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Inhibitory effects on the HMG-CoA Reductase in the chemical constituents of the *Cassia mimosoides* Linn

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Abstract

Cassia mimosoides Linn has been used from ancient times and used for treating hepatitis for its supposedly medically beneficial properties. In this study, different constituents of the Cassia mimosoides Linn (β-Sitosterol, Oleanolic Acid, Emodin, Carotene, Resorcinol, Luteolin, and α-L-Rhamnose) were evaluated for potential anti-HMG-CoA reductase effect. The inhibitory effects of HMG-CoA reductase of Cassia mimosoides Linn extracts and Pravastatin inhibitor at different concentrations (at doses of 1, 5, 25 or 125 μg/mL, respectively) in reaction system (70 mmol/L phosphate buffer, 200mmol/L NADPH, 5 μg HMG-CoA reductase, 2 mmol/L EDTA, 2 mmol/L cysteamine, 0.06% BSA) into 37°C preheat HMG-CoA for initiating this reaction, and then determined the change of HMG-CoA reductase activity (ΔAΔt) at 340 nm, the inhibition ratio of HMG-CoA reductase activity and its dynamic change of inhibitory effect within 15 min and the descent rate of NADPH. Emodin, Luteolin, β-Sitosterol, Oleanolic Acid, α-L-Rhamnose and Carotene showed good inhibition of HMG-CoA reductase activity. Among them, only the Emodin (1 and 5 μg/mL) groups showed a significant decrease of HMG-CoA reductase activity compared to the Pravastatin (1 and 5 μg/mL) groups respectively. In addition, the HMG-CoA reductase activity in the Emodin and Luteolin (25 and 125 μg/mL) groups was clearly lower than the Pravastatin (25 and 125 μg/mL) groups respectively. And the Emodin and Luteolin (1, 5, 25 or 125 μg/mL) groups exhibited a stable effect on inhibiting the HMG-CoA reductase within 15 min. These findings further support the exploration of Cassia mimosoides Linn as a potential agent for the treatment of hepatitis in future studies.

Keywords: *Cassia mimosoides Linn, 3-Hydroxy-3-methylglutaryl-CoA reductase, Emodin, Luteolin, Chinese herbal medicines.*

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Introduction

A recent study reported that hyperlipidemia is significantly associated with the pathogenesis of cardiovascular and cerebrovascular disease [1]. The improvement of people's living standards, lifestyle and diet structure play an important role in hyperlipidemia [2]. Long-time intake of high-fat, high-sugar and high-salt diets and sedentary lifestyle could ultimately cause a higher incidence of obesity, hypertension, diabetes mellitus, hyperlipidemia, coronary heart and cerebrovascular diseases [3]. Therefore, strict control of blood lipid may help to slow down the progression of cardiovascular disease.

Additionally, it has been proved that abnormally high levels of lipids in the blood are closely related to hyperlipidemia, including cholesterol and triglycerides. It is known that hyperlipidemia is a major risk factor of coronary artery disease, which remains the leading cause of death worldwide. It is also found that the most common type of hyperlipidemia was correlated with elevated serum low-density lipoprotein and triglycerides in the very low-density lipoprotein [4]. All of the above imply that serum low-density lipoprotein cholesterol levels seem to be the only reversible risk factor for cardiovascular disease [5].

3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) is a regulatory enzyme that catalyzes the committed step in cholesterol biosynthesis [6]. Statin drugs (such as pravastatin, atorvastatin and simvastatin) were used for preventing the process of HMG-CoA converting into mevalonate by HMG-CoA reductase, which is a rate-limiting step in cholesterol biosynthesis [7]. Another study displays that statin drugs could reduce "bad" plasma low-density lipoprotein cholesterol levels against hypercholesterolemia effectively via inhibiting the HMG-CoA reductase in the liver [8]. However, it has been proved that long-term use of these statin drugs would cause severe adverse drug reactions, including myopathy and disturbances in hepatic function and sexual disorders, drastically reducing the compliance and life-quality of patients [9]. Due to the side effects of statin drugs, natural HMG-CoA reductase inhibitors of plant origin are needed.

According to recent reports, the same genus and species of Cassia mimosoides Linn, Cassia alata Linn shows antidiabetic effects on inhibiting α-glucosidase, carbohydrate digestion and bile secretion [10]; Cassia auriculata Linn has hepatoprotective effects on ethanol and antitubercular drug-induced hepatotoxicity in experimental models [11]; Cassia sophera leaves have potent hepatoprotective action against carbon tetrachloride-induced hepatic damage in rats [12]; Cassia tora Linn presents a hypolipidemic effect in rats fed a high-cholesterol diet [13]. However, Cassia mimosoides Linn is a commonly used folk herbal that shows a protective effect on the liver, but its scientific evidence has been lacking until now. β-Sitosterol, Oleanolic Acid, Emodin, Carotene, Resorcinol, Luteolin, and α-L-Rhamnose are the chemical constituents of Cassia mimosoides Linn [14]. To determine whether the chemical constituents of Cassia mimosoides Linn can inhibit the HMG-CoA reductase activity and compare the inhibitory effect of the HMG-CoA reductase in these chemical constituents, we used Pravastatin as positive control drug and provided experimental basis for Cassia mimosoides Linn in the hyperlipidemia treatment or liver diseases.

Materials and methods

Chemical reagents and Drugs

Ethylene Diamine Tetraacetic Acid (EDTA), cysteamine, Bovine Serum Albumin (BSA), nicotinamide adenine dinucleotide phosphate (NADPH), 3-hydroxy-3-methyl glutar-

yl coenzyme A (HMG-CoA), 3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), and Pravastatin were purchased from Sigma, USA. Phosphate buffer (PBS) was purchased from HyClone, American. In this study, seven main chemical components of *Cassia mimosoides* Linn are: β-Sitosterol, Oleanolic Acid, Emodin, Carotene, Resorcinol, Luteolin, α-L-Rhamnose, which were screened for anti-HMG-CoA reductase activity and purchased from Sigma, USA.

The preparation of reaction system

The reaction system containing 70 mmol/L PBS (PH 7.0), 2 mmol/L EDTA, 2 mmol/L cysteamine, 0.06% BSA, 200 mmol/L NADPH, 30 μg HMG-CoA reductase, 6 μmol/L HMG-CoA was suitable for the evaluation of the inhibition of HMG-CoA reductase activity by Chinese herbal ingredients.

The preparation of different concentrations of drugs

To investigate the effects of the different components of *Cassia mimosoides* Linn on HMG-CoA reductase activity, we used the concentrations of HMG-CoA, HMG-CoA reductase and NADPH substrate as evaluation parameters in the 1mL reaction system. The drugs were first dissolved in dimethylsulfoxide, and then were made up into different concentrations with PBS as follows: β-Sitosterol (1, 5, 25 or 125 μg/mL), Oleanolic Acid (1, 5, 25 or 125 μg/mL), Emodin (1, 5, 25 or 125 μg/mL), Carotene (1, 5, 25 or 125 μg/mL), Resorcinol (1, 5, 25 or 125 μg/mL), Luteolin (1, 5, 25 or 125 μg/mL), α-L-Rhamnose (1, 5, 25 or 125 μg/mL), the positive control group of Pravastatin (1, 5, 25 or 125 μg/mL).

Inhibition of HMG-CoA Reductase Activity [15]

HMG-CoA reductase inhibitory activity of the main chemical components of *Cassia mimosoides* Linn was determined based on spectrophotometric measurements [16]. The HMG-CoA reductase was purchased from Sigma-Aldrich Co. (St Louis, MO, USA). The specific activity of the HMG-CoA reductase stock solution was 2-8 units/mg protein. The different concentrations of 7 chemical components of *Cassia mimosoides* Linn and Pravastatin were mixed, a reaction mixture we have prepared respectively. The reaction was incubated at 37°C, and absorbance was measured at 340 nm after 1 min. Pravastatin was used as a positive control, and distilled water as a negative control. The enzyme activity unit is expressed as $1U=1nmol•min^{-1}•mg^{-1}$. The activity of HMG-CoA reductase inhibition (%) was calculated using the following formula:

$$\text{Inhibition of HMG-CoA (\%)} = \frac{\text{The different drugs activity of different concentrations}}{\text{The activity of distilled water (0 μg/mL)}} \text{ X } 100\%$$

The change of HMG-CoA Reductase Activity [17]

1, 5, 25 or 125 μg/mL of β-Sitosterol, Oleanolic Acid, Emodin, Carotene, Resorcinol, Luteolin, α-L-Rhamnose, Pravastatin respectively were mixed into the reaction mixture for the HMG-CoA activity detection. The reaction without HMG-CoA reaction substrate was incubated at 37°C, and absorbance was measured at 340 nm after 1 min and recorded as $\Delta A_1\Delta t_1$. In addition, the reaction with HMG-CoA reaction substrate (0.6 μg/mL) was incubated at 37°C, and absorbance was measured at 340 nm after 1 min and recorded as $\Delta A_2\Delta t_2$. The difference is the change of HMG-CoA reductase activity ($\Delta A\Delta t=\Delta A_2\Delta t_2-\Delta A_1\Delta t_1$). And the $\Delta A\Delta t$ value is stand for the reaction velocity of HMG-CoA reductase.

The change of HMG-CoA Reductase Activity within 15 min

To further investigate the dynamic change of

HMG-CoA reductase activity, we detected the HMG-CoA reductase activity after Emodin, Luteolin and Pravastatin treatment, respectively within 15 min. The Emodin, Luteolin and Pravastatin (at the doses of 1, 5, 25 or 125 μg/mL, respectively) were mixed into the reaction mixture for the HMG-CoA activity detection. The reaction with HMG-CoA reaction substrate (0.6 μg/mL) was incubated at 37°C, and absorbance was measured at 340 nm every 30 s for 15 min and the HMG-CoA reductase activity was recorded. Pravastatin was used as a positive control, and distilled water (0 μg/mL) as a negative control.

Statistical analysis

The data were analyzed using SPSS 13.0 software and a one-way ANOVA analysis. The results are expressed as $\bar{x} \pm s$. P values < 0.05 were considered statistically significant.

Results

The inhibition ratio of HMG-CoA Reductase Activity in all groups

As shown in **Figure 1**, there was not statistically significant inhibition ratio of the HMG-CoA reductase activity among all the groups without chemical constituents of the *Cassia mimosoides Linn* or Pravastatin. As compared to the 1 μg/mL Pravastatin group, the inhibition ratio of HMG-CoA reductase activity was clearly higher in the doses of 1, 5, 25 and 125 μg/mL of Emodin groups (P<0.05), the doses of 5, 25 and 125 μg/mL of Luteolin and Pravastatin groups (P<0.05), and the doses of 25 and 125 μg/mL of β-Sitosterol, Oleanolic Acid, α-L-Rhamnose and Carotene groups (P<0.05). The inhibition ratio of HMG-CoA reductase activity was significantly higher in the doses of 5, 25 and 125 of μg/mL Emodin

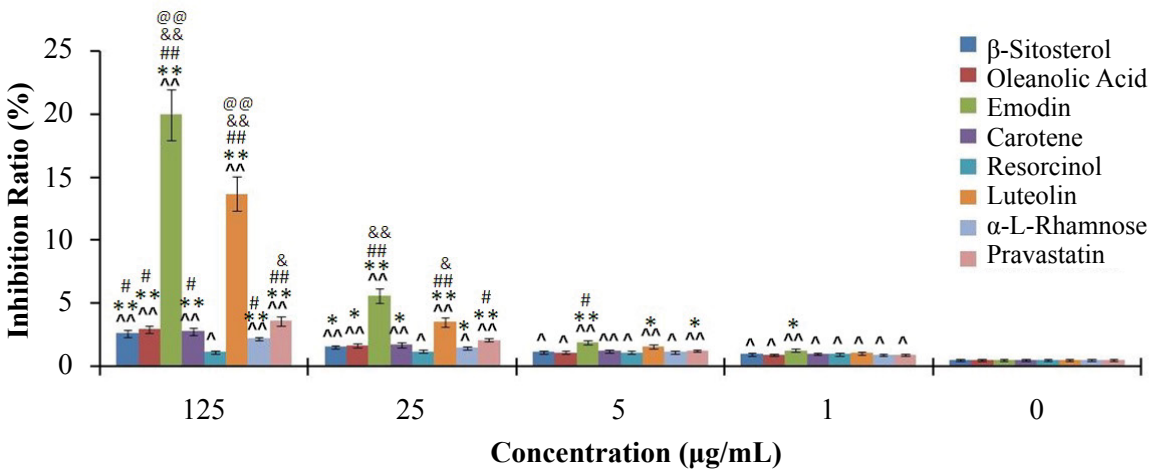

Figure 1. The inhibition ratio of the HMG-CoA Reductase Activity in all groups.

As compared to the 1, 5, 25 or125 μg/mL of Pravastatin groups respectively, the 1, 5, 25 or 125 μg/mL of Emodin groups all showed the highest inhibition ratio of the HMG-CoA reductase activity in the other drug-treated groups. Next, the second highest inhibition ratio of the HMG-CoA reductase activity was the 5, 25 and 125 μg/mL of Luteolin groups.

*Note: ^P<0.05, ^^P<0.01 compared to 0 μg/mL of Pravastatin group; *P<0.05, **P<0.01 compared to 1 μg/mL of Pravastatin group; #P<0.05, ##P<0.01 compared to 5 μg/mL of Pravastatin group; &P<0.05, &&P<0.01 compared to 25 μg/mL of Pravastatin group; @@ P<0.01 compared to 125 μg/mL of Pravastatin group.*

groups (P<0.05), the doses of 25 and 125 μg/mL of Luteolin and Pravastatin groups (P<0.05), and the dose of 125 μg/mL of β-Sitosterol, Oleanolic Acid, α-L-Rhamnose and Carotene groups (P<0.05) compared to the dose of 5 μg/mL of Pravastatin group. The inhibition ratio of HMG-CoA reductase activity was obviously higher in the doses of 25 and 125 μg/mL of Emodin and Luteolin groups (P<0.05), and the dose of 125 μg/mL of Pravastatin group (P<0.05) when compared to the dose of 25 μg/mL of Pravastatin group. In addition, the inhibition ratio of HMG-CoA reductase activity in the dose of 125 μg/mL of Emodin and Luteolin groups was greater than the dose of 125 μg/mL of Pravastatin group (P<0.01) (**Figure 1**).

The change of HMG-CoA Reductase Activity in all groups

As shown in **Table 1**, there was no significant difference of the change of HMG-CoA reductase activity among all the groups (do not contain the chemical constituents of the *Cassia mimosoides Linn* or Pravastatin). Also, there was no significant difference of the change of HMG-CoA reductase activity between the Resorcinol and the other groups, respectively. As compared to HMG-CoA reductase activity of Pravastatin (1 μg/mL) group, a significant decrease of HMG-CoA reductase activity was exhibited in the Emodin (1, 5, 25 and 125 μg/mL) groups (P<0.01), the Luteolin and Pravastatin (5, 25 and 125 μg/mL) groups (P<0.01), Oleanolic Acid and α-L-Rhamnose (25 and 125 μg/mL) groups (P<0.05), and the β-Sitosterol and Carotene (125 μg/mL) groups (P<0.01). As compared to the HMG-CoA reductase activity of Pravastatin (5 μg/mL), a significant decline of HMG-CoA reductase activity was showed in the Emodin (5, 25 and 125 μg/mL) groups (P<0.01), the Luteolin and Pravastatin (25, 125 μg/mL) groups (P<0.05), and the α-L-Rhamnose (125 μg/mL) group (P<0.05). As compared to the HMG-CoA reductase activity of Pravastatin (25 μg/mL), a clear drop of HMG-CoA reductase activi-

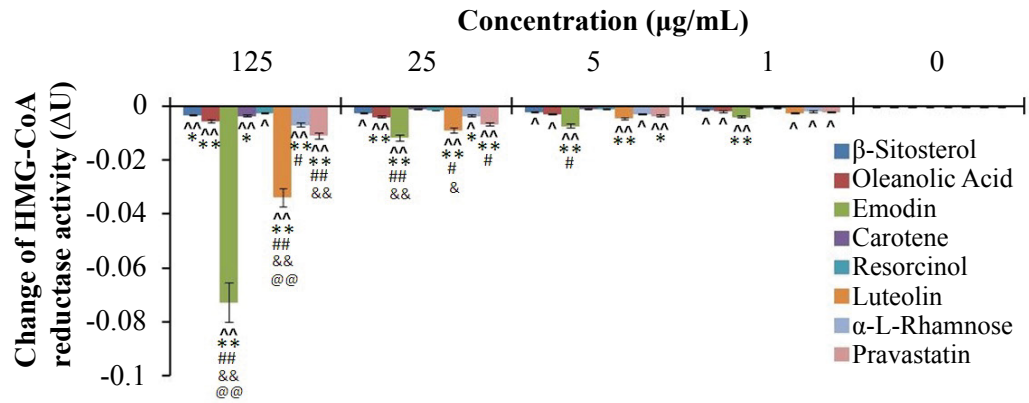

Figure 2. The change of the HMG-CoA Reductase Activity in all groups.

As compared to the 1, 5, 25 and 125 μg/mL of Pravastatin groups respectively, the 1, 5, 25 and 125 μg/mL of Emodin groups all exhibited the least activity of HMG-CoA reductase in the other drug groups.

***Note:** ^P<0.05, ^^P<0.01 compared to 0 μg/mL of Pravastatin group; *P<0.05, **P<0.01 compared to 1 μg/mL of Pravastatin group; #P<0.05, ##P<0.01 compared to 5 μg/mL of Pravastatin group; &P<0.05, &&P<0.01 compared to 25 μg/mL of Pravastatin group; @@P<0.01 compared to 125 μg/mL of Pravastatin group.*

Table 1. The change of the HMG-CoA Reductase Activity in all groups ($\Delta A \Delta t$, $*10^{-3}$)

Groups Dosages	β-Sitosterol	Oleanolic Acid	Emodin	Carotene	Resorcinol	Luteolin	α-L-Rhamnose	Pravastatin
125 μg/mL	-4.33± 0. 33^^*	-5.67± 0. 57^^**	-73.00± 7.30 ^^**##&&@@	-4.67± 0. 37^^*	-2.67± 0. 27^	-34.02± 3.40 ^^**##&&@@	-7.02± 0. 70^^**#	-11.01± 1.10 ^^**##&&
25 μg/mL	-2.87± 0. 27^	-4.03± 0. 40^^*	-11.67± 1.17 ^^**##&&	-3.17± 0. 12	-1.67± 0. 17	-9.01± 0. 90 ^^**#&	-3.67± 0. 36^*	-6.67± 0. 67 ^^**#
5 μg/mL	-2.33± 0. 23^	-3.10± 0. 31^	-7.33± 0. 73 ^^**#	-1.04± 0. 10	-1.07± 0. 11	-4.67± 0. 47 ^^**	-3.01± 0. 30^	-3.67± 0. 37^^*
1 μg/mL	-1.33± 0. 13^	-2.02± 0. 20^	-4.01± 0. 38^^**	-0. 67± 0.07	-0. 67± 0.07	-2.67± 0. 27^	-2.01± 0. 20^	-2.33± 0. 23^
0 μg/mL	-0. 33± 0.03	-0. 32± 0.03	-0. 34± 0.03	-0. 34± 0.03	-0. 33± 0.03	-0. 32± 0.03	-0. 34± 0.03	-0. 31± 0.03

Result showed that there was no significant difference of the change of HMG-CoA reductase activity among all the groups at the dose of 0 μg/mL. The HMG-CoA reductase activity increased significantly in the 1 μg/mL of Emodin group as compared to 1 μg/mL of Pravastatin group (P<0.01). As compared to the 5 μg/mL of Pravastatin group, the HMG-CoA reductase activity enhanced in the 5 μg/mL of Emodin group (P<0.05). When compared to the 25 μg/mL of Pravastatin group, the HMG-CoA reductase activity increased in the Emodin and Luteolin (5 μg/mL) groups (P<0.05). In addition, the HMG-CoA reductase activity increased significantly in the Emodin and Luteolin (125 μg/mL) groups as compared to 125 μg/mL of Pravastatin group (P<0.01).

*Note: ^P<0.05, ^^P<0.01 compared to 0 μg/mL of Pravastatin group; *P<0.05, **P<0.01 compared to 1 μg/mL of Pravastatin group; #P<0.05, ##P<0.01 compared to 5 μg/mL of Pravastatin group; &P<0.05, &&P<0.01 compared to 25 μg/mL of Pravastatin group; @@ P<0.01 compared to 125 μg/mL of Pravastatin group.*

ty was presented in the Emodin and Luteolin (25 and 125 μg/mL) groups (P<0.05) and the Pravastatin(125 μg/mL) group (P<0.05). However, an obvious decrease of the HMG-CoA reductase activity was exhibited in the Emodin and Luteolin (125 μg/mL) groups when compared with the HMG-CoA reductase activity of Pravastatin (125 μg/mL) (P<0.01) (**Table 1**, **Figure 2**).

The alteration of HMG-CoA Reductase Activity inhibition ratio within 15 min

Results showed that Emodin and Luteolin had a clear inhibitory effect on HMG-CoA reductase activity. In order to analyze the dynamic change of Emodin and Luteolin in HMG-CoA reductase activity, we investigated their inhibition ratio every 30 s for 15 min. The results showed that the HMG-CoA reductase activity inhibition ratio showed a stable trend within 15 min in the Emodin, Luteolin and Pravastatin (1, 5 or 25 μg/mL) groups. However, HMG-CoA reductase activity inhibition ratio displayed a sharp reduction trend during 15 min in the Emodin (125 μg/mL) group. In addition, the HMG-CoA reductase activity inhibition ratio also exhibited a stable trend in the Luteolin and Pravastatin (125 μg/mL) groups (**Figure 3**).

Discussion

Precursors of sterols, carotenoids, the prenyl groups of several proteins and other terpenoid compounds are synthesised via the acetate-mevalonate pathway [18]. One of the key enzyme of this pathway is the HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonate [19, 20]. Scientific literature shows that β-sitosterol and algal sterols reduced the plasma cholesterol levels by down-regulating the intestinal HMG-CoA reductase [21]. Emodin, the active component of Polygonum Multiflorum Radix, could inhibit the HMG-CoA reductase and contribute to the lipid metabolism [22]. β-carotene cannot be used together with cholesterol-lowering drugs. Evidence revealed that overexpression of 3-Hydroxy-3-methylglutaryl-coenzyme A reductase 2 (hmgR2) and 3-Hydroxy-3-methylglutaryl-coenzyme A re-

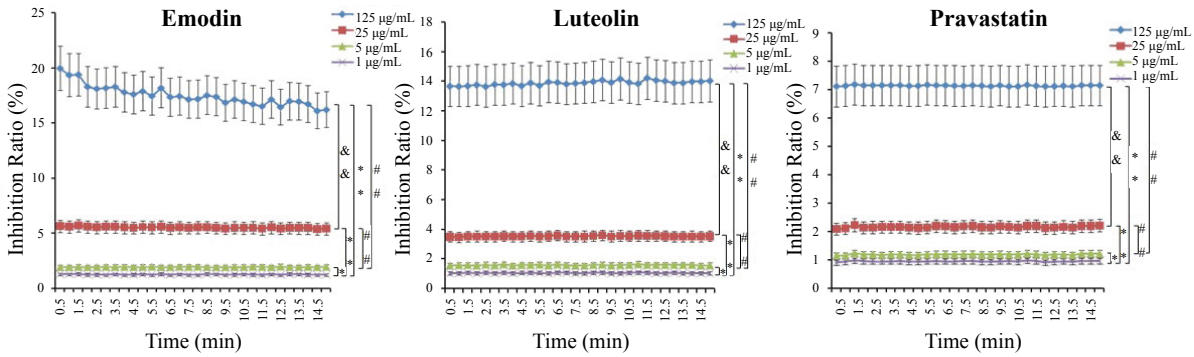

Figure 3. The dynamic change of HMG-CoA Reductase Activity inhibition ratio within 15 min.

There was no significant change of the inhibition ratio of HMG-CoA reductase activity among the 1, 5 and 25 μg/mL of Emodin, Luteolin and Pravastatin groups. In addition, the 125 μg/mL of Emodin group showed a marked decline of the inhibition ratio of HMG-CoA reductase activity during the 15 min, however, its inhibition ratio was higher than the 125 μg/mL of Luteolin and Pravastatin groups.

***Note:** *P<0.05, **P<0.01 compared to 1 μg/mL of Pravastatin group; #P<0.05, ##P<0.01 compared to 5 μg/mL of Pravastatin group; &P<0.05, &&P<0.01 compared to 25 μg/mL of Pravastatin group.*

ductase 3 (hmgR3) may be used to improve the carotenoid content [18]; Moreover, a recent study found that over-expression of HMG-CoA reductase coupled with addition of ergosterol biosynthesis inhibitors could increase the production of β-carotene in recombinant saccharomyces cerevisiae [23]. Furthermore, Echinocystic Acid exhibited no significant effect on the HMG-CoA reductase activity, however, it showed hypolipidemic activity by inhibiting cholesterol acyltransferase and diacylglycerol acyltransferase [24]. Luteolin is a representational and natural flavone, its distribution being very extensive in the vegetable kingdom [25]. The effects of α-L-Rhamnose, Oleanolic Acid, Resorcinol and Luteolin on HMG-CoA reductase inhibitory activity have been still unclear until now.

To study the effects on the anti-HMG-CoA reductase activity of the seven main chemical components of *Cassia mimosoides* Linn, we used the concentrations of HMG-CoA, HMG-CoA reductase and NADPH substrate as evaluation parameters in the 1mL reaction system for detecting the HMG-CoA reductase activity after drug intervention. Results of the change of the HMG-CoA reductase activity showed that all doses of Resorcinol groups showed no significant effect on the activity of HMG-CoA reductase compared to the Pravastatin groups. The HMG-CoA reductase activity decreased significantly in the β-Sitosterol and Carotene groups (at a dose of 125 μg/mL), the Oleanolic Acid and α-L-Rhamnose groups (at doses of 25 and 125 μg/mL), the Luteolin groups (at doses of 5, 25 and 125 μg/mL) and the Emodin groups (at doses of 1, 5, 25 and 125 μg/mL) compared to the dose of 1 μg/mL of Pravastatin. These results suggested that only Emodin could inhibit the HMG-CoA reductase activity at the dose of 1 μg/mL in all the drug groups. In addition, HMG-CoA reductase activity dropped clearly in the α-L-Rhamnose group (at a dose of 125 μg/mL), the Luteolin groups (at doses of 25 and 125 μg/mL) and the Emodin groups (at doses of 5, 25 and 125 μg/mL) compared to the dose of 5 μg/mL of Pravastatin. These results suggested that only Emodin could inhibit the HMG-CoA reductase activity at the dose of 5 μg/mL in all the drug groups. Besides, the HMG-CoA reductase activity declined clearly in the Emodin and Luteolin groups (at doses of 25 and 125 μg/mL) compared to the dose of 25 μg/mL of Pravastatin. This result suggested that only Emodin and Luteolin could inhibit the HMG-CoA reductase activity at the dose of 25 μg/mL in all the drug groups. Finally, the HMG-CoA reductase activity decreased clearly in the Emodin and Luteolin groups (at a dose of 125 μg/mL) compared to the 125 μg/mL of Pravastatin. This result suggested that the Emodin and Luteolin groups showed a lower HMG-CoA reductase activity than the Pravastatin group at a dose of 125 μg/mL. All of these results indicated that HMG-CoA reductase activity ranked from small to great as follows: Emodin < Luteolin < α-L-Rhamnose < Oleanolic Acid < Carotene < β-Sitosterol < Resorcinol.

We also found that the inhibition ratio of HMG-CoA reductase increased significantly in the β-Sitosterol, Oleanolic Acid, Carotene and α-L-Rhamnose groups (at doses of 25 and 125 μg/mL), the Luteolin groups (at doses of 5, 25 and 125 μg/mL) and the Emodin groups (at doses of 1, 5, 25 and 125 μg/mL) compared to the 1 μg/mL of Pravastatin. However, Resorcinol- and α-L-Rhamnose groups (at doses of 1, 5, 25 and 125 μg/mL) showed no significant difference in the inhibition ratio of HMG-CoA reductase. In addition, the inhibition ratio of HMG-CoA reductase elevated clearly in the β-Sitosterol, Oleanolic Acid, Carotene and α-L-Rhamnose groups (at a dose of 125 μg/mL), the Luteolin groups (at doses of 25 and 125 μg/mL) and the Emodin groups (at doses of 5, 25 and 125 μg/mL) compared to the 5 μg/mL of Pravastatin. Besides,

the inhibition ratio of HMG-CoA reductase in the Emodin and Luteolin groups (at doses of 25 and 125 μg/mL) was higher than that of the 25 μg/mL of Pravastatin. The inhibition ratio of HMG-CoA reductase increased clearly in the Emodin and Luteolin groups (at a dose of 125 μg/mL) compared to the 125 μg/mL of Pravastatin. These results indicated that the inhibition ratio of HMG-CoA reductase ranked from high to low as follows: Emodin > Luteolin > β-Sitosterol, Oleanolic Acid, Carotene and α-L-Rhamnose > Resorcinol.

We also found that the changes wave of HMG-CoA reductase activity inhibition ratio in Emodin and Luteolin groups (at doses of 1, 5, 25 and 125 μg/mL) were not big during 15 min. This result indicated that Emodin and Luteolin both showed a stable effect on inhibiting the HMG-CoA reductase within 15 min.

Conclusions

In conclusion, Emodin, Luteolin, β-Sitosterol, Oleanolic Acid, α-L-Rhamnose and Carotene could inhibit HMG-CoA reductase activity. Among them, the inhibitory effects of HMG-CoA reductase of Emodin and Luteolin treatment were even greater than the Pravastatin treatment. Thus, our studies could provide experimental basis for *Cassia mimosoides* Linn and support future studies regarding new potential treatments for hyperlipidemia or liver diseases.

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References

1. Kannel WB, McGee D, Gordon T. A general cardiovascular risk profile: the Framingham Study. Am J Cardiol. 1976; 38(1):46-51. DOI: 10.1016/0002-9149(76)90061-8.
2. Rodriguez-Fernandez R, Rahajeng E, Viliani F, Kushadiwijaya H, Amiya RM, Bangs MJ. Non-communicable disease risk factor patterns among mining industry workers in Papua, Indonesia: longitudinal findings from the Cardiovascular Outcomes in a Papuan Population and Estimation of Risk (COPPER) Study. Occup Environ Med. 2015;72(10):728-35. DOI: 10.1136/oemed-2014-102664.
3. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med. 1999;340(2): 115-26. DOI: 10.1056/NEJM199901143400207.
4. Zand Parsa A, Ashoori S, Abdollahi A. The Effect of Two Different Doses of Atorvastatin on Lipoprotein-a on Patients with Acute Coronary Syndrome. Iranian Journal of Pathology. 2012;7:101–6.
5. LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, et al. Treating to New Targets (TNT) Investigators. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med. 2005; 352(14):1425-35. DOI: 10.1056/NEJMoa050461.
6. Brown MS, Goldstein JL. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. J Lipid Res. 1980;21(5):505-17.
7. Carbonell T, Freire E. Binding thermodynamics of statins to HMG-CoA reductase. Biochemistry. 2005; 44(35):11741-8. DOI: 10.1021/bi050905v.
8. Friesen JA, Rodwell VW. The 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductases. Genome Biol. 2004; 5(11):248. DOI: 10.1186/gb-2004-5-11-248.
9. Maron DJ, Fazio S, Linton MF. Current perspectives on statins. Circulation. 2000;101(2):207-13. DOI: 10.1161/01.CIR.101.2.207.

10. Varghese GK, Bose LV, Habtemariam S. Antidiabetic components of Cassia alata leaves: identification through α-glucosidase inhibition studies. Pharm Biol. 2013;51(3):345-9. DOI: 10.3109/13880209.2012.729066.

11. Jaydeokar AV, Bandawane DD, Bibave KH, Patil TV. Hepatoprotective potential of Cassia auriculata roots on ethanol and antitubercular drug-induced hepatotoxicity in experimental models. Pharm Biol. 2014;52(3):344-55. DOI: 10.3109/13880209.2013.837075.

12. Mondal A, Karan SK, Singha T, Rajalingam D, Maity TK. Evaluation of Hepatoprotective Effect of Leaves of Cassia sophera Linn. Evid Based Complement Alternat Med. 2012; 2012:436139. DOI: 10.1155/2012/436139.

13. Cho IJ, Lee C, Ha TY. Hypolipidemic effect of soluble fiber isolated from seeds of Cassia tora Linn. in rats fed a high-cholesterol diet. J Agric Food Chem. 2007;55(4):1592-6. DOI: 10.1021/jf0622127.

14. Zhang JD, Hu YJ, Zhang W, Huang XA, Zhang HL. Chemical Constituents of Cassia mimosoides Linn. Journal of Tropical and Subtropical Botany. 2009;17:80-2.

15. Jung KA, Song TC, Han D, Kim IH, Kim YE, Lee CH. Cardiovascular protective properties of kiwifruit extracts in vitro. Biol Pharm Bull. 2005; 28(9):1782-5. DOI: 10.1248/bpb.28.1782.

16. Schointuch MN, Gilliam TP, Stine JE, Han X, Zhou C, Gehrig PA, Kim K, Bae-Jump VL. Simvastatin, an HMG-CoA reductase inhibitor, exhibits anti-metastatic and anti-tumorigenic effects in endometrial cancer. Gynecol Oncol. 2014;134(2): 346-55. DOI: 10.1016/j.ygyno.2014.05.015.

17. Soares RA, Mendonça S, de Castro LÍ, Menezes AC, Arêas JA. Major peptides from amaranth (Amaranthus cruentus) protein inhibit HMG-CoA reductase activity. Int J Mol Sci. 2015;16(2): 4150-60. DOI: 10.3390/ijms16024150.

18. Nagy G, Farkas A, Csernetics Á, Bencsik O, Szekeres A, Nyilasi I, Vágvölgyi C, Papp T. Transcription of the three HMG-CoA reductase genes of Mucor circinelloides. MC Microbiol. 2014;14:93. DOI: 10.1186/1471-2180-14-93.

19. Wang GY, Keasling JD. Amplification of HMG-CoA reductase production enhances carotenoid accumulation in Neurospora crassa. Metab Eng. 2002;4(3): 193-201. DOI: 10.1006/mben.2002.0225.

20. Burg JS, Espenshade PJ. Regulation of HMG-CoA reductase in mammals and yeast. Prog Lipid Res. 2011; 50(4): 403-10. DOI: 10.1016/j.plipres.2011.07.002.

21. Wang X, Guan L, Zhao Y, Lei L, Liu Y, Ma KY, Wang L, Man SW, Wang J, Huang Y, Chen ZY. Plasma cholesterol-lowering activity of dietary dihydrocholesterol in hypercholesterolemia hamsters. Atherosclerosis. 2015; 242(1):77-86. DOI: 10.1016/j.atherosclerosis.2015.06.046.

22. Wang W, He Y, Lin P, Li Y, Sun R, Gu W, Yu J, Zhao R. In vitro effects of active components of Polygonum Multiflorum Radix on enzymes involved in the lipid metabolism. J Ethnopharmacol. 2014;153(3):763-70. DOI: 10.1016/j.jep.2014.03.042.

23. Yan GL, Wen KR, Duan CQ. Enhancement of β-carotene production by over-expression of HMG-CoA reductase coupled with addition of ergosterol biosynthesis inhibitors in recombinant Saccharomyces cerevisiae. Curr Microbiol. 2012;64(2): 159-63. DOI: 10.1007/s00284-011-0044-9.

24. Han L, Lai P, Du JR. Deciphering molecular mechanism underlying hypolipidemic activity of echinocystic Acid. Evid Based Complement Alternat Med. 2014; 2014:823154. DOI: 10.1155/2014/823154.

25. Sá C, Oliveira AR, Machado C, Azevedo M, Pereira-Wilson C. Effects on Liver Lipid Metabolism of the Naturally Occurring Dietary Flavone Luteolin-7-glucoside. Evid Based Complement Alternat Med. 2015;2015:647832. DOI: 10.1155/2015/647832.