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Ex vivo inhibitory effect on tilapia LDL oxidation and hypolipidemia properties of *Glycine tomentella* root extract

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Abstract

The ethanolic extract of I-Tiao Gung (GT-E) (*Glycine tomentella* root extract) was found to reduce the oxidative rate and prolonged lag phase of LDL in human (*Homo sapiens*) and tilapia (*Oreochromis mossambicus*). The *in vivo* effect of GT-E was determined using tilapia as a model. Hyperlipidemia and hypercholesterolemia were induced in fish by feeding commercial feed daily at 2% body mass for 8 weeks, or at 1% body mass for 12 weeks. Thirty two adult male tilapia were randomly divided into two groups and fed with feed containing 1% (w/w) GT-E or control diet for 12 weeks. Specific growth rate was similar between the GT-E group and the control group. Total triacylglycerol, total cholesterol and low-density lipoprotein cholesterol (LDL-C) in plasma of the GT-E group were significantly lower, while plasma total antioxidant status was significantly higher than those of the control group. GT-E fed fish had longer lag phase of Cu^{2+}-induced LDL oxidation and retained more α-tocopherol in LDL particles than the control fish. LDL from the GT-E group had more monounsaturated fatty acids and less polyunsaturated fatty acids than the control group indicative of its effect on fatty acids metabolism. GT-E demonstrated hypolipidemic and hypocholesterolemic effects and inhibiting LDL oxidation in tilapia similar to the effects in mammals, thus tilapia can serve as a surrogate animal model for prescreening anti-atherosclerosis effect of natural products.

Keyword: Glycine tomentella; I-Tiao Gung; Isoflavone; Hypolipidemia; Hypocholesterolemia; LDL oxidation; tilapia; *Oreochromis mossambicus*

1. Introduction

High plasma LDL cholesterol is a singular risk of atherosclerosis (Mass and Böger, 2003; Hammad et al., 1998). The oxidation of human LDL is widely considered a pivotal event in atherosclerosis and the oxidized LDL is actively involved in atherogenesis (Steinberg, 1997, Chisolm and Steinberg, 2000). Thus measuring LDL oxidation *ex vivo* could predict the carotid artery disease status (Hendrickson et al., 2005).

Antioxidants have been used to inhibit LDL oxidation to significantly arrest atherogenesis in rabbits, hamsters, mice and non-human primates *in vivo* (Wu et al., 1998; Böger et al., 1998, Fruebis et al., 1995, Yasuhara et al., 2000, Iwai et al., 2002; Chen et al., 2005a,b; Mursu et al., 2005). Hamsters fed aqueous crude extract of I-Tiao Gung (*Glycine tomentella*) in high-fat diet for 10 weeks resulted in a decrease in plasma cholesterol (Ko et al., 2004). The 95% ethanolic extract had stronger antioxidant activity and free radical scavenging ability than the water extract, and inhibited LDL oxidation catalyzed by 15-lipoxygenase (15-LOX) and cyclooxygenase-2 (COX-2) *in vitro*, indicative of its potential preventive roles in human atherosclerosis (Pan et al., 2005; Chen et al., 2005b). Further *in vivo* animal studies are needed to confirm the bioactivities. Since test mammals are expensive, it is useful to look for surrogate animal models, such as fish to quickly screen the antioxidant and anti-atherosclerotic effects.

Atlantic salmon (*Salmo salar*) fed a high cholesterol diet stimulated artery wall lesions (Farrell et al., 1986) that showed positive correlations with the plasma LDL (Eaton et al., 1984; Farrell et al., 1986). The morphology of artery wall lesions of salmon was similar to that in vascular intima injury of human arteries (Gong and Farrell, 1995; Garcia-Garrido et al., 1993). Lipoproteins of ectothermic animals like fish contain high levels of polyunsaturated fatty acids that are susceptible to oxidative modifications. The oxidized or chemically modified LDL in fish are cleared via scavenger receptors on macrophage

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similar to the mechanism in human (Froystad et al., 2002). In addition, tilapia mast cell lysates enhanced neutrophil adhesion to cultured vascular endothelial cells contributed to the adhesion and transmigration of neutrophils in the inflamed tissues (Matsuyama and Iida, 2002). Fish have been considered an alternative for prescreening bioactivities of natural compounds or nutraceuticals *in vivo* (Bolis et al., 2001; Lee and Pan, 2003; Liu and Pan, 2004; Chen et al., 2005a,b). The plasma chemical indices for healthy fish have been established (Chen et al., 2003, 2004). Tilapia is a well-studied, fast-growing and widely-cultured fish species. But no study had been reported so far on the plasma lipid of tilapia and the potential of using this fish as a model for anti-atherosclerosis research.

The aim of this study was to build up a hypercholesterolemia and hyperlipidemia status in tilapia, which were then used to determine the *in vivo* hypolipidemic and hypocholesterolemic effects of I-Tiao Gung. This may provide a potential new cardioprotective use for this traditional remedy, while the tilapia may become a surrogate model for quick prescreening of novel antioxidative and anti-atherosclerotic herbs.

2. Materials and methods

2.1. I-Tiao Gung

G. tomentella Hayata was purchased from Kinmen, Taiwan. Fifty grams of dried roots were powdered by silent cutter, then added with 95% ethanol at a ratio of 1:10 (w/v) and refluxed for 2 h at 75 °C. The suspension was filtered. The solid material was refluxed again. The solvent extracts were combined and ethanol was removed under vacuum then freeze dried to powder (GT-E). The HPLC chromatogram of the isoflavones in GT-E is shown in Fig. 1. Daidzein and daidzin were the major isoflavones (Pan et al., 2005), while other antioxidants in GT-E are being identified.

2.2. Tilapia

Oreochromis mossambicus were captured from cultured ponds and transferred to the laboratory. Thirty two adult male fish with mean body mass of 59.5±9.5 g were acclimated for 2 weeks prior to experiments. They were kept in glass aquaria (eight fish per aquarium) with constant aerated fresh water (157 L) at a water temperature of 23–25 °C under a 12:12 light–dark cycle. The fish were fed ad libitum once daily in the evening (17:00–18:00) with commercial dry pellets for tropical fish (Tai Roum Company, Taichung, Taiwan, ROC). The feed contained protein (48.2%), carbohydrate (21.3%), lipid (4.6%), ash (13.5%), crude fiber (1.2%), and vitamin mix (0.1%) including α-tocopherol (20 ppm). The total energy was 3194 kcal/kg.

After acclimation, fish were weighed and maintained eight fish per aquarium. Two aquaria of fish were fed control diet, while 2 others were fed GT-E diet consisting of 1% GT-E. Daidzein was 9.4 mg/g GT-E. Both the control and GT-E groups were fed at two levels, 1% and 2% body mass per day.

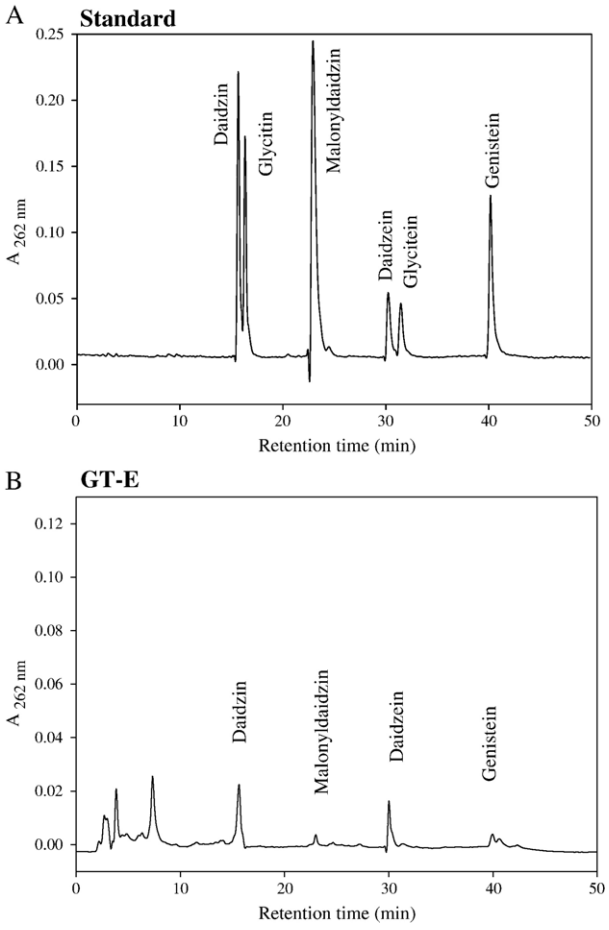

Fig. 1. RP-HPLC chromatogram of (A) authentic isoflavones, (B) 95% ethanol extract of I-Tiao Gung root (GT-E).

2.3. Blood samples

Blood samples were obtained from fasting tilapia for 24 h after feeding for 8 or 12 weeks. Fish were collected from aquaria by net. One milliliter blood sample was withdrawn from the caudal vein of each fish by heparinized syringes. After thorough mixing, each blood sample was transferred to a 2.0 mL microcentrifuge tubes containing 0.1 mL heparin (257 U.S.P unit/mL in PBS buffer, pH 7.4). The haematocrit was determined immediately. The blood samples were then centrifuged at 600×*g* for 15 min to obtain plasma for blood count.

2.4. Lipoprotein

Lipoprotein fractions were separated from plasma by ultracentrifugation (Hitachi SCP 85G, Koki, Japan) using a fixed rotor (RP55T) at 4 °C described by Paolucci et al. (1998). All reagents used were of analytical grade. The salt density intervals used were those currently used for humans. To obtain VLDL fraction, 3 mL of NaCl solution (density=1.006 g/mL) was layered on 3 mL plasma. Samples were then centrifuged at 44,000 rpm for 16 h. The VLDL fraction of 1 mL (density< 1.006 g/mL) floated on top of the sample was aspirated with a