

Original Article

Effects of total flavonoids extracted from *Polygonum perfoliatum* L. on hypolipidemic and antioxidant in hyperlipidemia rats induced by high-fat diet

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Abstract: The tubers of *Polygonum perfoliatum* L. was usually used as a folk herbal medicine due to its special biological activity. This research aimed to evaluate the bioactive composition and antioxidant and hypolipidemic effects of total flavonoids extracted from *Polygonum perfoliatum* L. (TFP) on high fat diet induced hyperlipidemic rats. HPLC analysis results showed the main flavonoids composition of TFP were quercetin and rutin. Investigation was performed by administering different oral doses (60, 120 and 240 mg/kg BW/day) of the TFP for four weeks. The levels of serum triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), as well as hepatic antioxidant enzyme activities and melondialdehyde (MDA) level were determined. Experiments showed that the levels of serum lipid, antioxidant enzyme activities and MDA in liver tissue of hyperlipidemia rats had obviously restored by the treatment with the different doses of TFP. These results suggest that TFP from *Polygonum perfoliatum* L. had demonstrated antioxidant and hypolipidemic effects *in vivo*, which could become a potential herbal medicine to prevent hyperlipidemia diseases.

Keywords: *Polygonum perfoliatum* L, hyperlipidemia rats, flavonoids, antioxidant effects, hypolipidemic effects

Introduction

The hyperlipidaemia is a lipid disorder disease, characterized by increased low density lipoprotein (LDL-C), serum total cholesterol (TC) and declined high density lipoprotein (HDL-C) levels [1], which is the main risk factors for the development of cardiovascular disease. Modern pharmacology and clinical research has showed that the large amounts of reactive oxygen species (ROS) declined the intracellular antioxidant defense system, causing protein oxidant, lipid peroxidation [2]. In recent years, some traditional medical plants have been reported to be used in hyperlipidaemia. And the antihyperlipidemic effect of the medical plants is mainly due to their ability to decline the cholesterol biosynthesis and increase the cholesterol excretion. However, investigating new antihyperlipidemic drugs from medical plants is still attractive because they have ingredients that may possess safe effects.

Polygonum perfoliatum L. is a well-known medicinal plant and commonly known as Gangbangui, which is widely distributed throughout the south of China, particularly in Guizhou province [3]. As a folk medicine, it is traditionally used for the treatments on fever, cough and bleb [4]. In recently years, modern pharmacology studies have demonstrated that *Polygonum perfoliatum* L. exhibits a great diversity of pharmacological activities, such as anti-tumor [5], antiviral [6], anti-inflammatory [7], and antifungal activity [8]. *Polygonum perfoliatum* L. is rich in flavonoids, including quercetin, rutin and quercetin-3-O-beta-D-glucuronide, had been isolated and identified from the 95% ethanol extracts of *Polygonum perfoliatum* L. tubers [9]. Rutin and quercetin have been demonstrated to be effective for treatment of hyperglycemia and dyslipidemia diseases, through attenuating the deficit of SOD and clearing the ROS [10, 11]. However, there is no research assessing antioxidant and hypolipidemic effects.

Table 1. Compositions of the experiment diets (%)

Ingredient	High-fat diet	Basic diet
Soybean meal	15.5	20
Corn meal	25	30
Wheat flour	15	15
Wheat bran	23	26
Fish meal	4	5
Bone meal	2	2
Cholesterol	3	0
NaCl	1	1
Sodium cholate	0.5	0
Lard	10	0
Yeast powder	1	1

cts of total flavonoids extracted from *Polygonum perfoliatum* L. Hence, the current work focused on the hypolipidemic and antioxidant effects of TFP on high fat diet (HFD) induced hyperlipidemia rats through detecting lipid peroxidation, lipid profile and antioxidant enzyme activities. In addition, the flavonoids composition of the *Polygonum perfoliatum* L. ethanol extracts were characterized using HPLC-DAD modes. This work aimed to further characterize the possible bioactive composition that responsible for its hypolipidemic and antioxidant effects.

Materials and methods

Chemicals and plant materials

Tubers of *Polygonum perfoliatum* L. were collected from Hunan province. Plant was authenticated and identified as *Polygonum perfoliatum* L. The assay kits for total protein, triglycerides, total cholesterol, HDL-C, LDL-C, ApoA and ApoB were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and the ELISA kits were used for determining antioxidant indicators (SOD, CAT, GSH-Px and MDA) was the same supplier. Xue Zhi Kang capsule was purchased from Luye Pharma (Beijing, China), it was acted as the positive control and the content of monascus was over 900 mg/g. Rutin and quercetin were purchased from Sigma Chemical Company. All other chemical reagents were of analytical grade that purchased from Sinopharm Chemical Reagent Co., Ltd.

Preparation of total flavonoids extracts

The dried samples of *Polygonum perfoliatum* L. were ground into powder and sieved through a

20-mesh sieve. The extraction process of TFP was referenced to the previous publications [7]. Briefly, 20 L of 80% ethanol was added to 1 kg dried powders. Then, the mixture was extracted twice in a hot water bath at 80°C for 60 min. The extract was then filtered and defatted with n-hexane. Afterwards, the defatted filtrate was added into the AB-8 macroreticular resin column for adsorption for 16 hours, and then in order to get rid of impurities, the column was washed with water and 20% ethanol, respectively. TFP was refined through eluting with 70% ethanol, the eluate was collected and condensed in a vacuum condition, then for further dried using a freeze-dryer [12]. Finally, the flavonoid content of extract was measured using rutin as reference by UV spectrophotometry [13]. The final TFP concentration was 825 mg/g.

Animal experiments

The animal experiments were approved by the ethics committee and conducted in accordance with local institutional. The male SD rats (190 ± 20 g) were purchased from the Experimental Animal Center (China Medical University) and housed under the conditions of humidity (45 ± 5%), temperature (21 ± 2°C) and a 12 h light/dark cycle.

Sixty rats were randomly divided into six groups (each group 10 rats). The normal group (NC) was fed with basic diets and water. Other rats were fed with high-fat diet as presented in **Table 1** for 42 days of experimental period after 7 days of accommodation [14]. These rats were randomly divided into five groups: model control group (MC), positive control group (Xue Zhi Kang, 60 mg kg⁻¹ BW/day, PC), TFP high dosage group (240 mg kg⁻¹ BW/day, HTFP), TFP medium dosage group (120 mg kg⁻¹ BW/day, MTFP), TFP low dosage group (60 mg kg⁻¹ BW/day, LTFP). The NC and MC rats were administered orally with the same amount of water, while the other rats were treated with the corresponding dosage of TFP once daily for 42 days [15, 16]. During the experiment, all rats were allowed freely access to water and fed with diets twice a day. At the end of experimental period, all rats were fasted for 12 h and anaesthetized with chloral hydrate by intraperitoneal injection. The blood samples were centrifuged at 9000 r/min for 15 min at 4°C after sampled from retrobulbar vein, the serums were collected and stored at -20°C until assayed. The liver tissue was

Table 2. Rutin, quercetin and total flavonoids content in TFP

Compound	Contents (mg/g in dry weight)
Rutin	348.73 ± 0.32 ^a
Quercetin	38.55 ± 0.11 ^a
Total flavonoids	825 ± 1.54 ^a

^aData were reported as mean ± SEM (n = 3).

quickly collected, washed with cold physiological saline wiped by filter paper and weighed. Thereafter, the liver tissue was homogenized with cold physiological saline and the homogenate was centrifuged at 4000 r/min for 15 min at 4°C. The supernatant was collected and stored at -20°C for further analysis [12].

Acute oral toxicity experiment

Another sixteen male rats were chosen for acute toxicity experiment. The rats were randomly divided into four groups (each group was four rats) and oral administrated TFP at increasing doses of 600, 1250, 2500 and 5000 mg/kg BW, respectively. All rats were observed frequently for the first three hours for any toxicological signs and until three days for mortality [17].

Biochemical analysis

The levels serum of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), SOD, GSH-Px, CAT, MDA, ApoA, ApoB and total protein were measured with commercial assay kits on the basis of the manufacturer's specification. Atherogenic index (AI) was calculated by following the formula: AI = (TC-HDL-C)/HDL-C.

HPLC analysis of TFP

TFP powder was accurately weighted and put into a 200 mL volumetric flask. Then the sample was dissolved with 80% methanol and diluted to volume (200 mL). Before HPLC analysis, all test samples were filtrated by a 0.45 µm membrane filter. HPLC analysis was performed on Waters series 2695 liquid chromatography, consisting of a vacuum degasser, a quaternary pump, and photodiode array detection (DAD). The HPLC column was Luna C18 column (250 mm × 4.6 mm, 5 µm). A gradient elution was conducted by adjusting the proportion of solvent A (methanol) to solvent B (0.05% phosphoric acid).

The linear gradient program was as follows: 0-5 min with 10% solvent A; 5-60 min from 10% to 70% A; 60-70 min with 70% A. The flow rate was 0.9 mL/min and column temperature was constant at 30°C. The injection volume was 20 µL and UV detection wavelength was set at 350 nm [18].

Statistical analysis

All experiment results were reported as the means ± SD and the experiment were replicated twice. The differences between groups were estimated by one-way ANOVA and using SPSS software (version 16.0); P < 0.05 was usually considered as statistically significant.

Results

Analysis of TFP

As shows in **Table 2**, the quercetin, rutin and total flavonoids contents of the TFP were tested. The total flavonoids content in TFP tested by UV spectrophotometry method was up to 825 mg/g. Quercetin and rutin contents in TFP were up to 38.55 mg/g and up to 348.73 mg/g, respectively. Identification of the peaks was carried out according to the UV spectra and retention time of rutin and quercetin standard available in our laboratory. From the **Figure 1**, we found that the main flavonoids composition of TFP were quercetin and rutin.

Acute toxicity experiment

No signs of toxicity or any changes in the behavior of rats during the acute toxicological study period. When up to the dose of 5000 mg/kg BW for three days, no mortality was observed.

Effects of TFP on liver index

All data of liver index in different groups were shown in **Figure 2**. Obviously, the liver index in MC group was significantly higher (P < 0.01) than those in NC group. However, the liver index of HTFP and MTFP groups were lower than the MC group (P < 0.01, P < 0.05), the increase of liver index could be mitigated by oral administration the TFP at the doses of 120 and 240 mg/kg BW/day on hyperlipidemia rats.

Effects of TFP on serum lipid profile

Usually, the serum levels of TG, TC, HDL-C and LDL-C were usually used as biochemical mark-

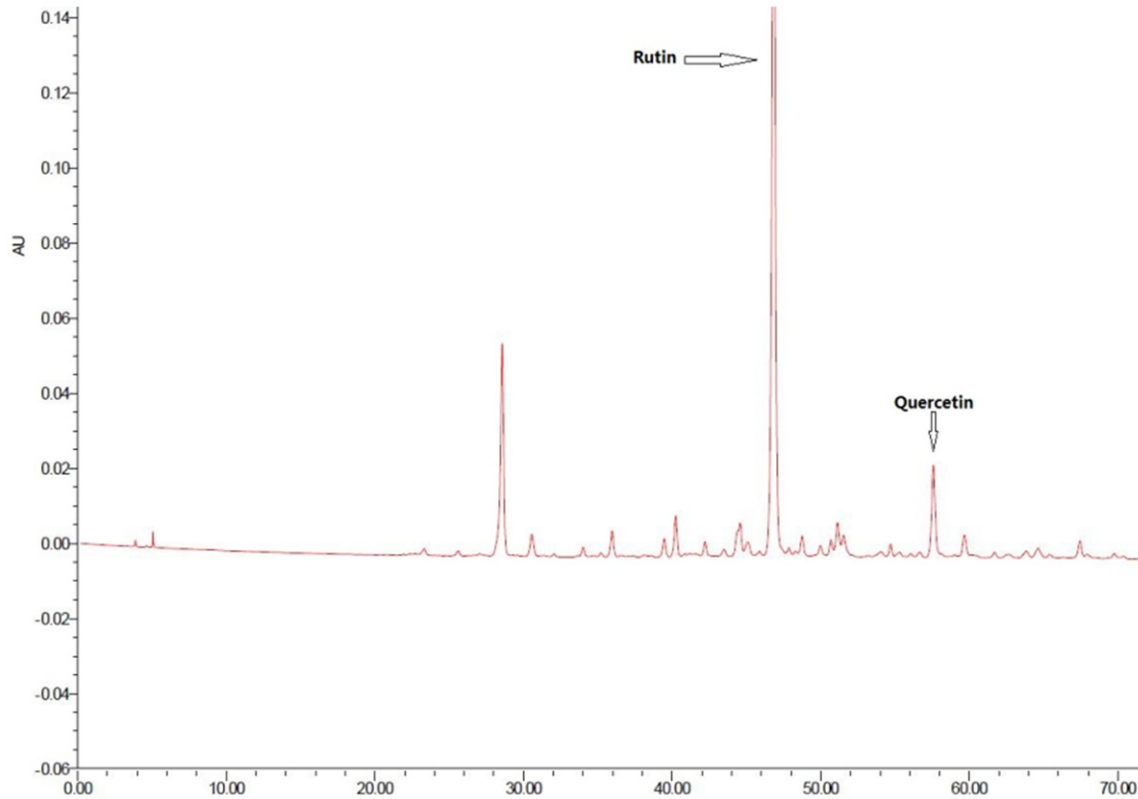


Figure 1. HPLC chromatogram for the TFP and chromatography analysis of rutin and quercetin.

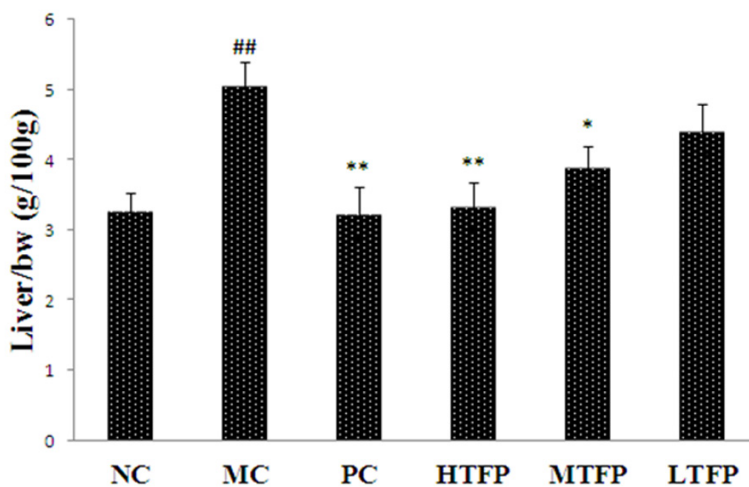


Figure 2. The liver index in HFD rats with different doses of TFP. The data were reported as the mean \pm SD of ten rats per group. ## $P < 0.01$ (vs NC group) and ** $P < 0.01$, * $P < 0.05$ (vs MC group).

ers for hyperlipidemia in clinical diagnosis. As shown in **Figure 3A** and **3B**), the levels of TC, TG and LDL-C in high-fat diet induced hyperlipidemia rats were significantly increased when compared with the normal rats ($P < 0.01$), whi-

ch suggested that the hyperlipidemia model was successfully induced by HFD. However, after oral administration the xue zhi kang ($60 \text{ mg kg}^{-1} \text{ BW}$) or different dosage TFP for six weeks, the increase of TG, TC, and LDL-C could be assuaged when compared with MC ($P < 0.05$ or $P < 0.01$). Change in the serum level of HDL-C was observed in all groups from the **Figure 3B**. The serum level of HDL-C was significantly decreased in MC group when compared with that in NC group. However, the decline of HDL-C could be mitigated by oral administration the Xue zhi kang ($60 \text{ mg kg}^{-1} \text{ BW}$) or TFP

at different dosage ($P < 0.05$ or $P < 0.01$). As shown in **Figure 4A**, the AI of MC group was obviously increased when compared with the NC group ($P < 0.01$). The increase of AI could be assuaged by treatment with TFP at the doses of

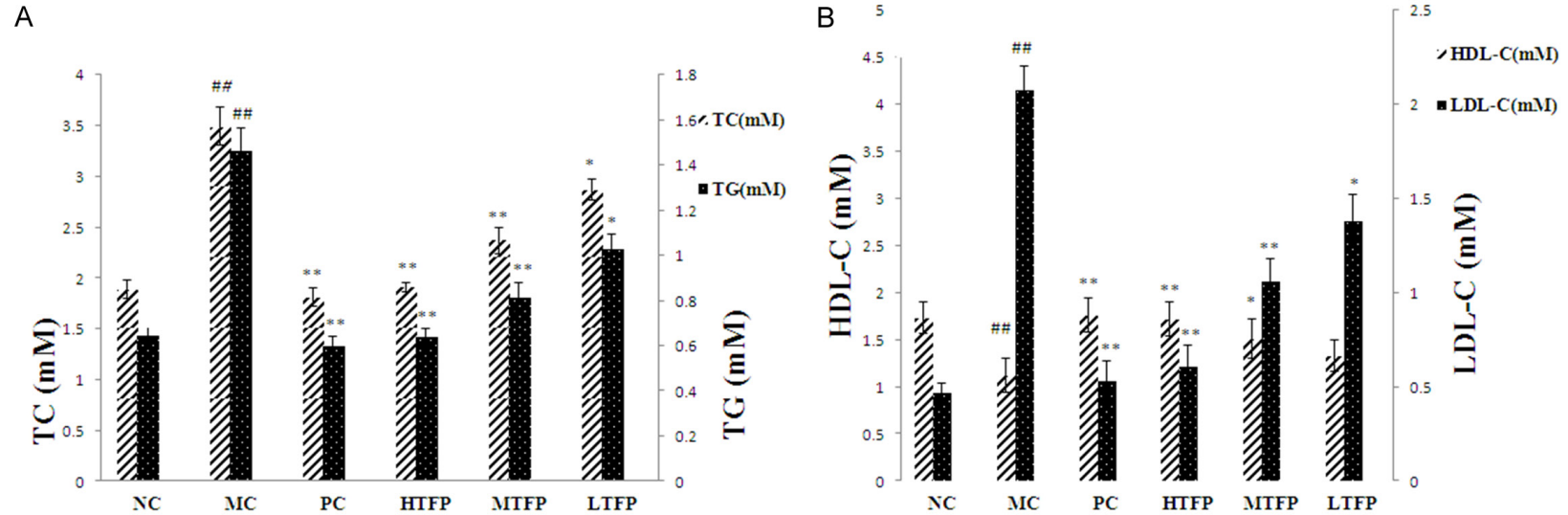


Figure 3. Effects of TFP on serum TC (A), TG (A), HDL-C (B) and LDL-C (B) in high-fat diet induced hyperlipidemia rats. The data were reported as the mean \pm SD of ten rats per group. ^{##}P < 0.01 (vs NC group) and ^{**}P < 0.01, ^{*}P < 0.05 (vs MC group).

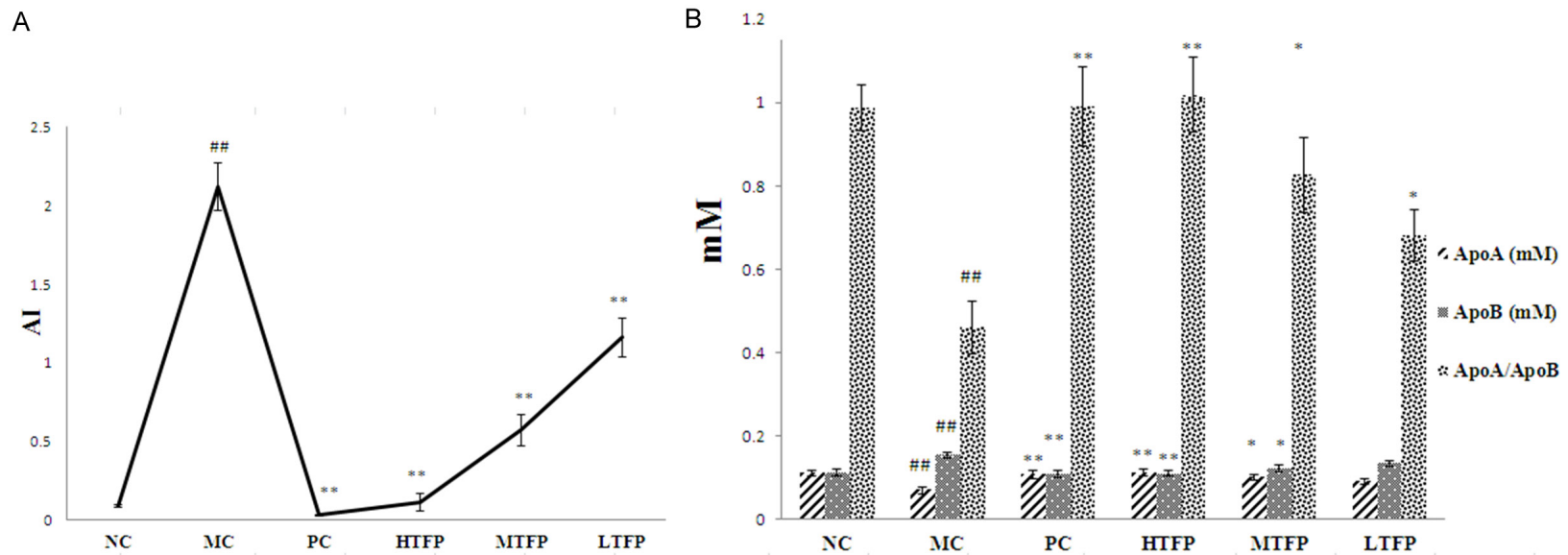


Figure 4. Effects of TFP on AI (A) and apoprotein (B) in HFD induced hyperlipidemia rats. The data were reported as the mean \pm SD of ten rats per group. ## P < 0.01 (vs NC group) and ** P < 0.01, * P < 0.05 (vs MC group).

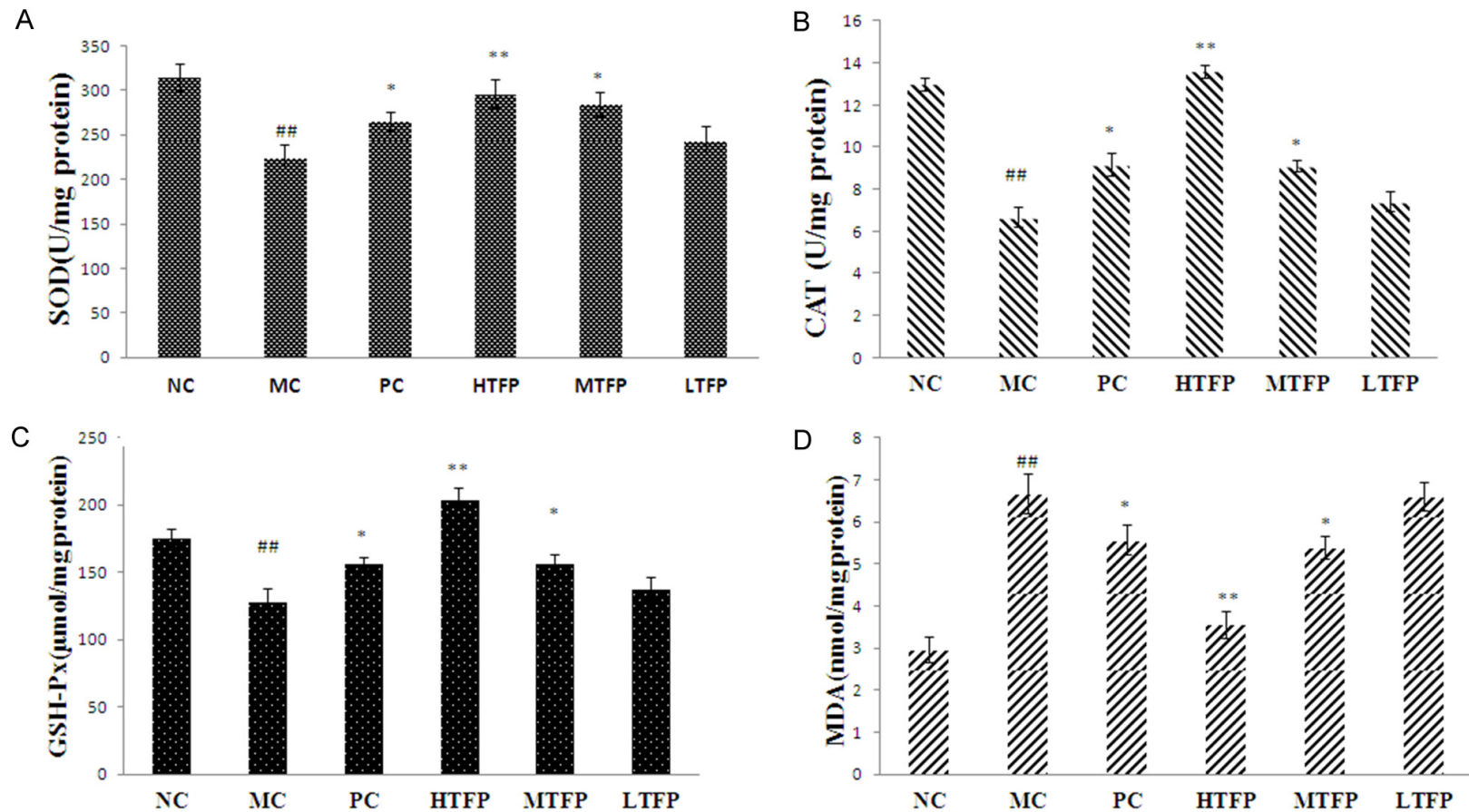


Figure 5. Effects of TFP on liver tissue SOD (A), CAT (B), GSH-Px (C) and MDA (D) in HFD induced hyperlipidemia rats. The data were reported as the mean \pm SD of ten rats per group. ## P < 0.01 (vs NC group) and ** P < 0.01, * P < 0.05 (vs MC group).

60, 120 and 240 mg/kg BW, respectively. The similarly result was observed in PC group ($P < 0.01$). Moreover, in **Figure 4B**, the level of ApoA was significantly lower in the MC group than that in the NC group ($P < 0.01$), while ApoB was apparently higher when compared to the NC group ($P < 0.01$). However, after the treatment with TFP at the doses of 120 and 240 mg/kg BW for six weeks, there was an obviously rise in the ratio of ApoA/ApoB and the level of ApoA ($P < 0.05$, $P < 0.01$) when compared with those in MC group. Meanwhile, the level of ApoB was significantly decreased in the HTFP group and MTFP group ($P < 0.01$, $P < 0.05$) when compared with the MC group. There was a similarly result has happened in the PC group and the trend was in accordance with the HTFP group. Therefore, the treatment of TFP at the doses of 240 mg/kg BW could exhibit obviously hypolipidemic effect. The experimental results imply that the HFD-induced hyperlipidemia could be improved to some extent by treatment with those TFP.

Effects of TFP on SOD, CAT, GSH-Px activities and MDA contents in liver tissue

In present work, the four biochemical indices that related to antioxidant activities were analyzed, in order to investigate the antioxidant activities of TFP in HFD-induced hyperlipidemia rats (**Figure 5**). As displayed in **Figure 5A-C**, the SOD, CAT and GSH-Px activities of HFD-induced hyperlipidemia rats were significantly decreased when compared with the normal rats ($P < 0.01$). However, after the six weeks of treatment, the decline of SOD, CAT and GSH-Px activities could be alleviated after the treatment with TFP at different dosages compared with that in MC group ($P < 0.01$). As shown in **Figure 5D**, the increased contents of MDA were observed in MC group when compared to the NC group ($P < 0.01$). However, the contents of MDA in liver were significantly decreased in HTFP and MTFP group, respectively ($P < 0.01$ and $P < 0.05$).

Discussion

Epidemiological researches have demonstrated the flavonoids play a positive role on cardiovascular and cerebral disease through alleviating oxidant stress and vascular dysfunction [19] and regulating the serum lipid metabolism [20]. In present research, two main flavonoids

constituent rutin and quercetin in TFP were identified and quantified through HPLC-DAD method in order to investigate the potential hypolipidemic and antioxidant effects of TFP.

The present study showed that hyperlipidemia modal was successfully established by high-fat diet. In the study, the increased of liver index was accompanied by serum lipid levels gain in high-fat diet rats, and the effect was mitigated by oral administration TFP at the doses of 120 and 240 mg/kg BW. Studies have reported that high-fat diet also cause the gather of cholesterol in liver tissue, possibly explaining the reason for the elevated of liver index [21]. Meanwhile, the serum levels of TG, TC, and LDL-C were increased, while HDL-C was decreased, which all were assuaged by treatment with HTFP and MTFP. Those results seemingly implied that the TFP could exert cardio-protective effect and regulate lipid metabolism by increasing the serum level of HDL-C on hyperlipidemia rats, because some researches have proved that lower coronary disease risk was associated with the increased levels of HDL-C [22]. Hyperlipidemia and the increased of serum lipid levels (TC, TG and LDL-C) were the main risk factors participated in the development of atherosclerosis diseases [23]. The atherogenic index (AI) was used to be an indicator for estimating the degree of atherosclerosis [12, 24]. In our research, the increased of AI in HFD induced hyperlipidemia rats, which was obviously declined by treatment with all doses of TFP. ApoA is an vital lipoprotein for removing superfluous cholesterol from tissue to the liver and it was related to high-density lipoprotein [25]. ApoB is the main lipoprotein of low-density lipoprotein, which taken charge of transferring the cholesterol to tissues. Some studies have exhibited that the ratio of ApoA/ApoB may be a priority risk indicator for cardiovascular diseases, and LDL-C alone was inadequate to define the cardiovascular risk [26, 27]. In this study, TFP could decline the level of ApoB and increase the level of ApoA, adjust the ratio of ApoA/ApoB and metabolic disturbance of lipoprotein by treatment with all doses of TFP in hyperlipidemia rats. These results demonstrated the potential of TFP as therapeutic agent against cardiovascular diseases and as preventive drugs against hyperlipidemia. These positive effects of TFP can be associated with its flavonoid constituent, namely, rutin and quercetin, which possess antioxidant activity [19].

Oxidative stress is one of causative factor that links dyslipidemia to the development of atherosclerosis [28]. Oxidative stress also occurs when the production of free radicals surpasses the capacity of the antioxidant system. Feeding rats with high-fat diet can facilitate production of free radicals, increasing the lipid peroxidation products and formation of ROS, which can cause irreversible injury to membrane lipids [29]. There are some essential antioxidants in biological tissue, including GSH peroxide (GSH-Px), superoxide dismutase (SOD) and catalase (CAT). The activities of those antioxidant enzymes indirectly reflect the capacities to remove free ROS. Malondialdehyde (MDA) is the main product of lipid peroxidation generated in the peroxidation of polyunsaturated fatty acid when lipids are assaulted by the free radicals, which indirectly reflect the degree of tissue injury [30]. In present study, TFP improved the activities of GSH-Px, SOD and CAT, decreased the content of MDA in liver tissue, indicating that TFP could exert antioxidant effects on hyperlipidemia rats. In addition, the TFP shows a considerable hypolipidemic activity that is associated with its antioxidant effect. Previously literatures have demonstrated that the flavonoid compounds could exert vitro antioxidant effect on LDL rely on their special functional hydroxyl groups [31]. What's more, the rutin and quercetin could exert the hypolipidemic effect and improve the antioxidant status *in vivo* [10, 11]. Those results were accordance with our researches, and the two main flavonoids constituent rutin and quercetin in TFP may representative of hypolipidemic and antioxidant effects.

In summary, our present investigation have showed that the hypolipidemic effects of TFP in hyperlipidemia rats, which may be relate to the adjustment of the serum lipid metabolism through regulation metabolic disturbance of lipoprotein and improved the oxidative stress as well as repressed development of a lipid peroxidant product. The observed hypolipidemic effects of TFP possibly owe to the presence of rutin and quercetin, which are the main constituents of TFP. However, the detailed mechanisms of these effects require to be expounded by further researches.

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Disclosure of conflict of interest

None.

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