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BIOMEDICAL SCIENCES

Hederagenin Exerts Potential Antilipemic Effect *via* p38MAPK Pathway in Oleic Acid-induced HepG2 cells and in Hyperlipidemic Rats

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Abstract: Hederagenin, a natural compound distributed in many medicinal plants, has a variety of pharmacological properties including anti-bacteria, anti-inflammation, anti-oxidation, and anti- apoptosis.. The aim of this study was to evaluate the effects of hederagenin on decreasing blood lipid and anti-oxidative stress in oleic acid-induced HepG2 cells and hyperlipidemic rats, and explore underlying mechanisms. *In vitro*, TG was used as the index to verify the lipid-lowering effect of hederagenin in oleic acid-induced HepG2 cells. *In vivo*, TC, TG, LDL-C, and HDL-C were used as direct indicators to study the antilipemic effect of hederagenin in hyperlipidemic rats. MDA, SOD, and GSH-PX were measured to analyze the anti-oxidative effect of hederagenin. The signaling pathways of anti-oxidation were evaluated using Western blot. Our results showed that hederagenin (250μmol/L) increased significantly TG clearance rate. In addition, treatment with hederagenin, XZK and simvastatin reduced effectively TC, TG, LDL-C and MDA content, and increased HDL-C, SOD and GSH-PX in HFD rats. Moreover, the phosphorylation level of p38 MAPK was inhibited after administration of hederagenin, XZK and simvastatin. Our results revealed that hederagenin possessed beneficial potentials for hypolipidemic effects, especially in TG clearance. The mechanism might be associated with inhibition of lipid absorption, reduction of lipid oxidation, and down-regulation of p38MAPK phosphorylation.

Key words: Hyperlipidemia, Hederagenin, p38MAPK phosphorylation, lipid-lowering.

INTRODUCTION

Hyperlipoidemia (HLP), a disorder of lipid metabolism, refers to the pathological state that the level of total cholesterol (TC) and/or triglyceride (TG) in serum or plasma increases independently or simultaneously in fasting condition (Liu et al. 2011).

According to the results of the 2012 Chinese lipid census, the morbidity of various types of abnormal lipid metabolism was 11.43% (Moran et al. 2010). Among patients with lipid metabolism disorder, the average TC and TG in adult serum were 4.50 mmol/L and 1.38 mmol/L, with prevalence rates of 4.9% and 13.1% respectively. Abnormal lipid metabolism greatly increased the occurrence of cardiovascular and cerebrovascular diseases (coronary heart disease, ischemic stroke, etc.) (Wu et al. 2006, Sarwar et al. 2007). As scientific investigation showed that about 4.4 million people died every year from ischemic stroke and heart disease induced by elevated cholesterol in serum (Conroy et al. 2003, Guilbert 2003).

Clinical treatment of high blood fat disease was broadly divided into cholesterol-lowering drugs (statins, cholic acid chelators etc. (H et al. 2001)) and triglyceride-lowering drugs (bate, niacin, etc.) (Rubins et al. 1999,

Keech et al. 2005, Das et al. 2010, Group et al. 2010). However, some hypolipidemic agents could lower both cholesterol and triglycerides, especially for severe hyperlipidemia. Although statins are effective in lowering lipids, they have serious side effects, such as liver damage and rhabdomyolysis.

Hederagenin, a pentacyclic triterpene are widely distributed in many medicinal plants, such as *dipsacus asperoids, clematis chinensis, radix pulsatillae, lonicerae japonicae*, etc. Studies have found that hederagenin has a variety of properties such as anti-tumor, anti-depression, anti-bacteria and anti-inflammation and anti-diabetes (Kim et al. 2017a, b). However, a function of hederagenin in lowering blood lipid or anti-hyperlipidemia is not clear.

Hyperlipidemia is often accompanied by lipid oxidation caused by oxygen free radicals (Omari-Siaw et al. 2016). In the process of producing oxidized lipids, malondialdehyde (MDA) is the product of reaction of polyunsaturated fatty acids and an active oxygen, whose changes in concentration reflects the level of lipid oxidation (Wang et al. 2015, Lykkesfeldt 2007). Superoxide dismutase (SOD) is an antioxidant enzyme with a level that reflects the ability of free radicals to remove oxygen in the body (Wang et al. 2015). As an important peroxidase decomposing enzyme, glutathione-peroxidase (GSH-PX) can transform the lipid peroxides into more stable hydroxyl compounds in time and block the free radical chain reaction in lipid peroxidation (Bednarek-Tupikowska et al. 2001).

It is well known that activated molecules can indirectly trigger a variety of stress-sensitive serine/threonine kinase signaling pathways including the p38 isoform mitogen-activated protein kinase (p38MAPK) (Klaunig et al. 2010). The current research had shown that hederagenin was documented to inhibit MAPK activation (Tian et al. 2020). Moreover, a high fat-diet (HFD) could lead to an increase of TC and TG in serum (Fu et al. 2019). Therefore, in this study, we investigated the lipid-lowering effects of using oleic acid-induced HepG2 cells and HFD-induced hyperlipidemia animal model. Furthermore, the p38MAPK signaling pathway related to lipid metabolism and antioxidant was evaluated and validated. Together, we provided evidence that hederagenin could lower the level of lipid, which might be associated with the inhibition of p38MAPK signaling pathway.

MATERIALS AND METHODS

Materials

Hederagenin (Shanghai Chunyou biotechnology co., LTD., p0207-201906); PBS powder (Sigma company, Lot : P2198); Oleic acid (Sigma company, Lot : 191P13759); Fetal bovine serum (FBS) (Hyclone, Lot : 19156); Bovine serum protein (BSA) (Jiangsu Kaiji biotechnology co., LTD., Lot : 20191121); DMSO (Nanjing chemical reagent co., LTD., Lot : 20190908); Western and IP cell lysates (Jiangsu Kaiji biotechnology co., LTD., Lot : 20191221); DMEM high sugar medium (Gibco, USA, Lot : 1989124); HEPES (Jiangsu Kaiji biotechnology co., LTD., Lot : 20191121); Penicillin (Solarbio, Lot : 119A0322); Streptomycin (Solarbio, Lot : 423A0520); Trypsin (Jiangsu Kaiji biotechnology co., LTD., Lot : 20191121); BCA protein kit (Nanjing Jiancheng biotechnology co., LTD., Lot : 20191229); Total cholesterol (TC) assay kit, total triglyceride (TG) assay kit, low density lipoprotein cholesterol (LDL-C) assay kit, high density lipoprotein cholesterol (HDL-C) assay kit (Nanjing Jiancheng biotechnology co., LTD. Lot: 20191229); Malondialdehyde (MDA) test kit, Total superoxide dismutase (SOD) assay kit, Glutathione peroxidase (GSH-PX) assay kit (Nanjing Jiancheng biotechnology co., LTD. Lot : 20191229).

Cell Culture and Treatment

HepG2 cells with good growth status were cultured in Dulbecco's modified Eagle's medium (DMEM) high sugar medium containing 1% fetal bovine serum (FBS) and 2% Bovine serum protein (BSA). Cells were grown in 37°C with a humidified atmosphere containing 5% CO_2. HepG2 cells was seeded in in 24-well plates when the concentration was adjusted to about 10^5 / mL. After incubation for 24 h, HepG2 cells were set as blank group, model group, XZK group, Simvastatin group, and different concentration hederagenin group, each with 3 parallel wells. The blank group was added to 1% FBS high glucose DMEM medium containing 2% BSA, and the model group was added 1% FBS high glucose DMEM medium containing a final concentration of 1 mmol/L oleic acid to each well. In the XZK group, 1% FBS high-sugar DMEM medium containing a final concentration of 1 mmol/L oleic acid and 200 μg/mL drug was added to each well. In the simvastatin group, 1% FBS high-sugar DMEM medium containing a final concentration of 1 mmol/L oleic acid and 20 μg/mL drug was added to each well. The hederagenin group was supplemented with 1% FBS high glucose DMEM medium containing a final concentration of 1 mmol/L oleic acid and different concentrations of drugs. Then place the 24-well plate in a constant temperature incubator and incubate for 24 hours, then remove the culture medium, wash it twice with PBS, and then use Western and IP cell lysate for ice bath for 30 min, collect the lysate, and centrifuge at 4 12000 r/min for 10 minutes, take the supernatant, and determine the triglyceride and protein content according to the operating steps on the kit, each group has 6 parallel holes, and the results are expressed in mmol/gprot, and the intracellular TG clearance rate of each administration group is calculated at the same time.

Animals and Treatment

Male Sprague–Dawley (SD) rats (180–220 g) were purchased from Comparative Medical Center in Yangzhou University, Jiangsu Province, China (Certificate No: SCXK2019-0117), and maintained on a 12-hour light–dark cycle, 22 ± 1°C,55 ± 3% humidity, with *ad libitum* feeding. All the animal experiments were carried out according to the Guidelines of Chinese Experimental Animal Administration Legislation, and were approved by the Animal Ethics Committee of China (Document No: 55, 2019).

After adaptive feeding for a week, seventy SD rats were randomly divided into 7 groups; there were blank group, Model group, Simvastatin group (2.1 mg/k/d), Xuezhikang group (XZK, 126 mg/kg/d), Hederagenin low-dose group (H-L, 9.0 mg/kg/d), Hederagenin middle-dose group (H-M, 27.0 mg/kg/d), and Hederagenin high-dose group (H-H, 81.0 mg/kg/d).The blank group was fed normal diet; the other groups were fed HFD (HFD, containing 8% soybean oil, 44% sweetened condensed milk and 48% basic diet comprised of 15.5% protein, 33.4% fat and 51.1% carbohydrates.) (Fki et al. 2005). After 6 weeks of feeding with HFD, the rats were administered intragastrically for next 8 weeks. The body weight of rats was recorded daily.

Blood Samples and Liver Tissue Preparation

After the last intragastric administration, blood samples were drawn from the abdominal aorta when rats were anesthetized deeply with 20% urethane, then the liver was excised from rats and was stored in ice-cold normal saline.

Determination of TC, TG, Low-Density Lipoprotein-Cholesterol (LDL-C) and High-Density Lipoprotein-Cholesterol (HDL-C)

The serum obtained from blood samples was operated according to the instructions of the TC, TG, LDL-C and HDL-C kits respectively, and

the absorbance was measured with a microplate reader.

Determination of SOD, MDA, and GSH-PX

The liver tissue was homogenized by cold normal saline (1 mg liver tissue in 9 ml of normal saline). The supernatants were separated in condition of 3,000 rpm, 20 min. The protein concentrations were detected by Coomassie blue protein binding using bovine serum album as a standard. Test kits were used to detect the levels of SOD, MDA and GSH-PX in supernatants, whose procedures followed strictly the manufacturer's specification (Wei et al. 1997, Katiyar et al. 2011).

Western blots

The liver samples were lysed with cold lysis buffer (Solarbio, Beijing, China), and the samples homogenates were centrifuged at 13,000 rpm for 15 min at 4 °C. The protein concentrations of supernatant were determined with a BCA protein assay kit (Beyotime, Jiangsu, China). The protein samples were mixed with loading buffer in a volume ratio of 4:1 and then boiled for 10 min. Subsequently, the protein samples were separated on 5% SDS-PAGE and transferred to a polyvinylidene fluoride (PVDF) membrane. The PVDF membranes were blocked for 1.5 h in 5% skim milk and subjected to immunoblotting using primary anti-MAPK antibody overnight at 4 °C. Then, the PVDF membranes were washed three time of 10 min each with TBST and incubated with secondary horseradish peroxidase (HRP)-conjugated antibody for 2 h at room temperature. The blotting of membranes was detected by chemiluminescence (ECL) kits (Millipore, Billerica, MA, USA) and Bio-Rad imaging systems. Relative intensities of protein bands were analyzed by Image J software.

Data analysis

Data are represented as means ±standard deviation ($\overline{X} \pm SD$). Statistical analysis of differences between groups was performed by one-way ANOVA using SPSS 19 statistical software. Differences were considered significant at $P < 0.05$.

RESULTS

Effect of Hederagenin on TG in HepG2 Cells Induced by Oleic Acid

Compared with the blank group, the TG in the model group increased significantly, indicating that the oleic acid-induced HepG2 cell fat accumulation model was successfully established. As shown in Table I, compared with the model group, XZK, simvastatin, and hederagenin (100 μmol/L, 250 μmol/L) significantly reduced the level of TG (P <0.01); TG clearance rates were: 30.84%, 41.94%, 24.76%, and 35.81%, respectively. Meanwhile, the result showed that hederagenin administration led to decreased TG in a dose-dependent manner.

Table I. Effect of hederagenin on TG content in HepG2 fat accumulation model($\overline{X} \pm SD$ n=10).

Group	Final concentration	TG content(mmol/gprot)	TG clearance rates (%)
Blank group	--	0.531±0.041	--
Model group	--	2.27±0.127**	--
XZK	200 μg/mL	1.57±0.039##	30.84
Simvastatin group	20 μg/mL	1.318±0.042##	41.94
Hederagenin	250 μmol/L	1.457±0.061##	35.81
	100 μmol/L	1.708±0.056##	24.76

Significant differences (P<0.01 *vs* blank group; ##P<0.01 *vs* model group) were analyzed using one-way ANOVA. TG clearance rate%=($TG_{model\ group}$-$TG_{dose\ group}$)/$TG_{model\ group}$ ×100%. TG: Triglyceride; XZK:XueZhiKang.**

Effect of Hederagenin on Body Weight

All animals survived in the duration of the treatment. The result showed that HFD-induced model group, simvastatin group, XZK group, H-L group, H-M group, and H-H group were heavier than blank group before administration (P<0.01) (table II). However, compared with the model group, simvastatin group, XZK group, H-L group, H-M group, and H-H group markedly improve the body weight induced by HFD at end of drug administration (P<0.01) (table II). The body weight of each group increased respectively before administration and the last administration: 32.8% in the blank group, 55.1% in the model group, 38.5% in the simvastatin group, 47.7% in the XZK group, 49.9% in the H-L group, and 39.8% in the H-M group and 37.5% in the H-H group.

Effect of Hederagenin on the Level of Lipid

Next, we tested whether the decrease of weight in HFD-induced models is related to hederagenin effect on the level of lipid. The levels of TC, TG and LDL-C were increased remarkably in the model group compared with the blank group, and the level of HDL-C decreased significantly, indicating that hyperlipidemia model was established successfully. The serum lipid profile markedly improved, especially in the hederagenin high-dose group (H-H) after drug administration for 8 weeks. The levels of TC, TG, and LDL-C in the H-H group were 24.2%, 27.3% and 23.1% lower than those in the model group, respectively (P<0.05), and the HDL-C level in the H-H group was 6.4% higher than that in the model group (Fig. 1a-d). The simvastatin treatment also significantly improved the serum TC, TG, LDL-C and HDL-C levels compared to the model group (P < 0.05).

Effect of Hederagenin on Response to Oxidative Stress

Furthermore, this study investigated whether hederagenin decreased the level of lipid in HFD-induced rats is associated with the effect of that on anti-oxidation. As shown as Fig. 2a, Compared with the blank group, it can be observed that the liver MDA level of the model group is 2.03 fold that of the blank group, which indicates that HFD significantly induced the increase of the liver MDA level (P < 0.01). XZK, Simvastatin H-L, H-M and H-H treatment could markedly reverse the HFD-induced increase of liver MDA level (36.2%, 44.5%, 24.6%, 31.8% and 36.9%(respectively) versus model group rats (P < 0.01, P < 0.05). Moreover, the levels of liver SOD and GSH-PX

Table II. Effect on body weight of rats fed a basic diet or a HFD from 6 weeks to 14 weeks($\overline{X} \pm$ SD n=10).

Section	Time	Blank group	Model	Simvastatin	XZK	H-L	H-M	H-H
Before administration	6 weeks	325.6±6.4	352.3±9.6**	349.4±10.3**	353±9.5**	351.6±10.7**	353.2±11.7**	355.2±10.5**
Begin administration	7 weeks	336.95±7.6	377±13.7**	369.8±13.4 #	376.8±12.3	375.6±14.5	376.6±13.3	374.2±12.9
	8 weeks	351.45±9.2	399.6±15.1**	388.6±16.5	399.5±14.4	399±15.3	397.3±16.4	393.7±15.1
	9 weeks	366.05±8.4	426.5±14.6**	406.2±15.9#	422.3±13.8	424.7±17.6	416.4±14.9	411.1±16.0#
	10 weeks	380.95±8.3	450.1±17.3**	423.1±19.2##	443.6±15.3	447.3±19.4	433.7±15.6#	431.8±18.2#
	11 weeks	394.2±10.5	474.3±20.5**	438.8±18.4##	464.8±17.8	468.8±19.8	449.6±17.2##	446.9±17.6##
	12 weeks	406.8±10.7	499.8±21.7**	454.1±20.7##	485.2±20.4#	489.3±21.3	464.4±19.7##	460.9±20.3##
	13 weeks	419.7±12.1	523.9±22.8**	469.8±19.3##	503.7±22.8#	508.9±24.2#	479.2±20.9##	475.1±22.8##
	14 weeks	432.3±14.5	546.4±25.3**	483.9±22.8##	521.4±24.1##	527.2±25.6##	493.8±22.1##	488.3±21.9##

****P<0.01 *vs* blank group; #P<0.05, ##P<0.01*vs* model group were analyzed using one-way ANOVA. HFD: high Fat-Diet; XZK:XueZhiKang; H-L: Hederagenin low dose group; H-M: Hederagenin medium dose group; H-H:Hederagenin high-dose group.**

in the model group rats were lower than blank group rats(42.3% and 48.0%)respectively (Fig. 2b-c, $P < 0.01$). However, XZK, simvastatin, H-L, H-M, and H-H groups increased dramatically the activity of SOD (36.7%, 48.4%, 25.1%, 31.2% and 38.0%(respectively) ($P < 0.01$) and GSH-PX (46.4%, 65.9%, 31.2%, 40.4% and 55.2%(respectively) ($P<0.05$) compared with model group. These results indicated that hederagenin treatment could improve the response to oxidative stress in rats induced by HFD.

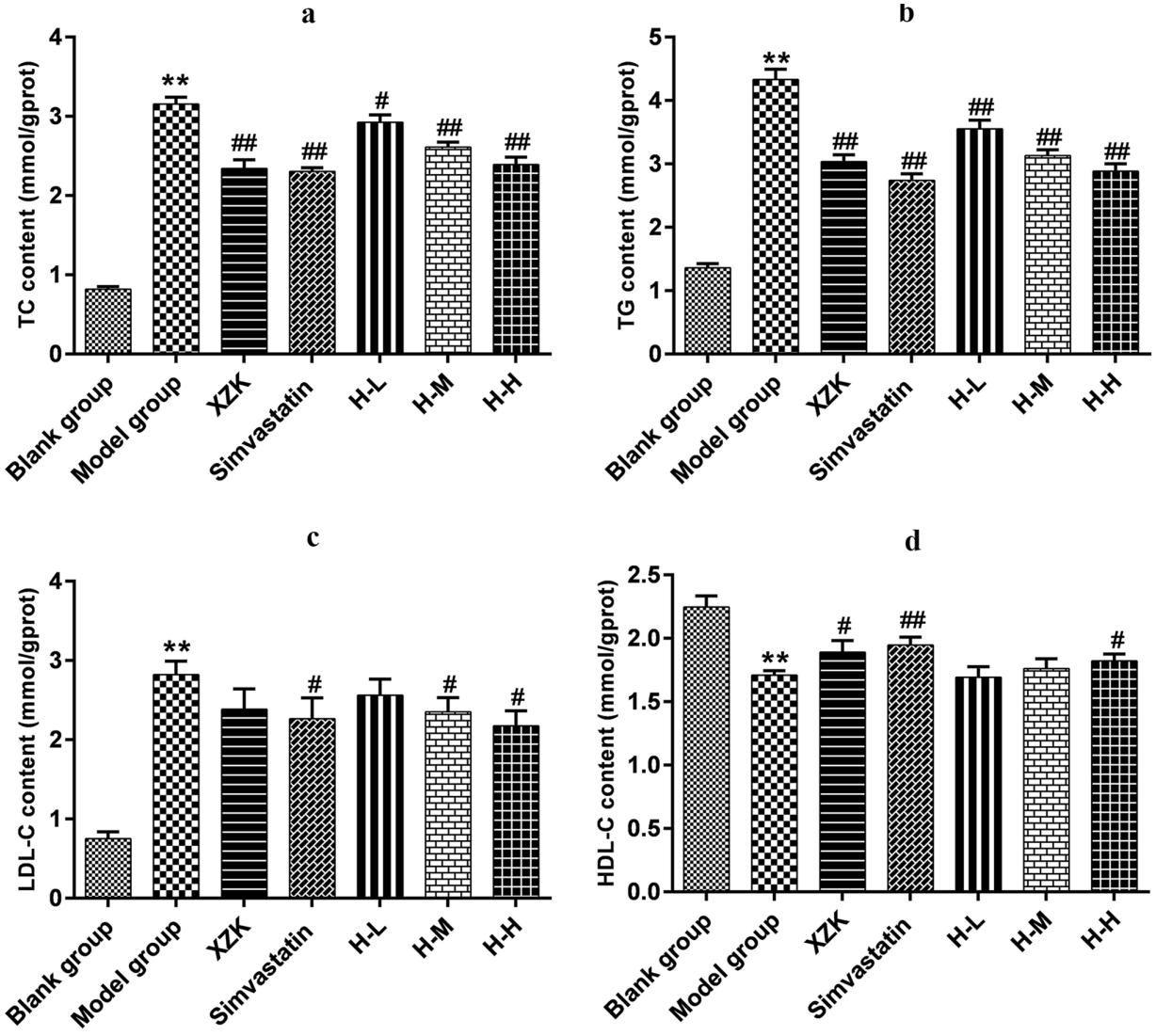

Figure 1. Effects of hederagenin on the level of lipid (n = 8). A: Effects of hederagenin on TC in serum. B: Effects of hederagenin on TG in serum. C: Effects of hederagenin on LDL-C in serum. D: Effects of hederagenin on HDL-C in serum. H-L: Low-dose of hederagenin H-M: Middle-dose of hederagenin; H-H: High- dose of hederagenin. The values are shown as the means ± sd, n=10 for each group. Significant differences ($^{**}P<0.01$ *vs* blank group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* model group) were analyzed using one-way ANOVA. TC: Total cholesterol; TG: Triglyceride; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; XZK: XueZhiKang; H-L: Hederagenin low dose group; H-M: Hederagenin medium dose group; H-H: Hederagenin high-dose group.

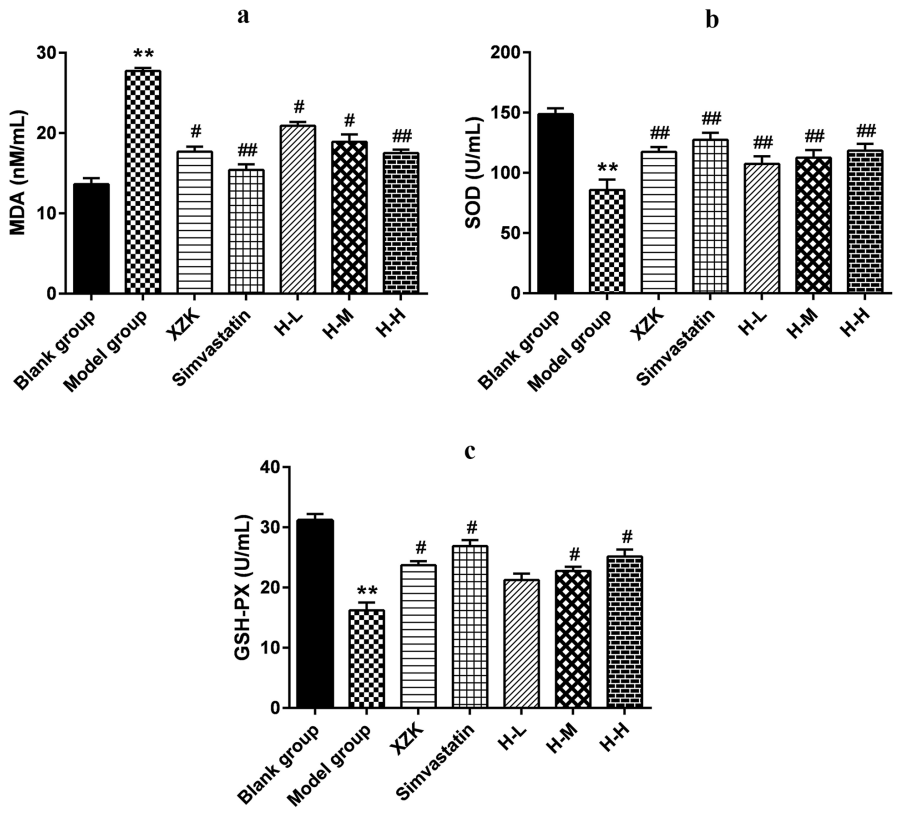

Figure 2. Effects of hederagenin on response to oxidative stress in liver (n = 8). a: Effect of hederagenin on SOD in serum. b: Effect of hederagenin on MDA in serum. c: Effects of hederagenin on GSH-PX in serum. H-L: Low-dose hederagenin H-M: Middle-dose hederagenin; H-H: High-dose hederagenin. Significant differences (***P*<0.01vs blank group; $^{\#}$*P*<0.05, $^{\#\#}$*P*<0.01vs model group) were analyzed using one-way ANOVA. MDA: Malondialdehyde; SOD: Total superoxide dismutase; GSH-PX: Glutathione peroxidase; XZK: XueZhiKang; H-L: Hederagenin low dose group; H-M: Hederagenin medium dose group; H-H: Hederagenin high-dose group.**

Effect of Hederagenin on the p38MAPK Pathway

How hederagenin improved the response to oxidative stress in rats induced by HFD is not clear. Therefore, the proteins related to p38MAPK pathway were tested with Western blot. Fig. 3 displayed the effects of hederagenin on the levels of total- p38 and p-p38. There was no striking difference in total-p38 expression between blank group and other groups. However, HFD induced p38MAPK phosphorylation, leading to p38MAPK signaling activation. Compared to the blank group, phosphorylation of p38MAPK in the model group significantly overexpressed. In treatment groups (simvastatin, XZK, H-L, H-M, and H-H), however, the expression of phosphorylation of p38MAPK significantly decreased. Interestingly, the hederagenin treatment groups were observed in dose-dependent manner in decrease of phosphorylation of p38MAPK.

DISCUSSION

Hyperlipidemia with TC, TG, LDL-C and HDL-C as main reference indicators is a leading cause of death from cardiovascular diseases throughout the globe (Le 2008). It is well known that hyperlipidemia is key risk factor of heart diseases, including atherosclerosis and coronary heart disease (Rosenson 2006, Le & Walter 2007, Das et al. 2010). Statins are the mainstay of lipid-lowering therapy in clinic which primarily act on LDL, also slightly lower the level of TG, and induce a moderate increase in HDL-C. However, statins have additional risk factors of increasing side effects and adverse drug interactions. In the meantime, previous studies had shown that hederagenin could effectively treat tumors through anti-oxidation (Kim et al. 2017a) and anti-inflammation (Lee et al. 2015). Lowering blood lipids is often closely related to properties of anti-oxidation (Stein et al. 2020, Gimeno-Mallench

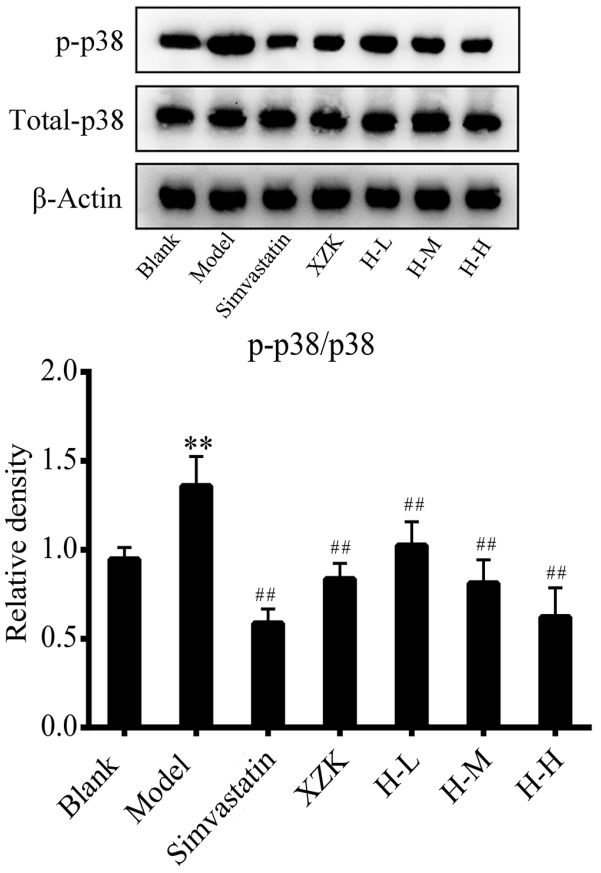

Figure 3. Effects of hederagenin treatment on the p38MAPK pathway in the rats (n = 3). *****P*<0.01*vs* **blank group; ##*P*<0.01 *vs* model group were analyzed using one-way ANOVA. p38MAPK: p38 isoform mitogen-activated protein kinase; p-p38: Phosphorylated p38 mitogen-activating protease; XZK: XueZhiKang; H-L: Hederagenin low dose group; H-M: Hederagenin medium dose group; H-H: Hederagenin high-dose group.

et al. 2019) and anti-inflammation (Canesi et al. 2019). However, there are no studies have reported that hederagenin can decrease blood lipid. Therefore, we hypothesized that hederagenin had the effect of reducing blood lipids and conducted a preliminary study on its mechanism. *In vitro* studies, preliminary results showed that hederagenin (100 mol/L, 250 mol/L) had beneficial effect on oleic acid induced-HepG2 cells. This proved that hederagenin could protect HepG2 cells from oleic acid by reducing intracellular TG level.

This study adopted a HFD to establish an animal model of hyperlipidemia similar to human disease, with highly reproducible results and easy operation. Previous studies had shown that rats who were fed a high cholesterol diet for 6 weeks showed a significant increase levels of TC and LDL-C in serum (Fki et al. 2005), which was consistent with the results of our study. Our study demonstrates that hederagenin could effectively slow the trend of weight gain in rats fed a HFD. In addition, this study also showed that hederagenin had a dose-dependent effect, the reasons for this were unclear, and we suspected that it might be related to the suppression of lipid absorption.

Our data demonstrated hederagenin notably improved lipid profile, thereby exerting hypolipidemic effect. Among them, hederagenin was effective in reducing TG which is a key risk factor of atherosclerosis, heart disease and fatty liver. If there is overabundance of TG, hoarding in the subcutaneous would lead to body obesity; hoarding in the blood vessel wall would cause atherosclerosis; hoarding in the heart would lead to heart hypertrophy; and hoarding in the liver would cause fatty liver. These were the leading causes of mortality throughout the globe (Le 2008). As we know, liver is one of the main organs of TG synthesis and metabolism. Liver cells are involved in many stages of lipid metabolism; therefore, it is possible that hyperlipidemia drives the development of fatty liver (Lee et al. 2014, Tessari et al. 2009). It is reported that most cholesterol is synthesized by the body itself, and a little is obtained from the diet (Kapourchali et al. 2016). On the contrary, TG are mostly obtained from the diet and a small part is made by the body itself (Alves-Bezerra & Cohen 2017). Therefore, the best way to reduce TG is to inhibit TG absorption. Our results show that Hederagenin has a good TG clearance rate and is dose-dependent, which may

be the reason why hederagenin has an effective anti-lipid effect.

Oxidative stress is the unifying mechanism for causing many cardiovascular diseases, especially in atherogenesis (Singh & Jialal 2006, Marchio et al. 2019, Peluso et al. 2012). In addition, we learned that the main characteristics of anti-oxidation was to reduce TG, increase HDL and adiponectin, and prevent LDL from being oxidized. Therefore, we selected SOD, MDA, and GSH-PX as anti-oxidative indicators to verify the antioxidant capacity of hederagenin. The results showed HFD induced oxidative stress reaction in rats, which were consistent with previous studies (Meng et al. 2011). Our results also showed that the blood lipid levels and anti-oxidative index of hederagenin showed a precise correlation (Fig. 1 and Fig. 2). Therefore, we believed that anti-oxidative stress may be one of the lipid-lowering mechanisms of hederagenin, which was consistent with other studies (Wang et al. 2020, Pengrattanachot et al. 2020) on lipid lowering and anti-oxidation results. Thus, hederagenin might be used as an antioxidant to treat hyperlipidemia.

Oxidative stress is well known to stimulate the MAPK pathway activation, especially the phosphorylation of p38MAPK. Furthermore, in order to further confirm hederagenin anti-oxidation activity, we conducted experiments from a molecular perspective and offered a plausible explanation for the molecular mechanism of hederagenin on decreasing blood lipid. The results of this study showed that HFD promoted phosphorylation of p38MAPK. The affections of oxidative stress on cellular signaling pathways and activation of p38MAPK pathways were inhibited by treatment with hederagenin, simvastatin and XZK.

CONCLUSION

In conclusion, the present study identified the scientific basis for the therapeutic effects of hederagenin in the treatment of hyperlipidemia. Our results found that hederagenin possessed the anti-oxidative potential for lipids-lowering in the rats received HFD, which might be related to suppression of p38MAPK phosphorylation. Hederagenin could inhibit MAPK pathway activation, thus exert anti-oxidation and lipids-lowering effect in rats induced by HFD.

Abbreviations

BSA	Bovine serum protein
DMEM	DMEM dulbecco's modified eagle medium
FBS	Fetal bovine serum
GSH-PX	Glutathione peroxidase
HDL-C	High density lipoprotein cholesterol
HFD	high fat-diet
H-H	Hederagenin high-dose group
H-L	Hederagenin low dose group
HLP	Hyperlipoidemia
H-M	Hederagenin medium dose group
LDL-C	Low density lipoprotein cholesterol
MDA	Malondialdehyde
p38MAPK	p38 isoform mitogen-activated protein kinase
PVDF	polyvinylidene fluoride
SD	Sprague–Dawley
SOD	Total superoxide dismutase
TC	Total cholesterol
TG	Triglyceride
XZK	Xuezhikang

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