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Hypolipidemic effects of total flavonoide extracted from the leaves of *Actinidia kolomikta* in rats fed a high-fat diet

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ABSTRACT

Objective(s): This study was to investigate the antihyperlipidemic and antioxidant effect of total flavonoid extract from *Actinidia kolomikta* (TFAK) in hyperlipidemia induced by a high-fat diet.

Materials and Methods: Male SD rats were randomly divided into 6 groups: normal group, model (hyperlipidemic diet) group, hyperlipedemic diet supplemented with TFAK (50, 100 and 200 mg/kg) and simvastatin (30 mg/kg) groups. The rats were administrated TFAK by oral for 28 days. Body weight, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were measured. The atherogenic index (AI) and coronary risk index (CRI) were calculated. The activity of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase in hepatic tissue was examined. Histopathologic changes were also checked.

Results: The levels of TC, TG and LDL-c were increased in model group. Compared to the model group, TFAK reduced significantly the body weight, TC, TG, LDL-c, AI, CRI and elevated the level of HDL-c. Moreover, the activity of SOD was elevated significantly, whereas the content of MDA decreased. The activity of HMG-CoA reductase was also decreased. Morphological evaluation found that rats in model group developed a severe steatosis, but the severity of liver steatosis was ameliorated in TFAK treated groups. The possible mechanism may be associated with decrease HMG-CoA reductase activity.

Conclusion: Our results suggest that TFAK exerts strong antioxidant and lipid-lowering effects, prevents hepatic fatty deposition and regulates the HMG-CoA reductase.

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Introduction

Hyperlipidemia is a serious epidemic disease involveing lipid metabolism disorder and is considered as the major risk factor associated with atherosclerosis and ischemic heart disease (1). Reducing cholesterol and lipid protein have been shown to be beneficial in patients for preventing stroke, cardiovascular disease, and acute cardiac events with statins, nicotinic acids and bile acid sequestrants. Although those drugs are effective in modulating hyperlipidemia in both preclinical and clinical studies, the toxicity of liver and kidney is not ignored. In recent years, chinese medical herbs and their extracts have received great attention for effective synergy and few side-effects (2, 3).

Many traditional Chinese medicines, including ginseng, coptis rhizome are used for prevention and treatment of hyperlipidemia (4). Several traditional food and condiments, such as wax gourd, bitter melon, ginger and celery have been proven to be beneficial for hyperlipidemia (5-7).

Hyperlipidemia and free fatty acids increase lipid-mediated oxidative stress (8). The oxidation of lipid, protein and other biological molecules by reactive oxygen species (ROS) can cause DNA mutation which resulting cell death (9). The biological effects of ROS are controlled by range of antioxidants enzymes, such as SOD, CAT and GSH-Px. Lots of plants have antioxidant effect, such as ginseng, grape and kiwifruit (10-12).

The kiwifruit is native to northern China, Korea, Siberia and Japan. The fruit of kiwifruit contains a number of nutritional compounds, such as vitamin C, vitamin E, folate, potassium, magnesium, ursolic acid, carotenoids and a range of polyphenols (13). Kiwifruit was reported have effect of antioxidant activity *in vitro* (14). Kiwifruit also protects against oxidative DNA damage or oxidative stress (15, 16). *Actinidia kolomikta* belongs to the family of kiwifruit. Recently published evidences have shown some beneficial effects of *Actinidia kolomikta*, which include antioxidant activity *in vitro* (17) and anticaner effects (16, 18). However, it remains unclear how total flavonoid extract from *A. kolomikta* (TFAK) exerts its hypolipidimic effects *in vivo*.

The present study was carried out to evaluate the hypolipedemic effect of TFAK on hyperlipidemia in

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rats. Results show that TFAK could prevent weight gain, reduced serum levels of lipids and lipoproteins, prevented hepatic fatty deposition, lowered the level of lipid oxidation product, increased the activity of antioxidant enzyme and decreased HMG-CoA reductase activity.

Materials and Methods

Chemical

Simvastatin, hydroxylamine hydrochloride, phosphatidate, trichloroacetic acid and dithiothreitol were purchased from Sigma (USA). Ethylene diamine-tetraacetic acid and ethylene glycol tetraacetic acid were obtained from Dingguo Changsheng Biotechnology (Changchun, China). TC, TG, LDL-c, HDL-c, SOD, MDA, CAT and GSH-Px kits were obtained from the Instituted of Jiancheng Biotechnology (Nanjing, China). All other chemicals were the analytical reagents.

Plant material

The leaves of *A. kolomikta* were collected at Jingyu County, Jilin Province of China. The specimens were identified by the professor Minglu Deng, who specializes in medicinal plants at Changchun University of Chinese Medicine. In brief, 3 kg of dried leaves were coarsely powered and was boiled in 30 l ethanol for 2 hr, and the extract was then filtered. The residue was extracted again and filtered. The combined filtrate was concentrated. Ethanol extract was 374 g. Ethanol extract was mixed with water. The concentration off the TFAK was approximately 0.2 g/ml. A high-perfomance liquid chromatographic method was used for the qualitative determination of the compounds in the extract (Figure 1).

Animals

All experiments were approved by the Laboratory Animals' Ethical Committee of Jilin University and followed national guidelines for the care and use of animals. Male SD rats (180-220 g) were obtained from the Experimental Animal Center of Jilin University. The rats were housed in an environmentally controlled room (22 ± 2 °C, humidity 40-65%, and a 12 hr light/dark cycle) and free access to rodent chow and water *ad libitum*. Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of Jilin University, and approved by the Ethics Committee.

Table 1. Composition (%) of the experimental diets

Ingredient	Basic diet	High-fat diet
Corn meal	30	26.3
Soybean meal	20	17.5
Wheat bran	25	21.9
Wheat flour	16	14
Fish meal	5	4.4
Bone meal	2	1.8
Yeast powder	1	0.9
NaCl	1	0.9
Cholesterol	0	2
Lard	0	10
Sodium cholate	0	0.3

Experimental design

Rats were fed a standard diet for 1 week before the experiment. Sixty rats were randomly divided into two groups: normal group (10 rats) fed with standard diet and hyperlipidemic diet group (50 rats) fed with high-fat diet for 2 weeks (19, 20) (Table 1). After the first 2 weeks fed with high-fat diet, the hyperlipidemic diet group 50 rats were took the blood sample from tail vein to measure the lipids and were randomly divided into 5 groups with 10 rats each group: model group (fed with high-fat diet), TFAK groups (fed with high-fat diet and TFAK 50, 100 and 200 mg/kg) and simvastatin group (fed with high-fat diet and

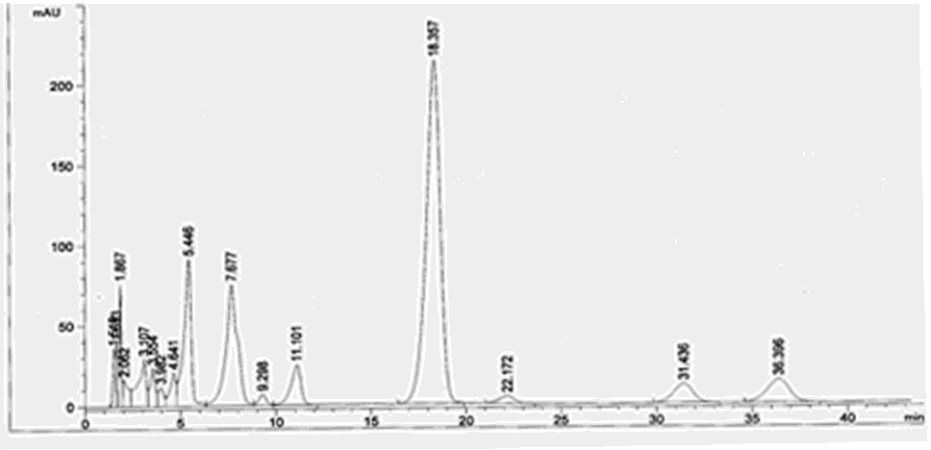

Figure 1. High-performance liquid chromatographic of total flavonoid extract from Actinidia kolomikta (TFAK)

simvastatin 30 mg/kg) as positive group. TFAK and simvastatin were administrated once a day by oral for 28 days. Normal and model groups were administrated with the same volume of distilled water. Body weight and food intake were recorded twice weekly. After 28 days, the rats were fasted for 12 hr and were anesthetized with chloral hydrate (350 mg/kg , IP).

Measurement of lipids in serum and hepatic tissue

After 28 days of treatment, rats were fasted for 12 hr. Blood samples were collected from the abdominal aorta. The samples were put at room temperature for to allow complete clotting, centrifuged to obtain the serum, and stored at -80 °C until analysis. The total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) were measured using commercial kits according to the manufactures' instructions on automatic biochemistry analyzer (Hitachi 7600, Japan). The atherogenic index (AI) and coronary risk index (CRI) were calculated as follows: AI=(TC-HDL-c)/HDL-c and CRI=TC/HDL-c, respectively (21). The lipids in hepatic was measured according to Folch's method (22).

Assay of antioxidant enzyme in serum

The activity of superoxide dismutase (SOD) and the content of malondialdehyde (MDA) were measured using commercial kits according to the manufactures' instructions.

Liver index

After the blood samples were taken, the livers were removed, rinsed with physiological saline solution and dried by tissue paper. The liver weights with respect to their body weight were recorded.

Hepatic lipid peroxidation and antioxidant profiles

After that a part of liver were weighted and homogenized in ice cold PBS. The homogenate was centrifuged and the supernatant was taken to measure the activity of SOD and the content of MDA by spectrophotometer according to the manufacturer's instruction.

Estimation of HMG-CoA reductase

Hepatic HMG-CoA reducatase activity was measured according to Visavadiya's method (23). Briefly, the liver tissue was removed and homogenated in saline arsenate solution. The homogenate was deproteinized and freshly prepared hydroxylamine reagent mixed with ferric chloride reagent was added. The absorbance was read after 10 min at 540 nm. The ratio of HMG-CoA/mevalonate was calculated (24). The ratio is inversely proportional to the enzyme activity, that is, the increase in ratio corresponds to a decrease in enzyme activity.

Histological analysis of the liver

At the end of the experiment, the rats were sacrificed. Livers tissue were removed quickly and fixed in the 4% paraformaldehyde solution for days and then embedded in paraffin. Five µm sections were cut, the liver tissue sections were stained with hematoxylin-eosin (H&E). The sections were examined under microscope and then photomicrographs were taken.

Statistical analysis

All data were reported as means ± SD. Statistical significance was determined by one-way analysis of variance (ANOVA), followed by Student's *t-test*. In all cases $P<0.05$ was considered statistically significant.

Results

Effect of TFAK on body weight

There was no difference in body weight among groups at the beginning of the experiment. But after 28 days treatment, the body weight in model group gained more than those in normal group ($P<0.05$). The TFAK treated groups (100 and 200 mg/kg) exhibited a remarkably reduced weight gain compared to model group ($P<0.05$). The lower dose of TFAK (50 mg/kg) also has some effect on body weight gain ($P>0.05$). Meanwhile, the food intake was no difference between the groups (Table 2). However, there was significant difference between groups in liver index. The TFAK treated groups (100 and 200 mg/kg) exhibited a remarkably reduced liver index compared to model group ($P<0.05$).

Table 2. Effect of total flavonoid extract from *Actinidia kolomikta* (TFAK) on body weight and food intake in hyperlipidemic rats (n=10)

Group	Final body weight(g)	Body weight gain(g)	Food intake (g/d)	Live index(g/kg)
Normal	400.5 ± 23.33	202.5 ± 27.45	23.05 ± 4.13	2.24 ± 0.49
Model	442.2 ± 33.51	243.2 ± 34.60	20.45 ± 3.67	3.77 ± 0.66
50 mg/kg	428.1 ± 26.15	229.1 ± 32.01	21.62 ± 4.54	3.58 ± 0.61
100mg/kg	412.2 ± 24.94	213.2 ± 31.98*	27.46 ± 4.63	2.94 ± 0.65*
200mg/kg	401.4 ± 21.34*	202.4 ± 25.59*	24.52 ± 4.17	2.8 ± 0.66*
Simvastatin	399.0 ± 17.98*	200.0 ± 19.20*	23.65 ± 5.12	2.69 ± 0.64*

All data are presented as the means ± SD. One-way ANOVA was performed. #$P<0.05$ compared with normal group. *$P<0.05$ compared with model group

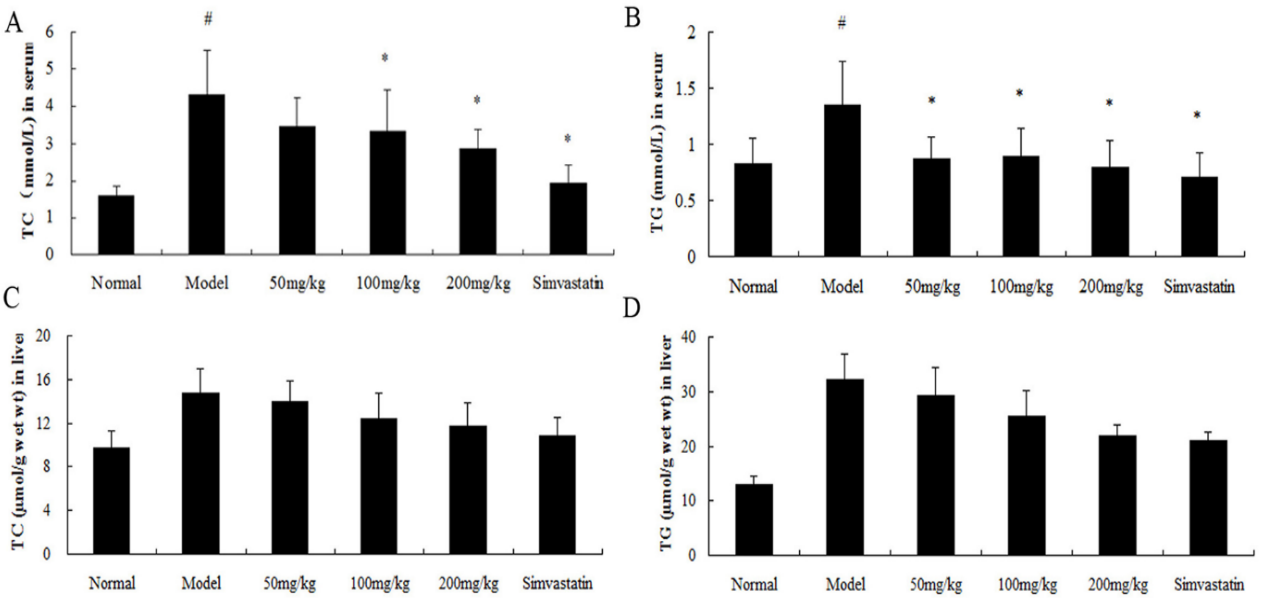

Figure 2. Effect of total flavonoid extract from *Actinidia kolomikta* (TFAK) on TC and TG in hyperlipidemic rats
(A) Level of TC in serum, (B) Level of TG in serum, (C) Level of TC in hepatic tissue and (D) Level of TG in hepatic tissue. Data are expressed as the means ± SD. #P<0.05 compared to the control group; *P<0.05 compared to the model group

Effect of TFAK on TC and TG levels

As shown in Figure 2, the levels of TC, TG were much higher in model group compared to normal group after 28 days treatment (P<0.05). The level of TC in rats fed with high-fat diet was no difference at the beginning of experiment. But at the end of experiment, the level of TC in serum treated with TFAK was significantly lower than those in model group (P<0.05). TC level dose-dependently was decreased by 19.96%, 23.03 % and 33.99% when treated with 50, 100 and 200 mg/kg, respectively (Figure 2A). These results indicated that TFAK could reduce the TC level in hyperlipidemic rats. A substantial reduction in TG level by 35.55%, 34.07% and 41.48% were observed in hyperlipidimic rats after treated with 50, 100 and 200 mg/kg, respectively (Figure 2B). The same tendency was also found in hepatic tissue (Figure 2C and 2D). The above results demonstrated that high dose of TFAK has more capacity to lower the levels of TC and TG in serum.

Effect of TFAK on serum lipoprotein profiles

Treatment of hyperlipidemic rats with TFAK increased serum levels of HDL-c compared to the model group (Figure 3A). After treatment for 28 days with 50, 100 and 200 mg/kg of TFAK, serum level of HDL-c increased by 2.81%, 8.17% and 15.41%, respectively. In contrast to HDL-c, the level of LDL-c was decreased by 21.02%, 29.23% and 49.41%, respectively (Figure 3B). The net benefit was evident when HDL-c was expressed as a percentage of all cholesterol or the atherosclerotic index (AI). As shown in Figure 3C and 3D, the CRI and AI were higher in model group compared to the normal group (P<0.05). After treatment for 28 days with TFAK, the AI and CRI were reduced. Those results indicated that TFAK could decrease the risk of coronary disease and atherosclerosis by raising the level of HDL-c.

Effect of TFAK on oxidative stress

As shown in Figure 4, hyperlipidemia induced significantly decrease the activity of SOD in serum and in hepatic tissue, decrease the activity of CAT and GSH-Px in serum, whereas the level of lipid peroxidation product MDA was increased compared to normal group (P<0.05). After treatment with TFAK for 28 days, the activity of SOD was increased by 6.25%,15.20% and 16.50% in serum, and 23.86%, 54.59% and 59.43% in hepatic tissue, respectively, compared to model group (P<0.05). The activity of CAT and GSH-Px were increased by 6.70%, 19.93%, 26.75%, and 7.50%, 23.484% and 32.50% in serum, respectively, compared to the model group (P<0.05). Consistent with the higher level of MDA in model group, the level of MDA was decreased in TFAK treated group (P<0.05). Those data indicated that TFAK could ameliorate the oxidative stress in hyperlipidemic rats.

Effect of TFAK on hepatic HMG-CoA reductase activity

As shown in Figure 5, the activity of HMG-CoA reductatse was increased in model group compared to normal group (P<0.05). After treatment with TFAK for 28 days, the activity of HMG-CoA reductase was decreased by 3.42%, 16.67% and 27.96%, respectively (P<0.05).

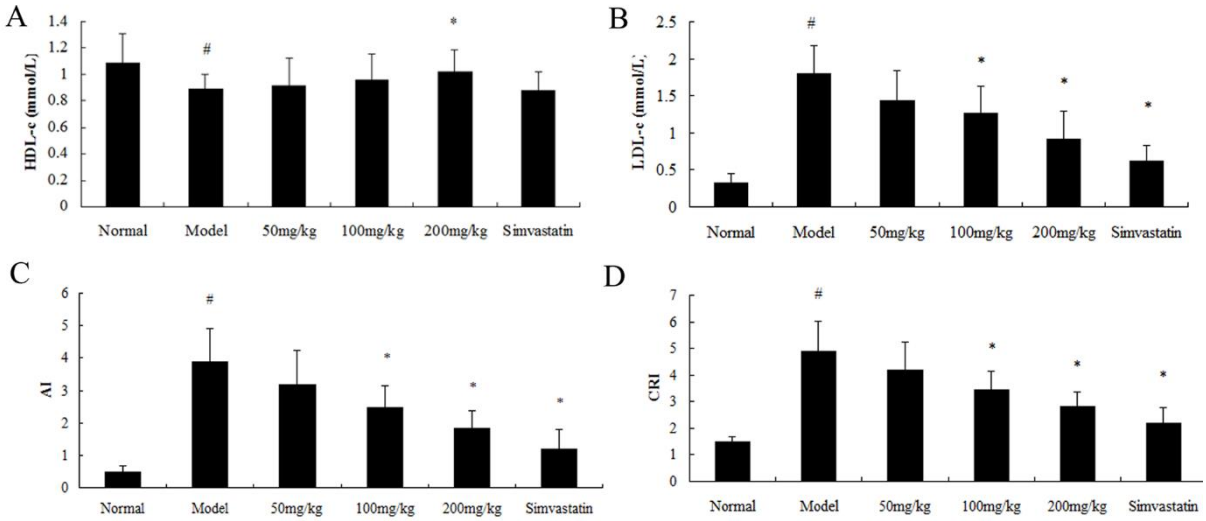

Figure 3. Effect of total flavonoid extract from *Actinidia kolomikta* (TFAK) on HDL-c, LDL-c, CRI and AI in hyperlipidemic rats
Level of HDL-c in serum, (B) Level of LDL-c in serum, (C) CRI and (D) AI. Data are expressed as the means± SD. #P<0.05 compared to the control group; *P<0.05 compared to the model group

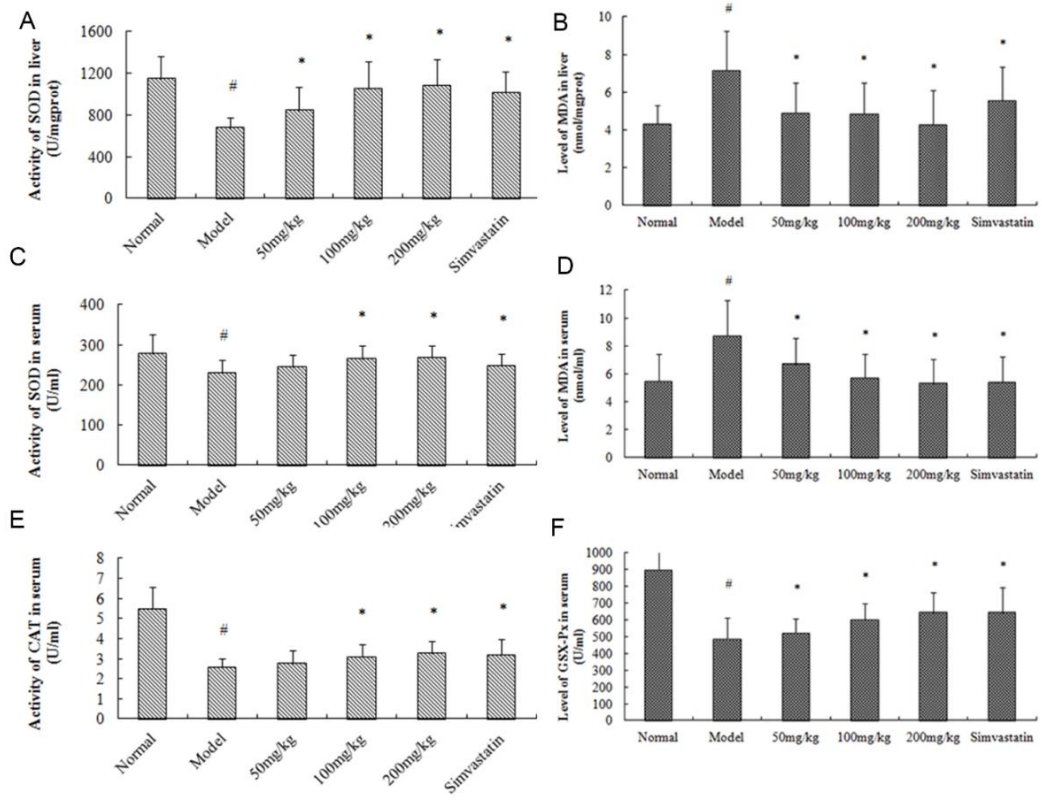

Figure 4. Effect of total flavonoid extract from *Actinidia kolomikta* (TFAK) on oxidative stress in hyperlipidemic rats
(A) Activity of SOD in serum, (B) Level of MDA in serum, (C) Activity of SOD in hepatic tissue, (D) Level of MDA in hepatic tissue, (E) Activity of CAT in serum and (F) Activity of GSH-Px in serum . Data are expressed as the means ± SD. #P<0.05 compared to the control group; *P<0.05 compared to the model group

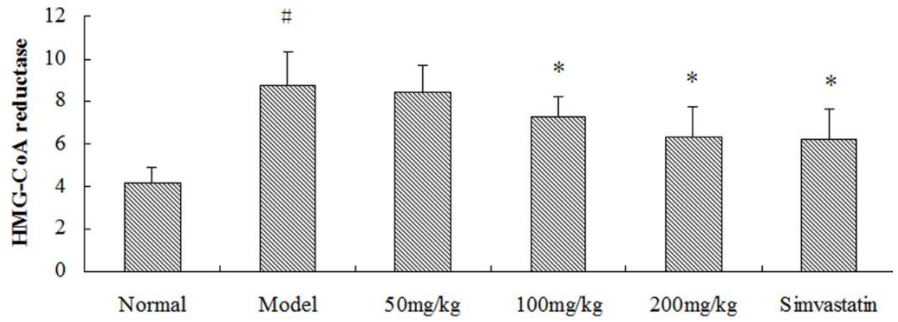

Figure 5. Effect of TFAK on HMG-CoA reductase activity in hyperlipidemic rats
Compared to the model group, the HMG-CoA reductase activity in the TFAK groups and simvastatin group was markedly decreased. Data are expressed as the means± SD. #P<0.05 compared to the control group; *P<0.05 compared to the model group

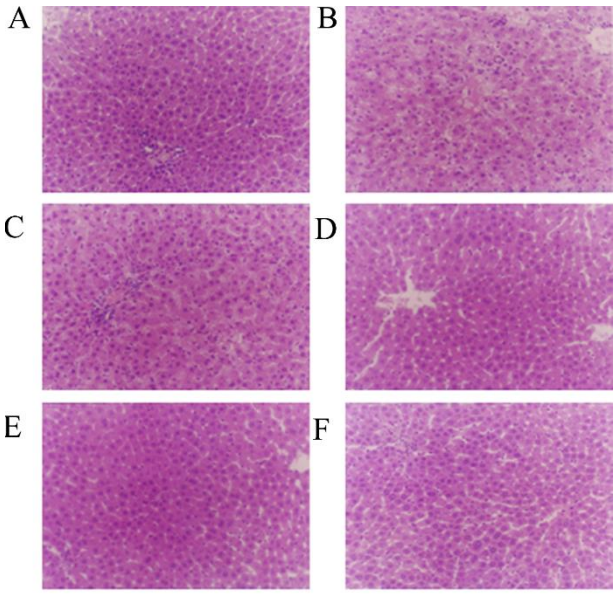

Figure 6. Histological image of rat liver tissues
Hyperlipidemic diet rats were treated with total flavonoid extract from *Actinidia kolomikta* (TFAK) (50, 100, and 200 mg/kg) for 28 days. The liver tissues were removed, subjected to histological examination by stained with H&E.
(A) Normal group, (B) Model group, (C) TFAK 50 mg/kg, (D) TFAK 100 mg/kg, (E) TFAK 200 mg/kg and (F) Simvastatin 30 mg/kg. The magnification in all panels is 200×

Effect of TFAK on fatty steatosis

The hepatocytes swelled and the volume increased significantly, and the cytoplasm was filled with a lot of fat vacuoles. The cell boundary was not clear. The hepatocytes showed severe steatosis in the model group (Figure 6B). Whereas the hepatocytes showed no steatosis in normal group (Figure 6A). Administration of TFAK could alleviate the fatty deposition in hepatocytes (Figure 6C-E).

Discussion

In the present study, we show that the total flavonoide extracted from the leaves of *A. kolomikta* (TFAK) has the property of decreasing the lipid profile. TFAK could reduce weight gain and lower the serum levels of TC, TG, LDL-c and ameliorated the fatty deposition in liver cells and, thus, has the potential to prevent onset of pathologies associated with hyperlipidimia. Furthermore, our results demonstrated that TFAK reduced oxidative stress in blood and in tissue.

Hyperlipidemia, especially high levels of LDL-c and TG, is considered to be one of the major risk factors for cardiovascular disease, such as atherosclerosis, myocardial infarction, heart attacks, stoke (25-27). Many recent studies have demonstrated that some chinese traditional herbs or fruits could lower the levels of serum triacylglycerol and total cholesterol (28, 29). Our results were consistent with previous studies, treatment with TFAK for 28 days was sufficient to decrease the levels of TC, TG, LDL-c and increase the level of HDL-c in hyperlipidemic rats. Those results strongly suggested that TFAK exerted a hypolipidemic effect and might have a protective effect against the atherosis. This effect is similar to the positive drug simvastatin.

Cholesterol in the serum is mainly produced by biological synthesis. The key enzyme for cholesterol biosynthesis is the HMG-CoA. If the enzyme is inhibited, the level of cholesterol in serum will lower. HMG-CoA is the target for statin drugs which is used widely in clinic for decreasing cholesterol. Our results showed that treatment with TFAK caused the reduction in hepatic HMG-CoA activity in a dose-dependent manner, suggesting that inhibition the activity of HMG-CoA was partially associated with the hypocholesterol effect. Reactive oxygen species (ROS), include free radicals, are normal generate in all aerobes. ROS elicit diverse biological effects depending on its concentration in cells. Oxidative stress is caused when the balance between the production of ROS and the ability of those antioxidant enzymes was broken (30). Oxidative stress can cause or aggravate several diseases such as atherosclerosis and coronary heart disease (31, 32). Antioxidant enzyme SOD plays a pivotal role in oxygen defense metabolism, following treatment of the TFAK

for 28 days, the activity of SOD was augmented in hyperlipidimic rats compared to model rats. MDA, the index of lipid peroxidation, was significantly increased in model group. TFAK treatment can abrogate MDA level, suggesting that TFAK might have antioxidant principles (33). In conclusion, this study has shown that the role the TFAK is capable of ameliorating the hyperlipidmia induced by a high-fat diet. The possible mechanism may be associated with decrease HMG-CoA reductase activity. Those results indicate that TFAK may be a potential drug for hyperlipidemia.

Conclusion

Our results suggest that TFAK exerts strong antioxidant and lipid-lowering effects, prevents hepatic fatty deposition and regulates the HMG-CoA reductase.

Acknowledgment

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Conflict of interest

We declare that we have no conflict interest.

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