### Materials

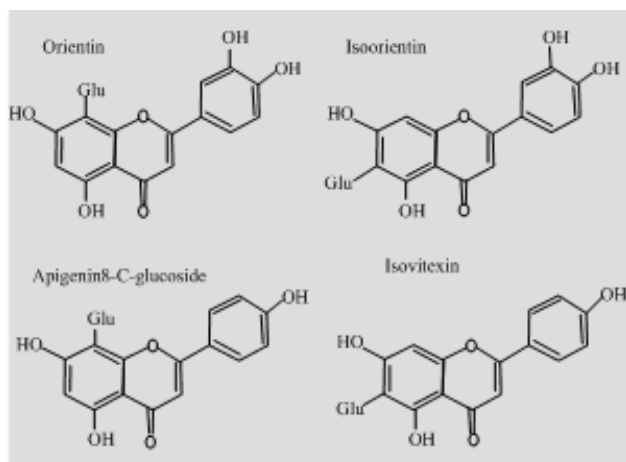
Ginkgetin, bamboo leaf flavonoid (EOB-f 01 (TF $\geq$ 50%), EOB-f02 (TF  $\geq$ 30%), EOB-f03 (TF $\geq$ 10%) etc.), Bailuda capsule (ginkgo leaf capsules), compound danshen (a kind of traditional Chinese medicine that can invigorate the circulation of the blood and regulate Qi to alleviate pain), lard oil, BHT (butylated hydroxytoluene), drying oven, 250 ml iodine flask, mixed liquid of trichloromethane and glacial acetic acid, sodium thiosulfate, starch, SD male rats (Hongmei *et al.*, 2002).

### Methods

#### Research of EOB-f03 on hyperlipidaemia

The blood fat regulation function of Zhukangning capsules was conducted clinical test with Bailuda capsules (ginkgo biloba extract preparations produced by Shanghai Xinyi Pharmaceutical Factory) as positive contrast. 78 cases of patients with abnormal lipid metabolism (serum cholesterol  $\geq$  5.98mmol/L or triglyceride  $\geq$  2.2mmol/L or high-density lipoprotein  $\geq$  1.04mm/L) and aging from 35 to 80 years old were

recruited in the study. These cases were divided into Zhukangning group (1 grain for one time and two times for every day) and Bailuda (ginkgo leaf capsule) group (2 grains for one time and three times for every day). The content of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), APOAI and APOB were detected before treatment and 6, 12 weeks after treatment.



**Fig. 1:** The chemical construction if four main carbon glycosides flavonoids in bamboo leaf.

**Research on the antioxygenation of bamboo leaf flavonoid on grease**

50.0 g warm lard oil was divided into 5 pieces and placed in beaker. 4 pieces were added EOB-f with 0.05%, 0.10% and 0.50% oil quantity and 0.02% BHT, respectively. The other piece of lard oil was taken as blank contrast. The 5 pieces of lard oil were placed in drying oven with (60±1) °C. Stir it every 12 h. The peroxide value (POV) of lard oil was detected at fixed time. Thus, we can measure the antioxidant activity of EOB-f.

2~3g of lard oil sample was weighted precisely and placed in 250ml iodine flask. Add 30 ml mixed liquid of trichloromethane and glacial acetic acid to make the sample completely dissolve. Add 1.00ml saturation KI solution and stopper the bottle cap tightly. Mix it lightly for 0.5 min and then place it in the dark place for 3 min. Take it out and add 100 ml water and mix. Immediately, use 0.002mol/L Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> standard solution for titration. When it turned to be yellow, then add 1 ml starch indicator until the blue disappear. Take mixed liquid of trichloromethane and glacial acetic acid, KI solution and water in the same amount for reagent blank test according to the same method.

$$POV = \frac{(V1 - V2) \times M}{m} \times 1000$$

POV of samples is

In the formula, POV refers to peroxide value of sample, mmol/kg;

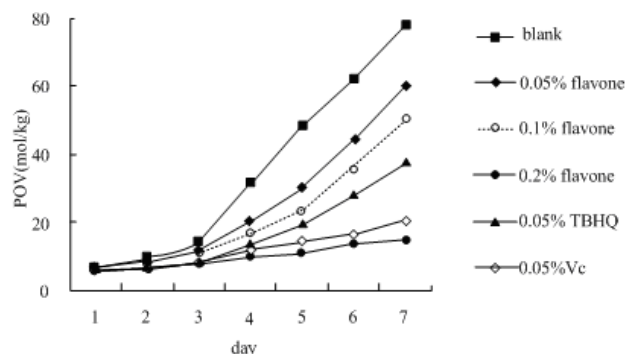
V1 refers to the volume of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> standard solution consumed by sample, mL ;

V2 refers to the volume of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> standard solution consumed in blank test, mL;

M refers to the molar concentration of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> standard solution, mol/L; m refers to the quality of sample.

**Animal test of EOB-f03 decreasing blood fat**

Adult male SD rats (Sprague Dawley rats) were taken as test objects for establishing high blood fat model. They were randomly divided into blank contrast group, ginkgetin (Tianbaoning) positive contrast group and (0.1, 0.2, 0.3g/kg) EOB-f03 group. Every week, the rats were weighted. 28 d later, the content of TG, HDL-c, LDL-c and total cholesterol (TC) were detected.



**Fig. 2:** Synergistic antioxidant effect

**STATISTICAL ANALYSIS**

Originpro8.0 (SR4 v.0951) was used for statistical analysis. The data was mean value ± standard derivation (x ± SD), α= 0.05.

**RESULTS**

**Effect of EOB-f03 on hyperlipidaemia treatment**

Research found that 3 months after Zhukangning treatment, TG in serum lipid single component from patient with lipid metabolic block decreased 33.3%, which was significantly lower than the level before treatment (P<.005); HDL-c increased 27.3%, which was significantly higher than the level before treatment (P<.005); Arterial stiffness index (AASI) gradually decreased to 3.3 12 weeks after treatment from 4.9 before treatment, which meant the increase of HDL-c and decrease of low-density lipoprotein (LDL) (as shown in table 1) and decrease of risk for arteriosclerosis. It had better function of blood fat regulation. After treatment by Zhukangning, there was no obvious side effect and it had no adverse effect on route of blood and urine, hepatorenal function and blood glucose. Clinical test proved again that Zhukangning had a clear curative effect and had no toxic and side effect. It was suitable for various abnormal metabolism of blood lipid and was a kind of safe and effective lipid regulation drug. It was worth to be applied and promoted in clinic. In addition, it had good clinical

**Table 1:** change of lipid level in serum before and after treating by EOB-f03

	Before treatment	6 weeks after treatment	12 weeks after treatment
TG (mmol/l)	3.3±1.9	2.2±1.9*	2.2±1.3*
HDL-c (mmol/L)	1.1±0.5	1.3±0.5	1.4±0.5*
AASI	4.8	4.2	3.3

\*P<0.05 Compare before treatment and 6 and 12 weeks after treatment

**Table 2:** The relative indexes of rats

Groups	Dose (g/kg.bw)	Weight		Triglyceride		High density lipoprotein cholesterol	
		0 day	28 day	0 day	28 day	0 day	28 day
high fat control group	0	216±20	356±34	60±14	130±25	33±5	31±5
EOB-f03	0.1	214±28	349±39	61±13	121±33	31±4	36±8
EOB-f03	0.2	215±19	316±39*	60±12	75±26**	32±6	35±8
EOB-f03	0.6	212±23	321±38*	59±14	77±42**	33±6	40±7**

\*p<0.05 and \*\*P<0.01 compared with control group E

application prospect for prevention of atherosclerosis and heart and cerebral vessel disease.

#### ***The antioxygenation function of EOB-f on grease***

Addition of different dose of EOB-f in blank contrast group showed different antioxidant activity (Shiyu *et al.*, 2004; Ying *et al.*, 2004). With the increase of addition of EOB-f, POV decrease, this indicated that the antioxygenation strengthened (fig. 2).

In addition, EOB-f and Vc was compounded in 1:1. It had significant synergistic antioxidant function on lard oil. POV of group of compound of EOB-f and Vc was the lowest. It indicated that its effect was superior to all groups of single antioxidant. Moreover, its synergistic antioxidant activity was stronger than the EOB-f with the same concentration, superior to the group with addition of 0.05% TBHQ (C10H14O2), slightly better than group with 0.05% Vc.

With the increase of standing time of lard oil, the slope of POV increased. It indicated that the oxidation rate of grease speed up; POV slope of the lard oil that added antioxidant was smaller than that of blank sample. It indicated that the increase was slow. In addition, with the increase of adding dose, the antioxidant performance gradually strengthened.

#### ***Animal test effect of EOB-f03 decreasing blood fat***

The result found that group of EOB-f in different dose could all decrease serum TG, TC and LDL-c of SD rats. Groups of medium and high dose could significantly increase HDL-c. Its lipid regulation function is similar to ginkgetin. AASI is the most important index for measuring lipid-lowering function and is expressed as LDL-c and HDL-c. AASI of the rats in contrast group obviously increased. Although low dose can lower the absolute content of Serum TG, TC and LDL-c, AASI did not decrease; AASI in groups of medium and high dose

obviously decreased and show dose dependence. It indicated that EOB-f had the function of preventing arteriosclerosis (table 2).

#### ***Effect of EOB-f on treatment of heart and cerebral vessels***

Epidemiological investigation and clinical research indicated that the leading risk factor of acute ischemia cerebral vessels disease and coronary heart disease is atherosclerosis (AI). Lipid metabolism block plays an important role in various factors that cause AI. Most scholars thought that, decreased HDL-c and increased TG were closely related to AI and heart and cerebral vessels diseases (Mahmood *et al.*, 2010). The clinical observation of EOB-f curing hyperlipidaemia indicated that, it had good effect on curing hyperlipidaemia. Researchers from Shanghai First People's Hospital used 78 cases of patients with abnormal lipid metabolism (serum cholesterol  $\geq 5.98$ mmol/L or triglyceride  $\geq 2.2$ mmol/L or high-density lipoprotein  $\geq 1.04$ mmol/L) aging from 30 to 75. They were divided into Zhukangning group (1 grain for one time and two times for every day) and Bailuda (ginkgo leaf capsule) group (2 grains for one time and three times for every day). And the content of TC, TG and HDL-c before treatment and 6 and 12 weeks after treatment were detected. Research found that after 3 months of treatment with EOB-f, TG from serum lipid single component of patients with lipid metabolism decreased by 33.3%, which was significantly lower than the level before treatment. HDL-c increased by 26.8%, which was significantly higher than the level before treatment and had better function of blood lipid regulation than Bailuda. After treatment with EOB-f, there was no obvious adverse effect. Through clinical test, it proved again that EOB-f had clear curative effect and no toxic and side effect. In addition, it is suitable to be applied in various kinds of abnormal blood lipid metabolism. It can be developed as a safe and effective lipid regulation drug and had a good

clinical application prospect in the prevention and treatment of cardiovascular and cerebrovascular diseases.

## DISCUSSION

EOB-f is a kind of plant flavonoids preparation with high mountain wild lophatherum gracile as raw material. Its functional factor is flavone glycoside, with the carbon glycoside flavone as majority. It plays important role in the treatment of hyperlipidaemia. Under the circumstance of the same intake of total flavone and comparison of ginkgo biloba extraction tablets, the content of TC, TG, HDL-c, ApoA1 and ApoB were detected before treatment and 6 and 12 weeks after treatment. Research indicated that, the content of TG in serum lipid single component from patient with lipid metabolism block decreased by 33.3% after 3 months of treatment with EOB-f, which was significantly lower than the level before treatment; the content of HDL-c increased by 26.8%, which was significantly higher than the level before treatment. This experiment proved that EOB-f had a good function of curing hyperlipidaemia.

Chinese scholar Zhang Ying (2014) found in test of EOB-f inhibiting the aggregation of rabbit blood platelet that, EOB-f had obvious inhibition function on the cruor process of rabbit (Ying *et al.*, 2004): in vitro test proved that groups of medium-dose, high-dose and high-dose EOB-f (2.5mg/kg, 5mg/kg, 10mg/kg) all had strong function on inhibiting platelet aggregation of rabbit. Among them, the inhibition function of groups of medium and high dose was stronger than Fufang Danshen tablets (0.1g/kg). It indicated that EOB-f has certain function on inhibiting platelet aggregation and preventing the formation of thrombus (Jie, 2004; Naifu, 2003; Jinquan *et al.*, 2012; Lianliang *et al.*, 2012). All these experiments proved that EOB-f was beneficial to maintain the normal physiological function of coronary artery and prevent cardiovascular diseases such as coronary heart disease, stenocardia, etc. to different degree.

EOB-f had a significant function in eliminating free radical. Its addition amount within test scope showed positive correlation with inoxidizability; meanwhile, it proved the synergistic antioxidant effect of EOB-f and Vc. Too much lipid per oxidase in human body can damage the cytomembrane of cells and promote aging. However, the free radical theory also points out that free radical can decrease the activity of enzyme, cause error on metabolism of nucleic acid and thus promote the aging of cells. Shen Jian *et al* (Lei and Jian, 1999) applied animal experiment method and found that EOB-f had the significant function on decreasing lipid per oxidation and increasing the activity of SOD (superoxide dismutase) and GSH -PX (glutathione per oxidase). Xu Gang, Zhang Hong *et al.* Applied NBT (nitroblue tetrazolium) photochemical reduction method and found that EOB-f

had strong function on eliminating free radical; bamboo leaf water bath extraction and ultrasonic extraction had O<sub>2</sub>- elimination rate of 77.52% and 79.68% on, which was stronger than ascorbic acid.

EOB-f can protect cardiovascular and ischemic injury and prevent atherosclerosis. The newest pharmacological experimental study proved that EOB-f had good function of lipid regulation and it was stronger than contrast group of ginkgetin. In the process, it had no adverse effect on blood and urine route, hepatorenal function and blood glucose and had no toxic and side effect; in addition, it can significantly inhibit the liposome peroxidation induced by AAPH (Lianliang *et al.*, 2012; Lei and Jian, 1999; Qizhou *et al.*, 2008; Hui, 2012; Xiaofang *et al.*, 2012; Jiajia *et al.*, 2014), prevent Cu<sup>2+</sup> mediated human serum LDL-c oxidation, decrease the animal lipid per oxidation and increase the activity of GSH-Px and SOD; thus, it can expand coronary vessels and increase the coronary flow, which performed protection function on cardiovascular and cerebrovascular diseases to a certain extent.

## CONCLUSION

Nowadays, the science and technology develops rapidly. The field of natural plant extraction possesses huge business opportunity. EOB-f is widely applied in fields such as natural functional food additives, natural drug, etc. because of its abundant sources, medical benefits, safety, etc., and it had multi-functions (Zhaolin, 2012): besides the anti-oxidation and block of nitrosation, it also had effect of antibiosis, bacteria inhibition, deodorization, and nutrition and health care. Zhou Yunbo (Yunbo, 2000) studied the antioxidation of EOB-f on grease with GB5538-85 method and found that the antioxidation of EOB-f was higher than the synthetic antioxidant BHT (butylated hydroxytoluene). Therefore, EOB-f has the development potential as natural anti-oxidation. In addition, researchers at home and abroad made further research on the its protection function for heart and cerebral vessels and found that EOB-f which focused on flavonoids compounds and coumarin lactone can prevent myocardial infarction, high blood pressure and hyperlipidemia, which had certain protection function on cerebral ischemia disease.

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