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ORIGINAL ARTICLE

Comparation of Hypolipidemic and Antioxidant Effects of Aqueous and Ethanol Extracts of *Crataegus pinnatifida* Fruit in High-Fat Emulsion-Induced Hyperlipidemia Rats

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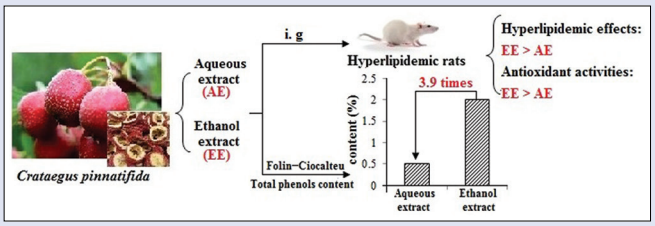
ABSTRACT

Background: Hawthorn (*Crataegus pinnatifida*) is a Chinese medicinal plant traditionally used in the treatment of hyperlipidemia. Recently, studies indicated free radical scavenging was one of the major pathways to alleviate hyperlipidemia. Moreover, hawthorn fruit is a rich source of phenols, which quench free radical and attenuate hyperlipidemia. However, the phenols vary with processing methods, especially solvent type. **Objective:** Our aim was to compare hypolipidemic and antioxidant effects of aqueous and ethanol extracts of hawthorn fruit in hyperlipidemia rats. **Materials and Methods:** After a 4-week treatment of high-fat emulsion, lipid profile levels and antioxidant levels of two extracts were determined using commercial analysis. Total phenols content in the extract of hawthorn fruit was determined colorimetrically by the Folin–Ciocalteu method. **Results:** Both ethanol and aqueous extracts of hawthorn fruit possessed hypolipidemic and antioxidant activities. Simultaneously, stronger activities were observed in ethanol extract. Besides, total phenols content in ethanol extract from the same quality of hawthorn fruit was 3.9 times more than that in aqueous extract. **Conclusion:** The obvious difference of hypolipidemic and antioxidant effects between ethanol extract and aqueous extract of hawthorn fruit was probably due to the presence of total phenols content, under the influence of extraction solvent.

Key words: Antioxidant activity, *Crataegus pinnatifida* fruit, hypolipidemic effect, total phenols content

SUMMARY

- Ethanol extract of hawthorn fruit exhibited more favorable hypolipidemic and antioxidant effects than aqueous extract. The higher effects could be due to the higher content of total phenols that varies with extraction solvent.



Abbreviations used: TC: Total cholesterol, TG: Triglyceride, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, GSH-Px: Glutathione peroxidase, SOD: Superoxide dismutase, MDA: Malondialdehyde, CAT: Catalase, NO: Nitric oxide, NOS: Nitric oxide synthase, SR-BI: Scavenger receptor Class B Type I.

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INTRODUCTION

Hyperlipidemia is predominant of cardiovascular diseases, which greatly rises morbid and mortality toll in the world.[1] It mainly reflects in dyslipidemia. Dyslipidemia, resulted from the modifications of serum/plasma lipid profile, is characterized by an elevation of serum total cholesterol, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) concentrations, and a marked decrease in high-density lipoprotein cholesterol (HDL-C) concentration.[2] Accumulating evidence showed that the initiation and progression of cardiovascular dysfunction including hyperlipidemia, hypercholesterolemia, and hypertension are closely related to oxidative stress.[3,4] A large amount of superoxide can be produced by various cells that implicated in the inflammatory responses to hypercholesterolemia.[3] As first-line drugs used for the treatment of hyperlipidemia, statins could reduce antioxidant vitamins, which are involved in the protection of LDL-C against oxidation.[5] In China, herbal medicines have been attracted a particular attention in hypolipidemic and antioxidant effects.[6,7]

Hawthorn (*Crataegus pinnatifida*) is a medicinal plant widely distributed in China. Its decoction has been used as antihyperlipidemics in the traditional Chinese medicine clinic for more than 400 years. Hawthorn fruit possesses hypolipidemic,[6] antioxidant,[8] ameliorate atherosclerosis,[9] antithrombotic,[10] and anti-inflammatory[11] activities. Especially to deserve to be mentioned, its extract is capable of quenching free radicals and inhibiting LDL oxidation.[9,12] At present, 82 compounds in over 150 compounds of hawthorn have been isolated and identified possessing phenolic structure,[13] such as epicatechin, hyperoside, and chlorogenic acid.[14] Phenols are generally acknowledged to obviously decrease serum total cholesterol (TC) and TG concentrations[15,16] and scavenge free radicals.[17] Therefore, phenols content in hawthorn fruit extract is an important, influential factor of hypolipidemic and antioxidant effects in high-fat emulsion-induced hyperlipidemia rats.

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Although aqueous and ethanol extracts of hawthorn fruit both have favorable therapeutic effects to hyperlipidemia and oxidative damage,[9,12,18] and knowledge about the comparison of hypolipidemic and antioxidant effects between them is scarce. In this study, our aim is to compare hypolipidemic and antioxidant effects of aqueous and ethanol extracts of hawthorn fruit in rats fed a high-fat emulsion diet. Simultaneously, it is necessary to investigate the difference of total phenols content between two extracts of hawthorn fruit.

MATERIALS AND METHODS

Materials and chemicals

Hawthorn (*C. pinnatifida* Bge. var. major N. E. Br.) fruits were collected from Pingyi County of Shandong Province, China, and identified by professor Kezhong Deng, School of Pharmacy, Jiangxi University of Traditional Chinese Medicine. A voucher specimen (No. 20121123) has been deposited in the Key Laboratory of Modern Preparation of Traditional Chinese Medicine, Ministry of Education, Jiangxi University of Traditional Chinese Medicine, China. Glutathione peroxidase (GSH-Px) assay kit (Lot No. 20130831), superoxide dismutase (SOD) kit (Lot No. 20130831), malondialdehyde (MDA) assay kit (Lot No. 20130831), catalase (CAT) assay kit (Lot No. 20130903), nitric oxide (NO) assay kit (Lot No. 20130827), and NO synthase (NOS) assay kit (Lot No. 20130903) were obtained from Nanjing Jiancheng Bioengineering company, China. TC assay kit (Lot No. ZG3001), TG assay kit (Lot No. ZG3001), LDL-C assay kit (Lot No. ZG9003), and HDL-C assay kit (Lot No. ZG3001) were offered by Sysmex Co., Japan. No. 3 bile salt (Lot No. 20130531-00) was purchased from Hangzhou Hongbo Biological Engineering Co., Ltd., China. Propylthiouracil (Lot No. 20130523) and cholesterol were obtained from Wuhan Sheng Tianyu Biological Technology Co., Ltd., China. Lard was purchased from Henan Zhumadian Dingsheng Food Co., Ltd., China. Distilled water was provided by the laboratory. Other chemicals were all analytical grade.

Preparation of extracts

A sample of hawthorn fruit (500 g) was extracted by distilled water under reflux successively (each 2 h, 1.5 L × 4 times) and filtered. The combined extracts were then rotary evaporated at 45°C and lyophilized. Aqueous extract (115 g) was stored at 4°C until the time of use. According to the above extraction process, 70% ethanol was used as extraction solvent. Ethanol extract (130 g) was stored at 4°C until the time of use.

Preparation of high-fat emulsion

High-fat emulsion diet was prepared as previously reported method[19] with some modifications. A volume of 10 g cholesterol and 1 g propylthiouracil were added to 25 g melted lard oil in a 100 ml beaker and fully mixed. Then, 25 ml Tween-80 was put into the mixture to make the oil phase. Meanwhile, the water phase was prepared by adding 30 ml distilled water and 20 ml propylene glycol to another 100 ml beaker and heated in an electric oven to 60°C, followed by the addition of 2 g No. 3 bile salt. Finally, the oil phase and water phase were mixed completely to prepare the high-fat emulsion. When using emulsion, added 50 ml distilled water and heated in water bath at 45°C.

Animals

A total of 72 male SD rats (230 ± 20 g, age 7–8 weeks) were supplied by Hunan Lake King of Laboratory Animal Co., Ltd. (Hunan, China). Rats were kept at room temperature (22–25°C, 55% ± 10% humidity, and 12/12 h light/darkness cycle) with commercial rat normal standard chow (Hunan SJA Laboratory Animal Co., Ltd., Hunan, China) and water *ad libitum*. After allowing 7 days for adaptation, all rats were assigned randomly into eight groups (n = 8). Group 1 rats (control) were intragastrically administered with 10 ml/kg body weight of distilled water twice a day. The other groups (Groups 2–8) rats were intragastrically administered with 10 ml/kg body weight of the high-fat emulsion once a day. After 6 h, Group 2 rats (model) were intragastrically given 10 ml/kg body weight of distilled water. Groups 3–5 rats were intragastrically administered with aqueous extract at low-, medium-, and high-dose (equivalent to about 0.6, 1.2, and 2.4 g/kg/day crude drug). Groups 6–8 rats were intragastrically administered with ethanol extract at low-, medium-, and high-dose (equivalent to about 0.6, 1.2, and 2.4 g/kg/day crude drug). After 28 days of administration by gastric gavage, the rats were fasted for 12 h and euthanized by decapitation. Blood was collected, left at room temperature for 15 min and then centrifuged at 3000 rpm (4°C, 10 min). The serum obtained was stored at −80°C until biochemical analysis. Livers were dissected, washed with saline, weighed, and homogenized (weighed 0.5 g, added 4.5 ml normal saline). The samples were centrifuged at 3500 rpm (4°C, 10 min). The supernatants were obtained and stored at −80°C immediately until enzyme activities analysis.

Plasma lipids and antioxidant enzyme activities

The plasma lipids levels (TC, TG, LDL-C, and HDL-C) were determined using commercial analysis kits from Sysmex Co., Japan. The antioxidant enzyme activities (SOD, CAT, and GSH-Px) and the levels of MDA, NO, and NOS in serum and liver were determined using commercial analysis kits from Nanjing Jiancheng Bioengineering Company, China.

Measurement of liver index

The liver index of rats was measured by the Zou's methods.[20] The liver index was gained via the following calculation:

Liver index = (wet weight of liver/body weight) × 100%

Measurement of total phenols content

Total phenols content of hawthorn fruit extract was determined colorimetrically by the Folin–Ciocalteu method with some modifications.[21] Gallic acid was used as the standard. The absorbance of samples was read at 760 nm, and the results were expressed as mg of gallic acid equivalents per 0.26 g ethanol extract or 0.23 g aqueous extract (equivalent to about 1 g crude drug) from a calibration curve of gallic acid (3.24–11.34 μg/ml). The equation of the calibration curve for gallic acid was

$Y = 61.675X - 0.0048$ ($R^2 = 0.9997$).

Statistical analysis

Data were presented as mean ± standard deviation. After validation of each parameter collected for homogeneity of variance, the statistical analysis was performed using one-way analysis of variance (ANOVA) with the SPSS software (version 18 for Windows, Chicago, IL, USA). Differences are considered to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

Comparing hypolipidemic effects of two extracts of hawthorn fruit

After 4 weeks of treatment, the TC, TG, and LDL-C levels in serum were markedly increased ($P < 0.01$) in rats fed a high-fat emulsion diet, but there is no significant effect on the level of HDL-C ($P > 0.05$), as shown in Figure 1. The elevation in serum TC, TG, and LDL-C are significant enough to indicate that the hyperlipemia model was successfully established since they play a significant role in atherosclerosis development and subsequent coronary heart disease.[22] In addition, the result of HDL-C may be associated with the compensatory mechanism of

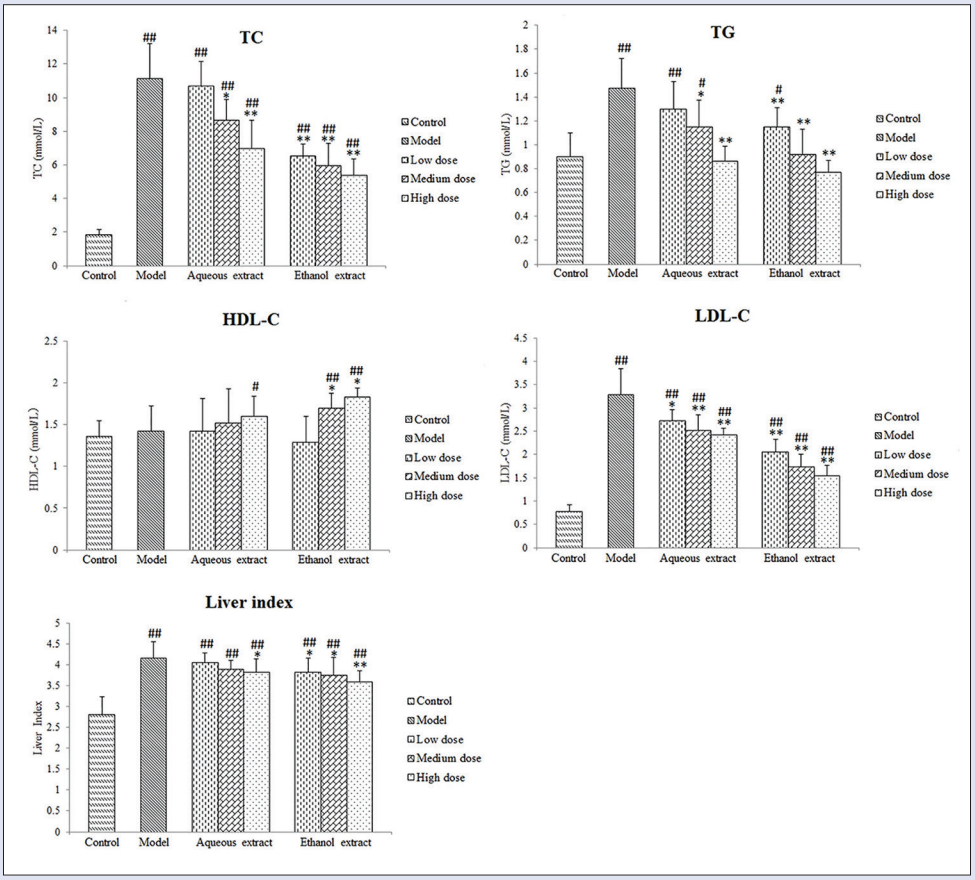

Figure 1: Effects of different groups on total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and liver index in rats fed on high-fat emulsion or normal diet. The low-, medium-, and high-doses were equivalent to about 0.6, 1.2, and 2.4 g/kg/day crude drug, respectively. Data are expressed as mean ± standard deviation, $n = 8$ replicates. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ represent significant difference when compared with the control group. $^{*}P < 0.05$ and $^{**}P < 0.01$ represent significant difference when compared with the model group

rats own to our knowledge. Rat is a kind of animal, which has the lower transport activity of plasma cholesterol ester transfer protein. Its HDL-C was directly metabolized primarily through scavenger receptor Class B Type I (SR-BI).[23] After rats fed a high-fat emulsion, SR-BI metabolizes HDL-C carrying exogenous cholesterol by the liver. Therefore, the HDL-C level in serum had no significant change.

In this study, both hawthorn extracts to the hyperlipidemia rats exhibited a dose-dependent manner in reducing TC, TG, and LDL-C levels and enhancing HDL-C level in serum. It is noteworthy that compared with aqueous extract at high-dose, ethanol extract at low-dose decreased more obviously in TC and LDL-C levels. The level of TG at low-dose of ethanol extract was approximately equivalent to that at high-dose of aqueous extract. Serum lipid profiles can be attenuated by ethanol extract of hawthorn fruit, which is abundant in phenols.[24] Phenols were considered to protect LDL-C from Cu^{2+} mediated LDL oxidation,[25] thus prevent endothelial dysfunction and atherosclerosis.[26] Figure 1 also shows compared with the model, ethanol extract at medium- and high-doses significantly elevated the level of HDL-C ($P < 0.05$). However, no significant difference in HDL-C level was observed between aqueous extract group and the model group ($P > 0.05$). Therefore, ethanol extract showed more significant ameliorative action than that of aqueous extract in the plasma lipids levels of rats fed a high-fat emulsion.

In addition, compared with the control group, the liver index in the model group significantly increased ($P < 0.01$). In contrast with the model, ethanol extract at three different doses and aqueous extract at high-dose to the hyperlipidemia rats caused a significant decrease of the liver index ($P < 0.05$). The liver index at low-dose of ethanol extract was approximately equivalent to that at medium-dose of aqueous extract in hyperlipidemia rats, as shown in Figure 1. Therefore, the protective effect of ethanol extract on livers of hyperlipidemia rats was more significant than that of aqueous extract.

Comparing antioxidant effects of two extracts of hawthorn fruit in serum and liver

Hyperlipidemia results in unbalance between oxidation and anti-oxidation and produces a large number of oxygen free radicals *in vivo*. Further, oxygen free radicals translate to MDA. Oxygen free radicals and MDA result in the injury of vascular endothelial cell and promote the formation and development of atherosclerosis.[27] The activities of GSH-Px, SOD, and CAT directly reflect the ability to scavenging oxygen free radicals.[28] The extract of hawthorn fruit presents antioxidant activity by increasing activities of GSH-Px, SOD, and CAT and decreasing level of MDA *in vivo*.[29] In this study, the lowered GSH-Px, CAT, and SOD activities ($P < 0.05$), and the elevated MDA level ($P < 0.01$) was recorded in the model group in serum and liver, respectively. Ethanol extract at three different doses and aqueous extract at high-dose remarkably lowered the elevated MDA level ($P < 0.05$) and increased the lowered GSH-Px and SOD activities ($P < 0.05$) in serum and liver of hyperlipidemia rats, the lowered CAT activity ($P < 0.05$) in

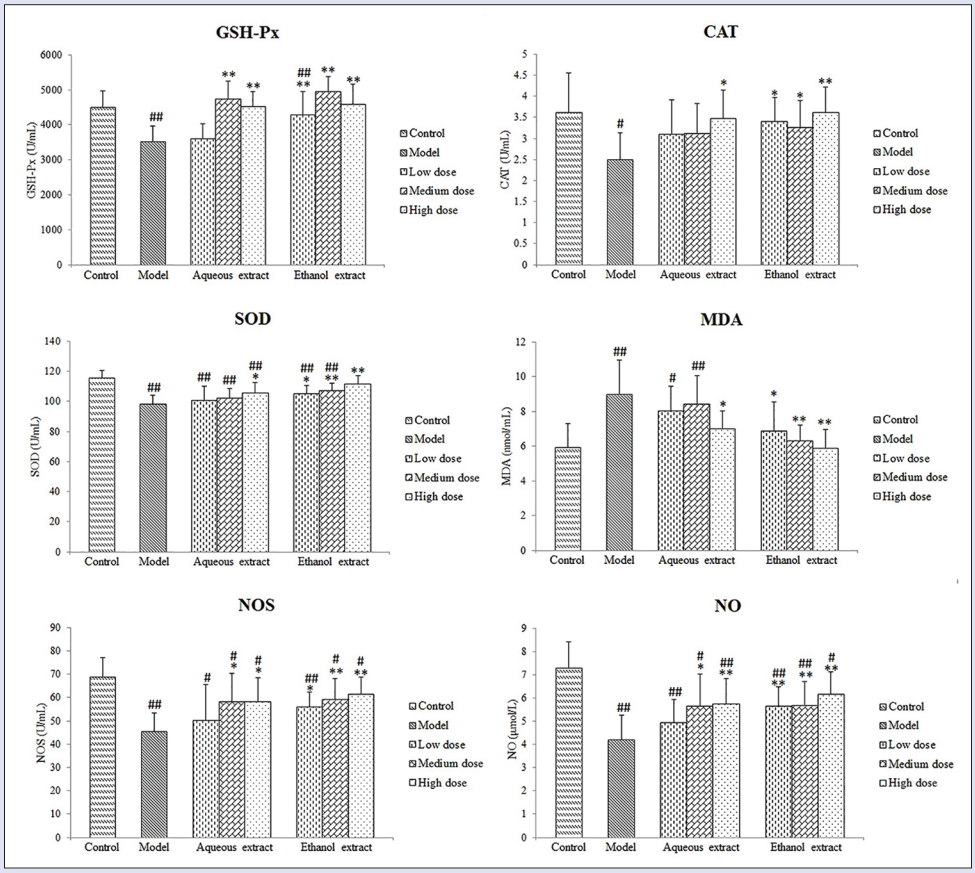

Figure 2: Effects of different groups on malondialdehyde, nitric oxide content and the activities of glutathione peroxidase, catalase, superoxide dismutase, and nitric oxide synthase of serum in rats fed on high-fat emulsion or normal diet. The low-, medium-, and high-doses were equivalent to about 0.6, 1.2, and 2.4 g/kg/day crude drug, respectively. Data are expressed as mean ± standard deviation, $n = 8$ replicates. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ represent significant difference when compared with the control group. $^{*}P < 0.05$ and $^{**}P < 0.01$ represent significant difference when compared with the model group

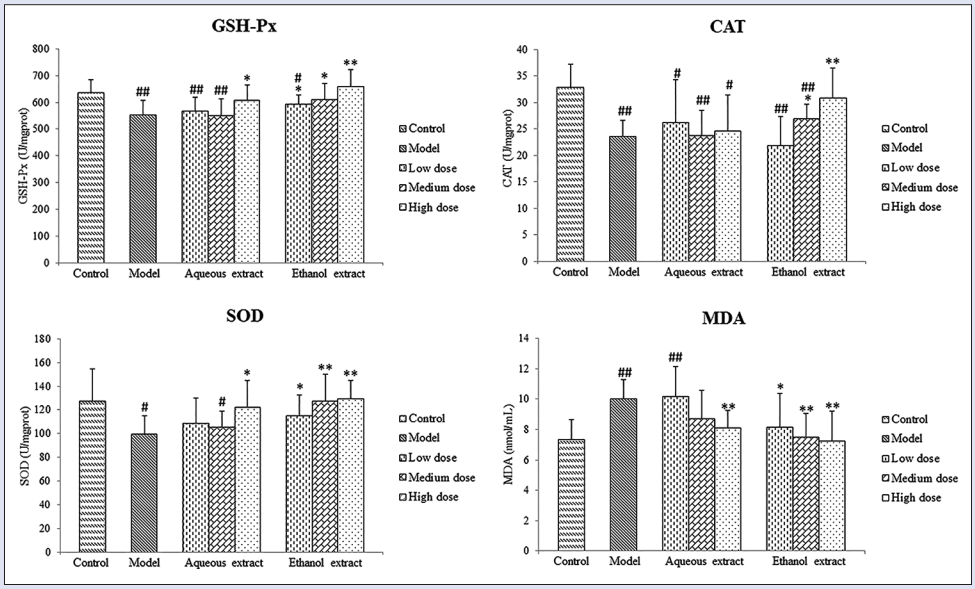

Figure 3: Effects of different groups on malondialdehyde content and the activities of glutathione peroxidase, catalase, and superoxide dismutase of the liver in rats fed on high-fat emulsion or normal diet. The low-, medium-, and high-doses were equivalent to about 0.6, 1.2, and 2.4 g/kg/day crude drug, respectively. Data are expressed as mean ± standard deviation, $n = 8$ replicates. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ represent significant difference when compared with the control group. $^{*}P < 0.05$ and $^{**}P < 0.01$ represent significant difference when compared with the model group

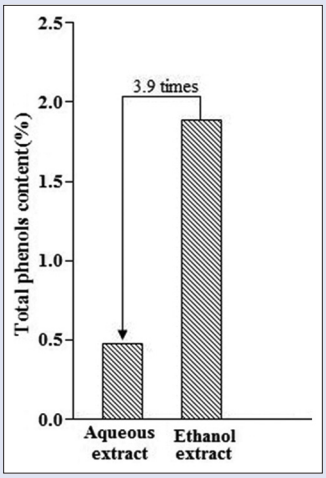

Figure 4: Comparation of total phenols content in two extracts from the same quality of hawthorn fruit

the serum of hyperlipidemia rats. Aqueous extract at three different doses did not significantly increase CAT, while ethanol extract at medium- and high-dose significantly increased the lowered CAT activity ($P < 0.05$) in the liver of hyperlipidemia rats as shown in Figures 2 and 3.

NO is the principal factor that inhibits vessels platelet aggregation and dilate vessels, so as to prevent vascular atherosis and thrombus formation.[30,31] When a vascular endothelial function in hyperlipidemia rats is injured, the content of NO, which is released from the endothelial cell, decreased. Simultaneously, the content of NO is affected by the activity of NOS in the cell.[32] In this study, ethanol extract at three different doses and aqueous extract at medium- and high-doses remarkably increased the lowered NOS activities and NO levels in serum of hyperlipidemia rats ($P < 0.05$) as shown in Figure 2. These data indicated that ethanol extract exhibited more significant antioxidant activities than aqueous extract in hyperlipidemia rats.

Comparing total phenols content in two extracts of hawthorn fruit

Phenols are recognized as hypolipidemic and antioxidant effect compounds of hawthorn fruits. As shown in Figure 4, ethanol extract and aqueous extract exhibited total phenols content at 1.99% and 0.51% in the same quality of hawthorn fruit, respectively. Total phenols content in ethanol extract was 3.9 times more than that in aqueous extract. These results suggested that total phenols content in different extract of hawthorn fruit was probably a key factor that resulted in the difference of hypolipidemic and antioxidant effects in hyperlipidemia rats.

CONCLUSION

By the whole, compared with aqueous extract, hypolipidemic and antioxidant effects of ethanol extract were more efficient in hyperlipidemia rats. Then, as hypolipidemic and antioxidant activities compounds of hawthorn fruits, total phenols content in ethanol extract was remarkably higher than that in aqueous extract through spectrum analysis. Accordingly, we suggested that extraction solvent of hawthorn fruits is of great importance to the research of combating hyperlipidemia drugs.

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Conflicts of interest

There are no conflicts of interest.

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