Int J Clin Exp Med 2018;11(5):4575-4584
www.ijcem.com /ISSN:1940-5901/IJCEM0055922

Original Article

Baicalin exerts lipid-lowering, fatty liver attenuating and anti-oxidation properties in hypercholesterolemic rats induced with a high-cholesterol diet

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Received April 21, 2017; Accepted December 24, 2017; Epub May 15, 2018; Published May 30, 2018

Abstract: Atherosclerosis and its associated oxidative stress and numerous complications are consequential to an aberrant increase in blood cholesterol generally known as hypercholesterolemia and constitute a fatal death cause. Lipid lowering drugs are the only promising option for decreasing the risk of death and morbidness related to cardiovascular disease (CVD). Treatment particularly designed for decreasing low-density lipoprotein cholesterol (LDL-C) such as statins are needed for limiting the risk of death from coronary heart disease (CHD) and stroke. However, for patients with a high risk of CVD, it is necessary to find alternative therapies. The present survey was designed to evaluate the lipid-lowering and antioxidative potential of baicalin, a flavonoid component of Traditional Chinese medicine (TCM) that is cognized for its therapeutic properties. SD rats were fed with a cholesterol-rich diet to induce hypercholesterolemia. Subsequently, the hypercholesterolemia rats were treated with different doses of baicailin. The levels of different types of lipids in serum and liver, as well as those of oxidative stress indicators such as thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD) and GSH-Px were examined. The cholesterol-rich diet increased the levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) in serum and hepatic samples HDL-C while the level of high-density lipoprotein cholesterol (LDL-C) was significantly decreased. The levels of TBARS, SOD and GSH-Px were equally increased. Liver index, coronary heart disease (R-CHD) value and atherosclerosis index (AI) were also increased by feeding with the high cholesterol diet. Baicalin administration controlled the weight gain in rats, and reversed all the above variables. Our present findings indicated that Baicalin is an efficient lipid-lowering, fatty liver attenuating and anti-oxidation agent that can be used for preventive and therapeutic purposes in atherosclerosis and other coronary heart diseases.

Keywords: Baicalin, lipid-lowering, antioxidant, hypercholesterolemia

Introduction

Hypercholesterolemia is an elevation of blood cholesterol and a risk factor for atherosclerosis, a principal cause of myocardial infarction, strokes and numerous other pathologies which can lead to severe disability or even death [1]. One of the cornerstones of research on atherosclerosis is centered on lowering cholesterol levels and mainly consists in the development of lipid-lowering molecules such as statins which allow reduction of about 30% blood cholesterol. Nevertheless, in some patients at high risk for cardiovascular disease, the drug efficiency is far from the expected result [2, 3]. Thus, the discovery of alternative efficacious lipid-lowering drug candidates is highly encouraged [4].

Baicalin is a flavonoid compound discovered in a number of species in the genus Scutellaria and is known as an emerging multi-therapeutic agent and an important component of Traditional Chinese Medicine (TCM) [5]. For instance, baicalin was reported to induce apoptosis in SW620 human colorectal carcinoma cells *in vitro* and exerts anti-tumor properties *in vivo* [6]. Baicalin was equally conveyed as a proteasome inhibitor that specifically target chymotrypsin-like catalytic activity [7]. Moreover, previous findings indicate that baicalin possesses antithrombotic activities and could be

applied for the development of new anticoagulant agents [8] and was shown to display anti-inflammatory effect on hypoxia/reoxygenation and TNF-alpha induced injury in cultured rat cardiomyocytes [9]. Furthermore, baicalin exerts anti-adipogenic function by modulating members of the Wnt/β-Catenin pathway to subsequently result in low-density lipoprotein receptor-related protein 6 [10]. Baicalin was also reported to exert therapeutic effect against the nonalcoholic fatty liver disease [11] and is able to alleviate high fat diet-prompted obesity and liver dysfunction by endorsing cholesterol efflux [12, 13]. Similarly, long-term baicalin treatment was reported to improve metabolic disorders as well as hepatic steatosis in high-fat diet fed-rats [14] and is able to reduce atherosclerotic lesions in *vivo* [15]. However, it remains unclear whether baicalin could exert lipid-lowering effects against hypercholesterolemia due to the limited number of studies apropos of this compound.

Previous clinical and preclinical investigations have indicated that oxidative stress plays a fundamental role in complications associated with hypercholesterolemia [16-21]. Meanwhile, emerging proof that baicalin exerts antioxidant effects has been conveyed [22-24]. Therefore, we hypothesize that baicalin could limit oxidative stress connected to hypercholesterolemia.

The present work was thus aimed to investigate the lipid-lowering and anti-oxidation efficiency of baicalin in a rat model of hypercholesterolemia in order to evaluate its use for possible treatment or prevention of atherosclerosis and other cardiovascular diseases.

Material and methods

Chemicals

The LabDiet's standard rat diet 5012 was purchased from YoungLi (Shanghai) Biotech Co., LTD. Cholesterol (C8667 SIGMA, purity ≥99.0%) and baicalin (≥99.0% (HPLC), optical activity of [α]/D -83±3°, c=1 in DMSO) were purchased from Sigma-Aldrich. Triglyceride (TG) reagent (T2449 SIGMA) used for quantitative enzymatic determination of triglyerides was obtained from Sigma Aldrich. The colorimetric total cholesterol (TC) assay kit (Catalog Number STA-384) and HDL-cholesterol (HDL-c) assay kit (Catalog Number STA-394) were purchased from Cell Biolabs (Biolabs IND., San Diego, CA) whereas LDL-cholesterol (LDL-c) assay kit (Catalog# 80069) was bought from Crystal Chem (Crystal Chem, Chicago, IL). The colorimetric superoxide dismutase (SOD) activity assay kit (ab65354), glutathione peroxidase (GSH-Px) assay kit (ab102530), lipase activity assay kit (ab102-524) and BCA protein quantification kit were all obtained from Abcam (China). Thiobarbituric acid reactive substance (TBARS) assay kit was obtained from Cayman Chemical (China).

Rat model of hypercholesterolemia

All animal studies, as well as protocols, conformed to the recommendations defined in the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health 85-23, revised 1996). The study was reviewed and approved by the Institutional Animal Care and Use Committee of Inner Mongolia Medical University.

At the start, 60 male Wistar rats (age: 10 weeks, body weight: 241±14 g) were housed with free access to water and standard rat diet in an environment under temperature (22-24°C), humidity (55%-65%) and light (12 hours day/night) controlled conditions. The rats were arbitrarily assigned to one of the following two groups: normal diet (ND; n=20) or high cholesterol diet (HCD; n=40). The normal diet group was fed with the LabDiet's standard rat diet 5012 whereas the HD group received the LabDiet's standard rat diet 5012 plus 5% cholesterol (Sigma-Aldrich). All the rats had access to water and food ad libitum until the end of the experiment. After four weeks of experiments, rats were put under ether anesthesia and venous blood withdrawn to assess the serum levels of TC and LDL-C. The hypercholesterolemia model was considered fruitfully established at the only condition that there was a significant difference in the increase of serum levels of TC or LDL-C between the ND and the HCD groups.

Baicalin administration

After confirmation of model establishment, rats in HCD group (without significant difference in weight and cholesterol level) were divided into four groups (n=10 in each group) and then orally treated with 10 mg/kg Baicalin (Baicalin low dose, BL), 20 mg/kg Baicalin (Baicalin medium dose, BM), 40 mg/kg Baicalin (Baicalin high

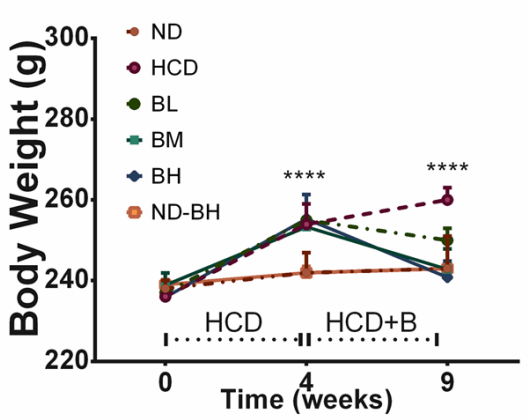

Figure 1. Effect of Baicalin on body weight of hypercholesterolemia rats. The body weight of rats in each group was measured at 4 and 9 weeks. Rats in HCD group had a markedly increased body weight compared with rats in ND group, while rats treated with baicalin (BL, BM and BH) had a significantly reduced body weight compared to the HCD. ****p<0.0001.

dose, BH), water (model, HCD) once/day for 5 weeks. The ND group of rats were distributed into two groups (n=10 in each group): the group treated with high concentration of Baicalin (ND-BH) and that given water (untreated control, ND), respectively. The dose of baicalin was decided according to a previous report [25]. Baicalin was mixed into sterilized normal or high-cholesterol diet at indicated doses.

Sample collection and storage

After 9 weeks (four weeks for model and 5 weeks drug administration), the rats were anesthetized using ketamine hydrochloride 10% (40 mg/kg; sigma) and Xylazine (4 mg/kg; sigma) by intraperitoneal (IP) injection. Blood samples were withdrawn and loaded directly into the syringe prior to distribution into EDTA tubes. After resting for at least 2 hours at ambient temperature, tubes were centrifuged at 2000 × g for 15 minutes (Beckman model L centrifuge) and serum samples collected, aliquoted and stored at -20°C. Following the collection of blood samples, the rats were subjected to perfusion with saline and then decapitated for the removal of Liver, kidney and spleen. Finally these organs were washed with saline and stored at -70°C pending subsequent experiment.

Biochemical measurements

The serum and liver extract levels of TC, TG, HDL-C, LDL-C, SOD, GSH-Px and TBARS were measured using corresponding kits as instructed. The liver extracts were obtained by preparing 1 mL of 95% ethanol tissue homogenates from 100 mg of the tissue collection of supernatant by centrifugation at 7000 × g for 10 minutes at ambient temperature. Supernatants were pooled, air dried and rehydrated using a solution containing 484 µl of phosphate-buffered saline (PBS) and 16 µL of 95% ethanol for solubiliztion of the sterols content.

Assessment of atherosclerosis index, coronary heart disease index (R-CHD) and viscera index

Atherosclerosis index (AI), coronary heart disease index (R-CHD) and viscera index were calculated using the equations below:

Eq(1): AI=(TC-HDL-C)/(HDL-C)

Eq(2): R-CHD=(LDL-C)/(HDL-C)

Eq(3): Viscera Index=(Viscera weight)/(Body weight) ×100

Measurement of fatty liver index

Liver tissue (0.2 g) was accurately weighed, grinded with 2 mL saline and then centrifuged at 2500 rpm for 10 min. Supernatant was collected and measured for LPL, HL, SOD and GSH-Px as instructed. Another 0.1 g liver was accurately weighed, grinded with the same volume of chloroform-methanol solution and centrifuged at 4000 rpm for 10 min. Supernatant was collected and measured for liver TC and TG level as described in the manufacturers' guidelines.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism software version 6.01. One-way ANOVA or Two-way ANOVA was followed by multiple comparison tests to evaluate the significance between groups. The difference was significant at p<0.05.

Results

Baicalin regulates the body weight of hypercholesterolemia rats

After 4 and 9 weeks high cholesterol diet or high cholesterol diet and baicalin administration, the body weight of rats in the HCD group was significantly increased compared to rats in

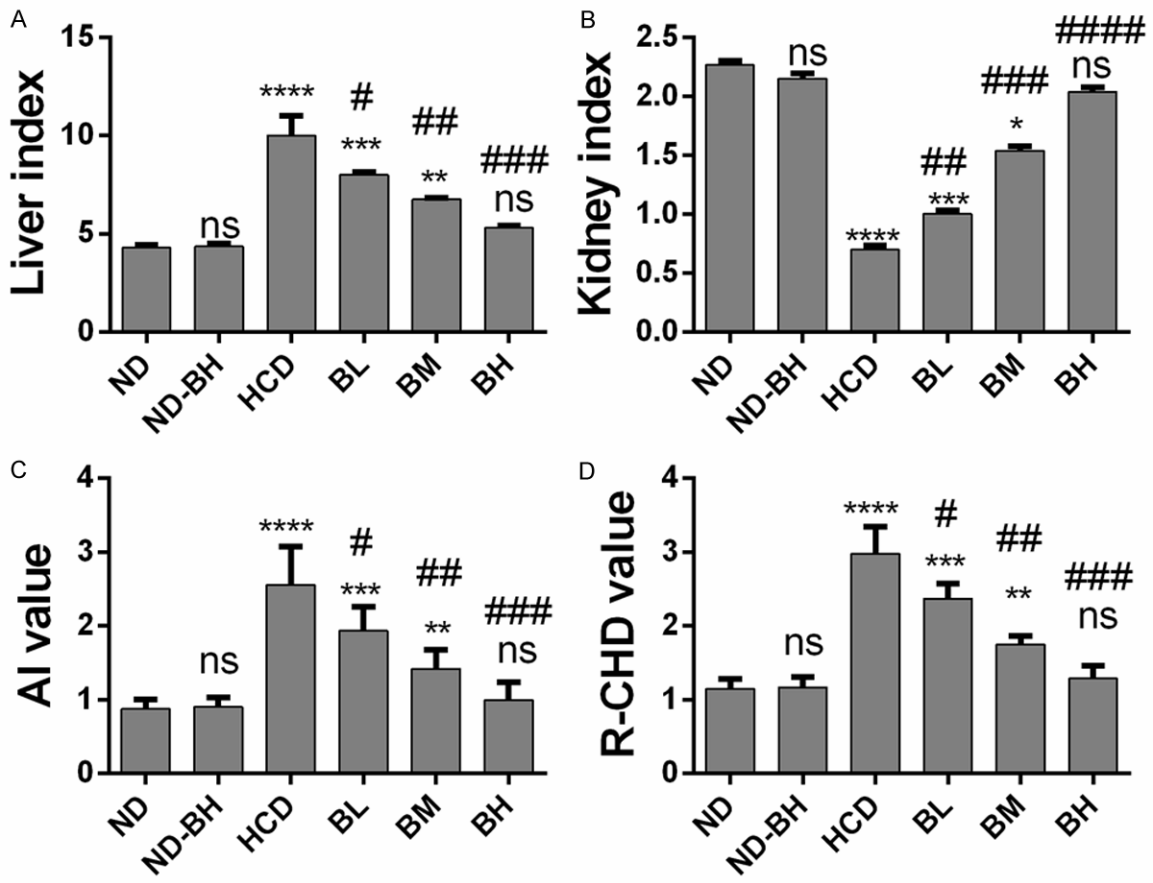

Figure 2. Baicalin attenuates the risk of cardiovascular disease in hypercholesterolemic rats. A. Liver index of HCD rats was markedly icreased compared to ND rats, whereas rats administered with Baicalin (BL, BM and BH) displayed significantly reduced liver index relatively to HCD rats. B. Kidney index of HCD rats was notably decreased when compared to ND group whereas BL, BM and BH rats had improved kidney index in a significant manner compared to HCD rats. C. Effects of Baicalin on AI in rats serum. AI value in HCD rats was higher compared with that in ND rats, while Baicalin markedly lowered AI value in a dose-dependent manner. D. Effects of Baicalin on R-CHD value in rats serum. R-CHD value in HCD rats was higher compared with that in ND rats, while Baicalin markedly lowered R-CHD value in a dose-dependent manner. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 compared with the ND group, #p<0.05, ##p<0.01 and ###p<0.001 compared with HCD group. ns=non-significant.

the ND group. The body weights of rats in BL, BM and BH groups were significantly decreased compared with rats in HCD group (P<0.05, **Figure 1**). There was no significant difference between BH group compared to the ND and ND-BH groups (p>0.05). This observation suggested that baicalin could reduce body weight of hypercholesterolemic rats.

Baicalin attenuates the risk of cardiovascular disease in hypercholesterolemic rats

After 9 weeks high cholesterol diet, the fatty liver index in the HCD rats was significantly increased though the kidney index was decreased comparatively to the ND group (**Figure 2A**, **2B**). This result clearly suggested cholesterol-induced damage of renal and liver functions. Interestingly, treatment with baicalin was followed by a significantly decreased liver index (**Figure 2A**, P<0.05) relatively to the HCD group. All these data indicated a baicalin-induced improvement of liver and kidney functions.

We also found that atherosclerosis index (AI) was markedly increased in HCD group compared with ND as well as ND-BH groups, but baicalin administration significantly decreased this increased AI value (**Figure 2C**). This suggested the protective effect of baicalin on atherosclerosis. As shown in **Figure 2C**, R-CHD in

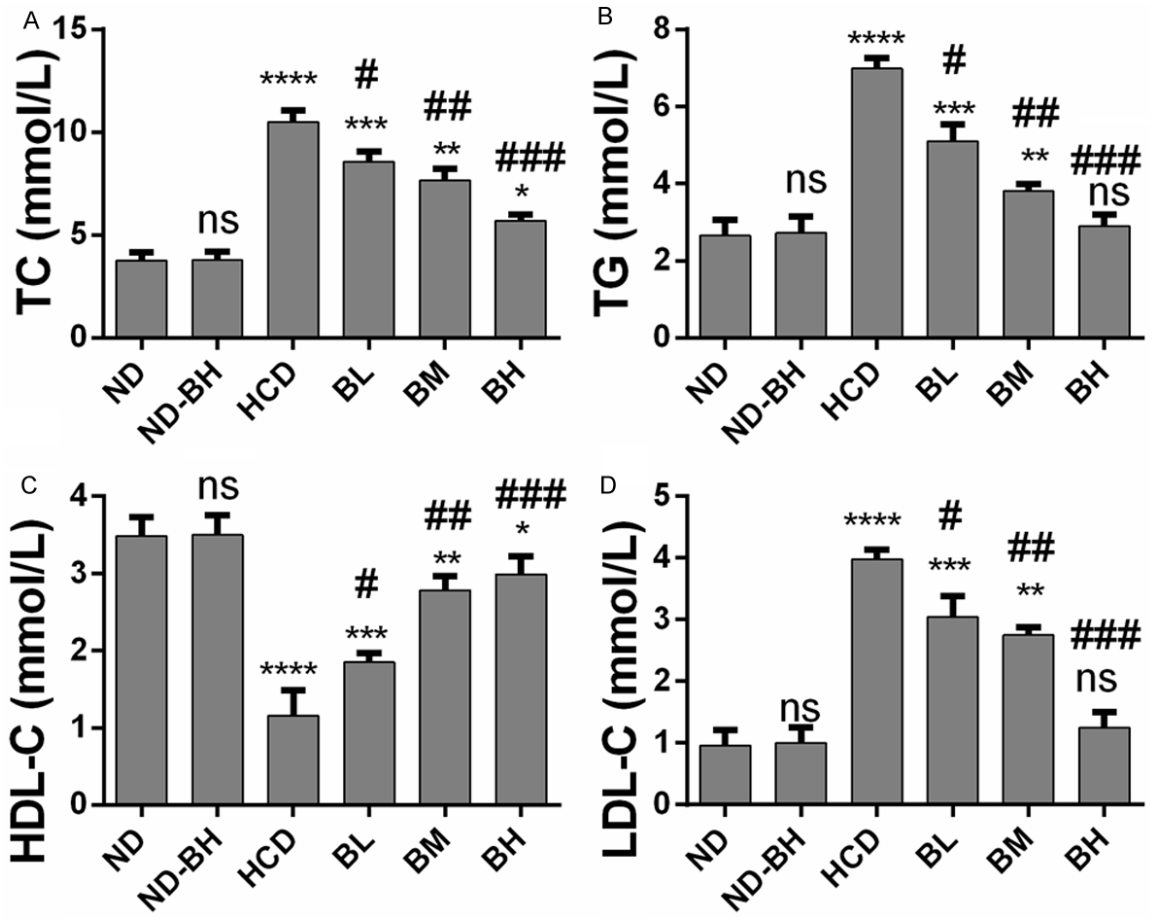

Figure 3. Baicalin exerts lipid-lowering properties in hypercholesterolemic rats. Both TC (A) and TG (B) level in HCD rats were significantly increased compared to rats in ND group, whereas Baicalin administration notably lessened TC and TG levels in a dose-dependently relatively to HCD rats. (C) Baicalin administration significantly reversed the reduced HDL-C in HCD rats. (D) Baicalin reduced dietary cholesterol-induced LDL-C in a dose-dependently. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 compared with the ND group, #p<0.05, ##p<0.01 and ###p<0.001 compared with HCD group. ns=non-significant.

HCD group was equally significantly increased compared with ND and ND-BH groups. On the contrary, after Baicalin treatment, R-CHD was significantly reduced compared with HCD group, showing that Baicalin significantly reduced the risk of coronary heart disease.

Baicalin exerts lipid-lowering properties in hypercholesterolemic rats

After baicalin administration, TC and TG levels in rats from BL, BM and BH were significantly and dose-dependently lowered relatively to the HCD group (**Figure 3A**, **3B**, P<0.0001), showing that baicalin has the potential to lessen TC and TG levels in hypercholesterolemic rats. The serum levels of TC and LDL-C in HCD rats were remarkably increased comparatively to the levels obtained in ND groups (P<0.0001, **Figure 3C**, **3D**). On the contrary, HDL-C level was significantly reduced (P<0.0001, **Figure 3C**). As indicated in **Figure 3C**, the HDL-C level in rats from BL, BM and BH groups was considerably higher than that in hypercholesterolemic rats in HCD group (P<0.01). Furthermore, LDL-C level was remarkably decreased in baicalin administration rats compared to HCD group (**Figure 3D**, P<0.01). These results indicated that Baicalin has the potential to concomitantly lower LDL-C and induce HDL-C levels. This suggested the lipid lowering properties of baicalin and suggested its protective ability against cardiovascular diseases such as atherosclerosis and coronary heart disease.

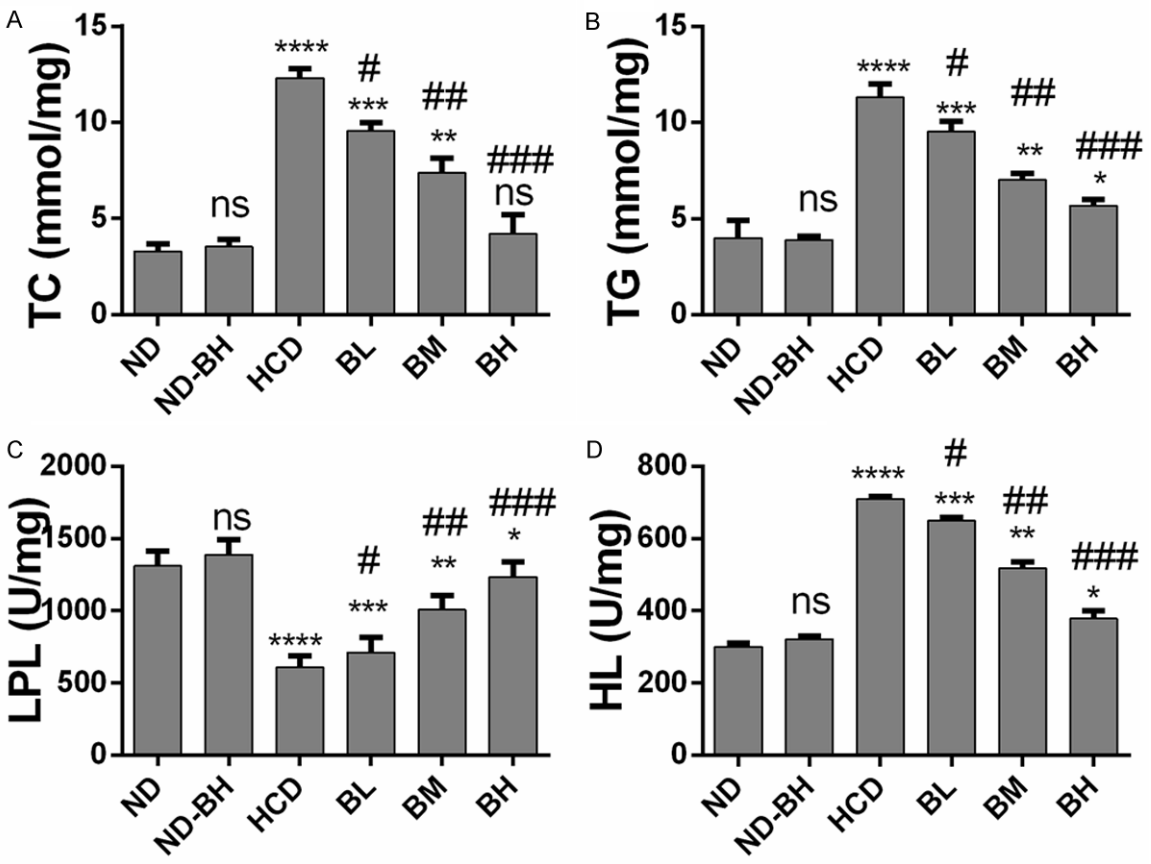

Figure 4. Effects of Baicalin on lipid levels, LPL and HL activities in liver of hypercholesterolemia rats. TC (A) and TG (B) level in HCD liver were significantly increased compared with that in ND rats, but reduced in those treated with Baicalin treated. Baicalin reversed the suppression of both LPL (C) and LDL-C (D) activities caused by HCD. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 compared with the ND group, #p<0.05 ##p<0.01 and ###p<0.001 compared with HCD group. ns= non-significant.

Effect of baicalin on lipid levels and LPL and HL activities in the liver of hypercholesterolemia rats

Similarly to results obtained in serum, TC and TG levels were significantly increased in the liver of HCD rats compared with that in ND group (**Figure 4A**, **4B**, P<0.0001). The levels of TC and TG in the liver of rats in the BL, BM and BH groups were markedly lowered by baicalin treatment (**Figure 4A**, **4B**, P<0.0001), which is indicative of a protective effect of baicalin against fatty liver. We equally found that dietary cholesterol inhibited LPL and HL activities in the liver of HCD rats by comparison with the ND group (P<0.0001, **Figure 4C**, **4D**). Baicalin significantly improved LPL and HL activities in a dose-dependent manner compared with HCD rats.

Baicalin exerts antioxidant properties in hypercholesterolemic rats

Cholesterol diet increased the TBARS in the HCD group when compared with rats in the normal group. Baicalin administration was followed by a dose-dependent decrease in liver TBARS compared with rats in the HCD group (P<0.01, **Figure 5A**). The detection of superoxide dismutase (SOD) activity in HCD rats was remarkably lessened compared with ND and ND-BH rats (P<0.01, **Figure 5A**), whereas SOD activity in serum and liver of Baicalin-treated rats was significantly elevated compared with HCD rats (P<0.05, **Figure 5B**). Additionally, SOD activity in BH rats did not exhibit significant difference compared with ND and ND-BH rats. All these data showed that baicalin was remarkably effective on promoting SOD activity.

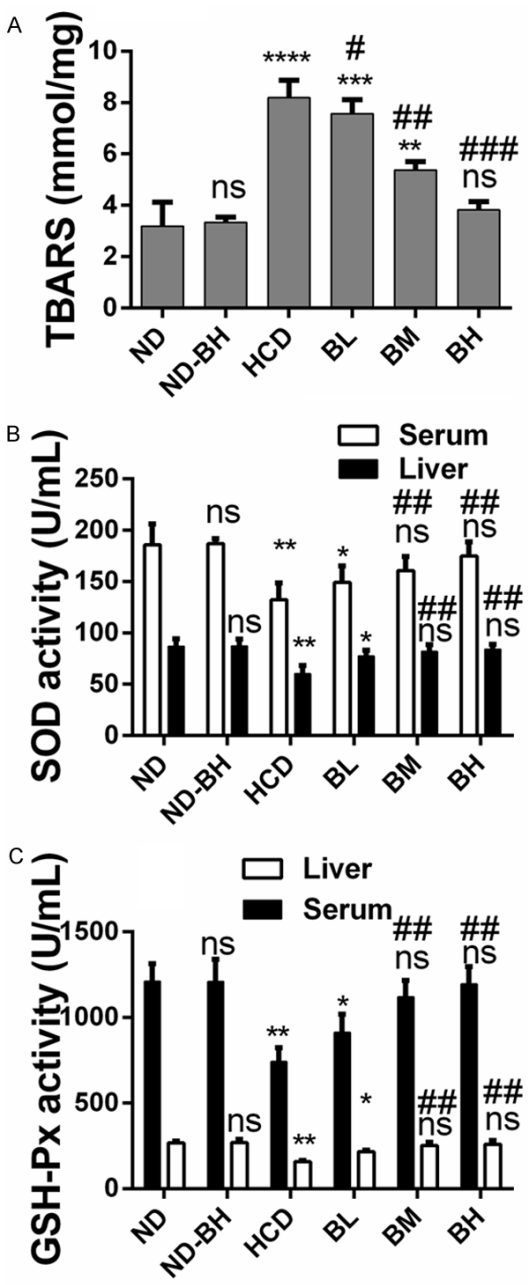

Figure 5. Baicalin exerts antioxidant properties in hypercholesterolemic rats. A. Baicalin reduced TBARS in the liver of HCD rats. B. SOD activity in serum and liver of HCD rats were markedly reduced compared to ND rats, but improved in Baicalin treated rats relatively to that that in HCD rats. C. Baicalin reversed the decreased levels of GSH-P activity in both serum and liver of HCD rats. *p<0.05, **p<0.01 and ****p<0.0001 compared with the ND group, #p<0.05, ##p<0.01 compared with HCD group. ns=non-significant compared with the BH group.

GSH-Px activity was measured in serum and liver of HCD rats was notably reduced relatively to ND and ND-BH rats (P<0.01, **Figure 5C**). Contrariwise, GSH-Px activity was enhanced in serum and liver of Baicalin administration rats compared to HCD rats (P<0.05, **Figure 5B**) except for BL group. Similarly to SOD activity, the activity of GSH-Px in BH rats had no difference with ND rats. All these observations indicated that Baicalin had clear improvement effect on serum and hepatic GSH-Px activity (P<0.05, **Figure 5B**). These results indicated that baicalin exerts anti-oxidation properties.

Discussion

Atherosclerosis is an extremely common disease, particularly in industrialized countries, with a wide range of clinical consequences, mainly cardiovascular diseases which constitute a complex of disorders affecting the heart and blood vessels, including coronary heart disease, embolism, thrombosis and more acute forms such as infarcts and strokes. Atherosclerosis is generally due to the presence of several associated risk factors such as hypercholesterolemia. Therefore, there is an absolute consensus on the need to lower cholesterol in people with high cardiovascular risk. Baicalin, a compound found in herbaceous plants of the genus Scutellaria, is particularly used in TCM owing to its multi-therapeutic properties. Previous studies have suggested the potential of baicalin to exert lipid-lowering and antioxidation properties.

The findings reported in this, to the best of our knowledge, is the extensive evidence exploration of the effective inhibitory action of baicalin in hypercholesterolemia and oxidative stress in high cholesterol fed rats. By analyzing the effects of baicalin on the serum levels of diverse type of lipids in the high-cholesterol-fed rats, we recorded increased levels of serum TC, TG, and LDL-C with decreased HDL-C level. The increase in serum total TC and TG levels recorded in HCD animals are in conformity with previous studies [26, 27]. The highly increased LDL-C levels found in HCD rats can be explained by the down-regulation of LDL receptors by dietary cholesterol [28]. Treatment of HCD-fed rats with baicalin led to significant decrease in TC, TG, and LDL-C levels but improved HDL-C levels compared to HCD group. These effects of baicalin were somehow similar to findings of other researchers [11-15]. The ability of baicalin

to induce HDL-C is a very important property due to the correlation of HDL-C with a reduced risk of cardiovascular diseases owing to its role in easing the serum cholesterol carriage to the liver, an organ responsible for catabolism and excretion of toxic compound from the body.

Moreover, we found that the dietary cholesterol induced an increase of liver weights, which suggested a deposition of lipids (cholesterol and triglycerides) in the liver. Contrarily, liver weight was reduced by baicalin treatment, which indicated that this compound could induce lipid degradation in liver and its elimination from the body. This observation was further supported by the decreased levels of TC, TG, and LDL-C and the decreased HDL-C level in the liver by baicalin treatment. The results obtained in our study corroborated with previous findings [11, 12, 29].

The AI (ratio of LDL-C to HDL-C), is a key atherosclerotic risk factor. Our data clearly demonstrated that baicalin significantly decreased AI comparatively to the HCD group. Thus, we hypothesized that baicalin exerted antiatherogenic and/or anti-coronary heart disease properties by increasing the HDL-C levels and decreasing the LDL-C levels in HCD-fed rats. Viscera index is an indicator of the health or damage grade of a given organ [30]. Herein, we found that baicalin markedly improved kidney index and reduced fatty liver index, suggesting that baicalin protects these organs from hypercholesterolemia-induced swelling or atrophy.

Numerous studies have demonstrated an amplified oxidative stress in clinical and experimental hypercholesterolemia [17-21, 31, 32]. The *in vitro* and *in vivo* antioxidative activities of baicalin have been equally conveyed previously [33]. In our study, the high cholesterol diet induced a remarkable increase in TBARS levels and decreased activities of SOD, CAT and GSH-Px. Baicalin administration showed significant reduction of TBARS and increased the levels of SOD, CAT and GSH-Px. These results suggest that baicalin reduces oxidative stress by preventing the generation of free radicals and finally inhibits development of atherosclerosis.

In conclusion, our results showed that baicalin is efficient in the protection against hypercholesterolemia by decreasing serum TC, TG, and LDL-C and increasing HDL-C, thus decreasing the AI. Moreover, baicalin also improve antioxidant status by lowering lipid peroxidation and enhancing antioxidant enzymes. Baicalin is a candidate lipid-lowering drug to against cardiovascular diseases.

Acknowledgements

This research was supported by the Chinese National Natural Science Foundation: The basic research on the Inner Mongolia allergic constitution family based on epigenetics (gjzr14688).

Disclosure of conflict of interest

None.

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