Hypolipidemic and antioxidant activities of volatile oils from fresh leaves of *Michelia martini* Levl

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Abstract: This study aimed to investigate the hypolipidemic and antioxidant activities of volatile oils from *Michelia martini* Levl. The antioxidant property of volatile oils from *Michelia martini in vitro* was investigated by establishment of various systems. High fat diet induced rats were used to assess the hypolipidemic and antioxidant activities of *Michelia martini* volatile oils *in vivo*. The level of total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, alanine transaminase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase in serum, and the activities of catalase, malondialdehyde, super oxide dismutase and glutathione in liver of rats were assayed by standard procedures. Our results showed that *Michelia martini* exhibits strong hypolipidemic and antioxidant activities both *in vitro* and *vivo*. Our data were also supplemented with histopathological studies on liver tissues and aorta sections of rats.

Keywords: Hyperlipidemia activity, antioxidant, volatile oils, *Michelia martini* Levl.

INTRODUCTION

Cardiovascular disease, a serious disease with high morbidity, mortality and disability rate, has become the main leading cause of death in many parts of the world (Fang *et al*., 2010). Atherosclerosis (AS) is one of the most common and serious cardiovascular diseases affecting the health of people seriously, while the morbidity and mortality of AS are still increasing ((Fang *et al*., 2010; Clemente *et al*., 2015). Dyslipidemia is always considered to be the most important risk factor of AS (Ji *et al*., 2013). Meanwhile, hyperlipidemia is a main cause responsible for pathological changes of AS, and disorder in lipid metabolism or abnormalities in lipoprotein also plays an important role in the pathogenesis of AS.

Plants of the genus *Michelia* (*Magnoliaceae*) contain many volatile oil constituents which has strong antioxidant activity (Dominique *et al*., 2007; Wang *et al*., 2010; Vivek *et al*., 2011; (Umadevi 2012). The main compositions of volatile oil isolated from the dried flower bud of *Michelia martinii Levl.* included α-pinene, β-pinene, camphene, β-myrcene, 2-carene, α-phellandrene, 1,8-Cineole and so on (Xu and Zhao 1989). All these volatile compounds of alcohols and terpenes have been reported to be beneficial for patients with cardiovascular diseases through aiding the circulation of the blood (Xie and Ma 2011). Moreover, the volatile oil constituents isolated from the fresh leaves of *M. martini* mainly β-myrcene, camphene, 2-carene, α-phellandrene showed synergistic hypolipidemic effects (Pan 1990). The present study was performed to explore the effects of volatile oil isolated from the fresh leaves of *M. martini* on plasma lipid level and antioxidant activity in hyperlipidemic rats, intending to provide a reference for industrialized exploitation and utilization of *M. martini*, and also for research on pharmacology, healthy function and landscape application.

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MATERIALS AND METHODS

Plant material

M. martini is a kind of evergreen arbors belonging to the genus *Michelia* (*Magnoliaceae*). The fresh leaves of *M. martini* were collected from wild forest in Yuanling, Huaihua, Hunan in April 2014 and identified by Professor Xianjin Wu in College of biology and food engineering of Huaihua University. All the voucher specimens (HHTC-D56) were preserved in herbarium of Dong minority in Huaihua University.

Preparation of samples of volatile oil constituents from fresh leaves of M. martini

The extraction methodology and chemical profiling of volatile oil in the study have previously been reported (Lei and Li 2016). The fresh leaves of *M. martini* were firstly cut into pieces, and put into 5000ml distilling flask. These leaves were boiled after addition of 3000-3500ml deionized water for 5 hours. The condenser pipe was rinsed by ether until complete delamination and the ether layer was collected. After complete volatilization, the left yellow oil was identified to be *M. martini* volatile oil. Collected samples were tightly sealed and kept in a refrigerator at 4°C.

Detection of anti-oxidative activity of M. martini volatile oil in vitro

DPPH radical scavenging assay. DPPH radical-scavenging assay was performed as previously described (Oyedemi and Afolayan 2010). Briefly, *M. martini* volatile oil was firstly diluted to a concentration of 20% by Tween 80. Then total of 3.5ml DPPH in methanol solution (0.06mmol/l) was added into 10-100µl 20% volatile oil, respectively. Thirty minutes after incubation, reactions were detected at optical density (OD) of 517nm. Each sample was operated for 3 times in parallel during this experiment. The scavenging rate of reactive DPPH was calculated by a formula as follow:

Scavenging rate (%) = [(Ac-As)/Ac]×100%

Ac represented the OD of 3.5mL DPPH mixed with Tween 80; As represented the OD of 3.5mL DPPH mixed with *M. martini* volatile oil samples.

2) ABTS-radical-scavenging assay. ABTS-radical-scavenging assay was performed as previously described (Wu *et al*., 2006). Briefly, sample of *M. martini* volatile oil was firstly diluted to a concentration of 4% by Tween 80. Then 2.85 ml ABTS solution was added into 10-100µl 4% *M. martini* volatile oil, respectively. After 10 min for incubation, reactions were detected at OD at 734 nm. Each sample was operated for 3 times in parallel during this experiment and the scavenging rate of reactive ABTS was calculated by a formula as follow:

Scavenging rate (%) = [(Ac-As)/Ac]×100%

Ac represented the OD of 2.85ml ABTS mixed with Tween 80; as represented the OD of 2.85 ml ABTS mixed with *M. martini* volatile oil samples.

Effects of M. martini volatile oil on cholesterol biosynthesis in Chinese hamster oocytes (CHO) cells

The effects of *M. martini* volatile oil on cholesterol biosynthesis were analyzed as presented previously (Sun *et al*., 1989; Xue *et al*., 2007). CHO were firstly digested into cell suspension with 0.25% trypsin. Then these cells were diluted to a concentration of 2×10^4/ml by solution A (RPMI1640 culture medium with 300µg/ml glutamine and 10% calf serum), and inoculated in a 96-well plate at 100µl each well. After attachment, cells were incubated in 200µl solution B (RPMI1640 with 300µg/ml glutamine and 10% lipoprotein free serum) and cultured for 24 h. Then cells were changed to incubate with solution C (solution B + 0.25 mmo1/l mevalonolactone) and treated with different concentrations of *M. martini* volatile oil (0.15, 0.3 and 0.6 ml/ml Tween 80) for another 24 h. In this experiment, cells cultured in medium without amphotericin B was set as control group and cultured in medium with amphotericin B was set as negative control group, and treated with simvastatin (0.5 mg/ml) were set as positive controls. After washing with PBS (pH 7.4) for twice, 200µl solution D (solution B+ 300µg/ml amphotericin B) was added into the cells and cultured for 8 h. After washing with PBS (pH 7.4)10µl MTT (5mg/ml) was added into cells and the mixture was incubated for another 4 h. Finally, 100µl 10% SDS was used to terminate the reaction and OD value was read at a wavelength of 570nm detected by enzyme-linked immune detector DG-5031. A higher OD value represented more living cells and stronger inhibitory effects on cholesterol biosynthesis in CHO cells.

Experimental animals

Male Sprague-Dawley rats (180±25g) were purchased from the animal house of the Xiangya School of Medicine, Central South University, China. These rats were kept in departmental animal houses under strict temperature control (25±2°C) with a relative humidity of 50±5% and a 12-h light/dark cycle. All the animals were provided with free access to standard laboratory food and tap water prior to the experiment. This study was performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee.

High fat diet induced hyperlipidemia in rats

Hyperlipidemia rats were induced by maintaining a high fat diet for 45 days (table 1). Then rats were randomly divided into 5groups (10rats each group) based on different treatments. Group I (normal control): 45 days of normal diet + 15 days (31th to 45th day) of 1% W/V CMC (1 ml/100g BW); Group II (model control): 45 days of high fat diet + 15 days of 1% W/V CMC; Group III (positive control): 45 days of high fat diet + 15 days of 10 mg/kg BW simvastatin; Group IV, V and VI: 45 days of high fat diet + 15 days of 0.15, 0.3 and 0.6 ml/kg BW *M. martini* volatile oil, respectively. Forty-five days after treatment, all rats were fasted for 12 h and the blood was collected by cardiac puncture under light ether anesthesia. Afterwards, rats were sacrificed, and liver tissues were excised immediately, followed by washing with ice-cold normal saline. A part of liver tissues were preserved in 10% buffered formalin for histopathological examination, and serum lipid and antioxidant parameters in liver tissues were also determined.

Assessment of the activities of liver marker enzymes in serum

The serum contents of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) were assayed by special assay kits, which were obtained from Nanjing Jiancheng Biological Engineering Institute (Nanjing, China).

Assessment of antioxidant activity in liver

The activities of the activities of catalase (CAT), malondialdehyde (MDA), super oxide dismutase (SOD)

and glutathione (GSH) in liver tissue were determined by special assay kits, which were obtained from the Nanjing Jiancheng Biological Engineering Institute (Nanjing, China).

Histopathology

Liver tissue and pulmonary artery were firstly fixed in 10% buffered formalin for at least 24h before being embed ded in paraffin, and cut into 5μm-thick sections by a rotary microtome. Then these sections were stained with hematoxylin-eosin (HE) and observed under a microscope (IX51, Olympus, Japan) to detect the histopathological changes.

RESULTS

Effects of M. martini volatile oil on DPPH-radical-scavenging

The radical scavenging ability of *M. martini* volatile oil on DPPH was firstly analyzed in this study. As shown in fig 1A, DPPH free radical was obvious scavenged by *M. martini* volatile oil. In addition, the scavenging rate of DPPH was significantly increased with increased concentrations of *M. martini* volatile oil, which exhibited a positive correlation.

Table 1: Compositions of high fat diet used for inducing of hyperlipidemia rats

No.	Contents	Food pellet per 100g
1	Fat	20g
2	Cholesterol	5g
3	Glucose	5g
4	Fructose	5g
5	Glutamine	5g
6	Methylthiouracil	1g
7	Egg yolk	9g
8	Wheat flour	30g
9	Corn flour	20g

Effects of M. martini volatile oil on ABTS-radical-scavenging

ABTS can be oxidated into green $ABTS^+$ by suitable oxidant and the generation of $ABTS^+$ could be inhibited by antioxidant, so the antioxidant capacity was always evaluated by detection of ABTS and $ABTS^+$ at OD value of 414 nm and 734 nm. In this study, ABTS+ was significantly scavenged by the intervention of *M. martini* volatile oil, and the scavenging efficiency is proportional to the concentrations of *M. martini* volatile oil (fig 1B). When the concentration of *M. martini* volatile oil was 2.5 g/l, the scavenging rate of ABTS could reach 53.48%.

Protective role of M. martini volatile oil on CHO cells from amphotericin B

Obvious protective effects of *M. martini* volatile oil on CHO cells from amphotericin B-induced cell damage were observed in our study. As shown in table 2, the OD value at 570nm of CHO cells with amphotericin B (negative control) was significantly lower than normal CHO cells without amphotericin B (normal control) (P< 0.001). However, simvastatin (positive control) could significantly protect the damage induced by amphotericin B, with a higher OD value than the negative control group (P<0.001). Notably, the intervention of *M. martini* volatile oil could also induce a higher OD value than the negative control group (P<0.001) and the protective effects were positively correlated with the concentrations of *M. martini* volatile oil.

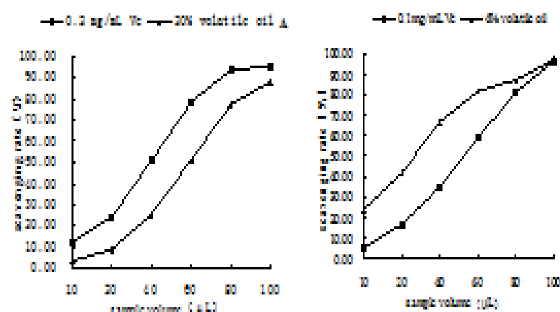

Fig. 1: The radical scavenging ability of *Michelia martinii* (Levl.) volatile oil on dpph and abst.

DPPH free radical scavenging activity ABTS free radical scavenging activity.

Effects of M. martini volatile oil on serum lipid in hyperlipidemia rats

When compared with hyperlipidemia rats in the model group, the serum contents of TC and LDL-C were significantly reduced by *M. martini* volatile oil (p<0.05), and the differences were extremely remarkable in mid and high concentration group (V and VI, p<0.01). In addition, the level of TG was significantly reduced in mid and high concentration group (V and VI, p<0.05), while no significantly different was revealed in low concentration group (IV). Similarly, the value of AI was also significantly reduced by *M. martini* volatile oil (p< 0.05). Besides, the content of HDL-C was significantly increased by *M. martini* volatile oil at mid and high concentration (V and VI, p<0.05), and no significant difference was revealed in low concentration group (IV). When compared with model rats treated by simvastatin (III), the contents of TC, TG, LDL-C and AI were also found to be significantly increased and HDL-C was significantly reduced by *M. martini* volatile oil (p<0.05)

Effects of M. martini volatile oil on activities of liver marker

Enzymes in serum of hyperlipidemia rats

When compared with normal rats (I), the serum contents of ALP, AST, ALT and GGT were all significantly increased in hyperlipidemia rats (model group II, p<0.01). However, simvastatin could significantly reduce the level of ALP, AST, ALT and GGT in hyperlipidemia rats (III, p<0.001). In addition, the level of ALP, AST, ALT and

GGT could also be significantly inhibited by the intervention of *M. martini* volatile oil in a dosage-dependent manner (IV, V and VI, p<0.01). To pay attention to, the effects of *M. martini* volatile oil in high concentration (VI) could be nearly equivalent to simvastatin.

Table 2: Effects of different concentrations of *Michelia martini* (Levl.) volatile oil on CHO cells treated by amphotericin B

Group	Dose (mL/mL)	OD570
Normal control (without amphotericin B)	-	0.85 ± 0.08
Negative control	-	0.27 ± 0.02###
Positive control (simvastatin)	0.5	0.79 ± 0.04***
MVO-0.15 volatile oil	1.0	0.45 ± 0.06**
MVO-0.3 volatile oil	1.5	0.65 ± 0.03***
MVO-0.6 volatile oil	2.0	0.75 ± 0.04***

** and *** represented significantly at P<0.01 and 0.001 when compared with negative control; ### represented significantly at P<0.001 when compared with normal control.

Effects of M. martini volatile oil on antioxidant activity in liver of hyperlipidemia rats

The antioxidant activity of *M. martini* volatile oil in liver of hyperlipidemia rats was analyzed by the activities of CAT, SOD, MDA and GSH. As shown in table 5, the activities of CAT, SOD, MDA and GSH were obviously more deteriorated in hyperlipidemia rats when compared with normal rats (P<0.01). *M. martini* volatile oil at mid and high concentrations could significantly improve these changes in CAT, SOD, MDA and GSH activities (V and VI, p<0.01). However, low concentration of *Michelia martinii* (Levl.) volatile oil only significantly reduce the activity of MDA (IV, p<0.01) and the activities of CAT, SOD and GSH were not significantly changed. Besides, the activities of CAT, SOD and GSH in rats treated by *M. martini* volatile oil (IV, V and VI) were all significantly lower than rats treated by simvastatin (III) (p<0.01), while the activities of MDA in IV, V and VI group were close to simvastatin group (III).

Effects of M. martini volatile oil on histopathological changes of liver tissue in hyperlipidemia rats

The liver tissue was basically normal in the normal control group, characterized by complete and distinct histological structure, clear liver sinusoid and polygonal regularly arranged liver cells with abundant homogeneous cytoplasm, distinct membranes and large nucleus in the middle of the cytoplasm in normal morphology. Meanwhile, the central vein of liver tissue was surrounded by cords of liver cells that radiate out in all directions. In high fat diet induced hyperlipidemia rats, enlarging liver with roundness edge was observed (such as nutmeg liver with greasy feeling). The surface of liver tissue turned yellow with a point distribution. In addition, most of hepatic sinus disappeared, the arrangement of hepatic cells was disordered, and severe steatosis

Table 3: Effect of *Michelia martini* (Levl.) volatile oil on serum TC, TG, LDL-C, HDL-C and AI level in hyperlipidemia rats (mean ± SD, n=10)

Group	TC (mmol/l)	TG (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)	AI (LDL-C/HDL-C)
I	10.85±0.21##	0.79±0.12##	1.29±0.16#**	0.62±0.1##	0.48±0.06
II	15.56±1.09**&&	1.34±0.12**&&	1.83±0.17*&	9.53±0.40&&**	5.23±0.24**&&
III	10.98±1.01##	0.92±0.05##	2.24±0.18#&&	6.78±0.27##&	3.03±0.12##
IV	13.39±1.76#*&	1.15±0.17**&	2.08±0.25&*	8.04±0.20#**	3.91±0.37#**&&
V	12.10±1.72##&	1.04±0.15#*&	2.15±0.14#&&	7.43±0.16##*	3.46±0.15##*
VI	11.78±1.48##	0.95±0.11#*&	2.36±0.20#&&	7.05±0.28##	2.99±0.13##

*and ** represented significantly at P<0.05 and 0.01 when compared with positive control (III); # and ## represented significantly at P < 0.05 and 0.01 when compared with model control (II); & and && represented significantly at P<0.05 and 0.01 when compared with normal control (I).

Table 4: Effect of *Michelia martini* (Levl.) volatile oil on serum ALP, AST, ALT and GGT in hyperlipidemia rats (mean ± SD, n=10)

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)
I	65.4±2.4	87.6±1.7	78.7±1.4	52.3±1.7
II	184.2±10.8###	248.3±12.4###	256.5±7.8###	168.2±3.9###
III	73.6±2.34***	923±4.4***	82.5±2.1 ***	65.8±1.2***
IV	155.7±4.8**	216.5±7.6**	218.4±4.5**	142.9±3.6**
V	108.5±2.7***	158.7±4.5***	135.4±7.6**	87.6±2.4***
VI	81.5±1.6***	102.5±2.3***	89.5±1.8***	70.3±3.5***

** and *** represented significantly at P<0.01 and 0.001 when compared with model control (II); ### represented significantly at P <0.001 when compared with normal control (I).

appeared in most of hepatic cells, especially in peripheries of the lobule. Hepatic cells were extremely swollen in a circular form, and a large number of lipid droplet vacuoles (steatosis) in different size and quantity were observed in cytoplasm. Meanwhile, the number of hepatic cells containing lipid droplet vacuoles accounted for more than 2/3 of the total hepatic cells, and the lipid droplets were always fused with each other in severe stage. After treated by *M. martini* volatile oil, the degree of liver steatosis was significantly relieved in Group IV, V and VI. In macroscopic view, the liver tissue was pink and enlarged, and no other obvious differences were observed. Under low magnification, the structure of hepatic lobule and sinusoid was clear and the distribution of hepatic cells was arranged in order. When compared with the positive control group with the treatment of simvastatin, the number of lipid droplet vacuoles was significantly reduced in hepatic cells treated by *M. martini* volatile oil, and only small vacuoles around the nucleus were found but no fusion occurred. Meanwhile, nucleus with normal form and structure were found to be located in the middle of hepatic cells, which were similar with cells in the normal control group. Besides, regenerated hepatic cells were also observed and no interstitial fibrosis was revealed in liver tissue of rats treated by *M. martini* volatile oil. The ameliorative effects of *M. martini* volatile oil on liver of hyperlipidemia rats were revealed to be similar with positive control group (fig. 2).

Effects of M. martini volatile oil on histopathological changes of pulmonary artery in hyperlipidemia rats

In normal rats, pulmonary artery always exhibited smooth and intact intima, no fatty streak, closely connected endothelial cells and clear cell contour (fig. 3A). However, significantly thickened vascular wall, irregular and loosely cell arrangement, and damaged and dropped endothelial cells were induced by high fat diet in rats of model group (fig. 3B). In the positive control group treated by simvastatin, only small part of vascular was thickened, small amount of endothelial cells were damaged and dropped and the cell arrangement was regular (fig. 3C). By the intervention of *M. martini* volatile oil, the thickness of vascular wall was gradually returned to normal level, which positively associated with increased concentrations of *M. martini* volatile oil (fig. 3D-F). Meanwhile, cell arrangement gradually became irregular and loosely, while small amount of endothelial cells were damaged and dropped in low concentration group (IV, fig. 3D). Furthermore, the protective effects of *M. martini* volatile oil were revealed to be better in high concentration group with smooth intima when compared with the positive control group (fig. 3F).

Table 5: Effect of *Michelia martini* (Levl.) volatile oil on activities of CAT, MDA, SOD and GSH in hyperlipidemia rats (mean ± SD, n=10)

Group	CAT (U/mg·prot)	MDA (U/mg·prot)	SOD (U/mg·prot)	GSH (U/mg·prot)
I	77.42±11.95	1.58±0.29	260.65±40.19	9.80±1.07
II	40.30±9.20	3.48±0.45	149.30±25.58	3.78±0.82
III	68.00±19.31	2.60±0.94	201.10±40.17	7.60±1.74
IV	42.54±11.62	2.82±0.98	142.02±28.70	4.10±1.29
V	52.30±12.04	2.68±0.94	162.87±30.02	4.43±1.55
VI	55.09±15.17	2.54±0.93	174.17±37.36	5.52±1.64

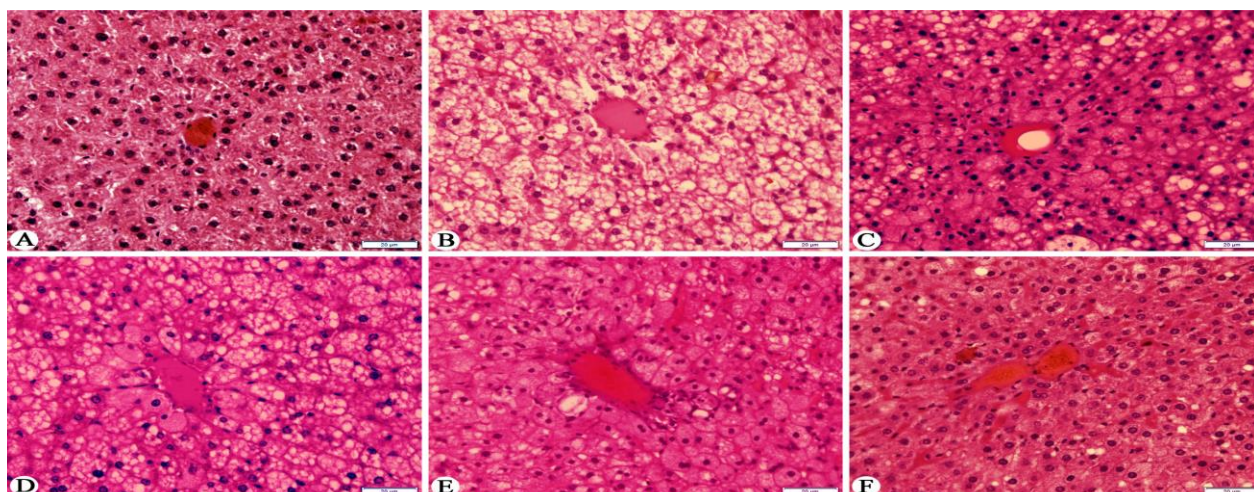

Fig. 2: HE staining of liver tissue in rats (×40). A: Group I (normal control); B: Group II (model control); C: Group III (positive control); D: Group IV (*Michelia martini* (Levl.) volatile oil in low concentration, 0.15mL·Kg^{-1}·d^{-1}); E: Group V (*Michelia martini* (Levl.) volatile oil in mid concentration, 0.3mL·Kg^{-1}·d^{-1}); F: Group VI (*Michelia martini* (Levl.) volatile oil in high concentration, 0.6mL·Kg^{-1}·d^{-1})

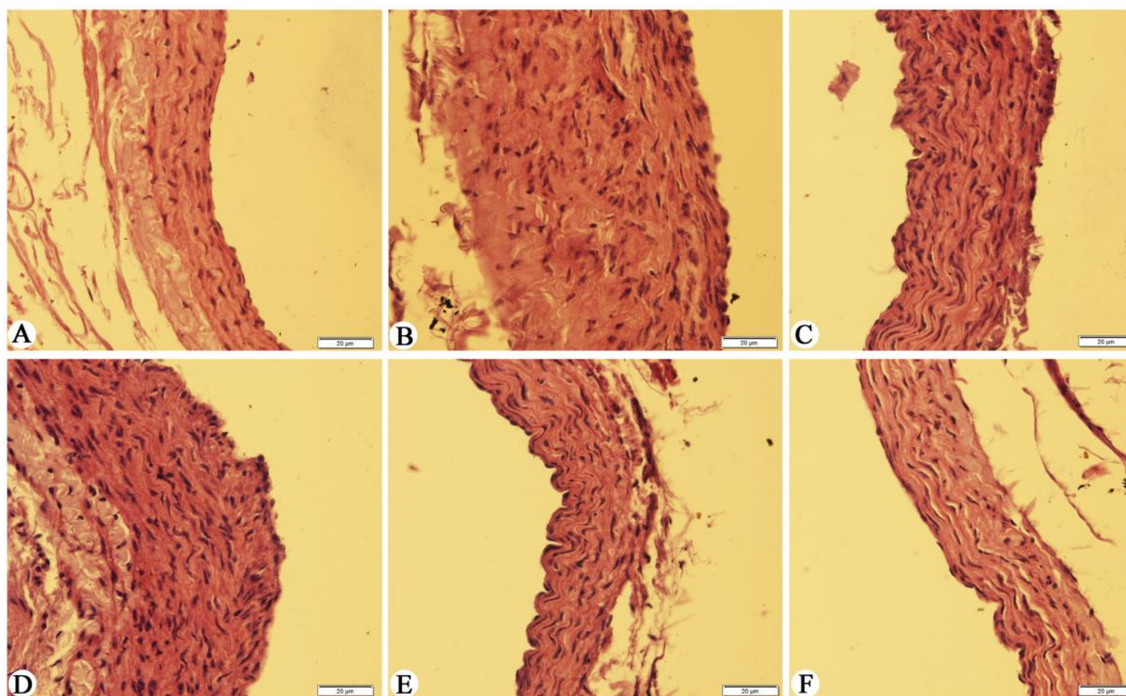

Fig. **3**: HE staining of pulmonary artery in rats (×10). A: Group I (normal control); B: Group II (model control); C: Group III (positive control); D: Group IV (*Michelia martini* (Levl.) volatile oil in low concentration, $0.15mL \cdot Kg^{-1} \cdot d^{-1}$); E: Group V (*Michelia martini* (Levl.) volatile oil in mid concentration, $0.3mL \cdot Kg^{-1} \cdot d^{-1}$); F: Group VI (*Michelia martini* (Levl.) volatile oil in high concentration, $0.6mL \cdot Kg^{-1} \cdot d^{-1}$)

DISCUSSION

Amphotericin B cell model was a simple and effective method for screening of endogenous cholesterol synthesis inhibitors. The combination of Amphotericin B with cholesterol lead to the formation of compound and the appearance of micropores, followed by the disruption of cell membrane and spillover of cytoplasmic contents. In the absence of exogenous cholesterol supply, application of endogenous cholesterol synthesis inhibitors could suppress endogenous cholesterol biosynthesis by blocking the formation of cholesterol complex, thereby relieve the damage of Amphotericin B on cells ((Sun *et al*., 1989; Ou *et al*., 2003). In Amphotericin B cell model, a higher "A" value represented more live cells and less cellular damages, illustrating the inhibition of endogenous cholesterol biosynthesis in the absence of exogenous cholesterol supply. In this study, a significant protective role of *M. martini* volatile oil on Amphotericin B-induced damage was observed on CHO cells in a dose-dependent manner, indicating endogenous cholesterol biosynthesis could be significantly inhibited by *M. martini* volatile oil. This process may be one of the most important mechanisms in the regulation of blood lipid.

Total cholesterol and triglyceride in serum were important indexes of hyperlipidemia. The main function of Low density lipoprotein was transporting liver-synthesized cholesterol to all tissues for utilization in the whole body, and its level was positively correlated with the occurrence of atherosclerosis. On the other hand, high density lipoprotein was an effective anti-atherogenic factor, which was negatively correlated with the morbidity of atherosclerosis. *In vivo*, high density lipoprotein can transport extrahepatic cholesterol to liver tissue for metabolism, thereby preventing deposition of cholesterol in arterial wall (Silveira *et al*., 2015). In this study, the content of TC, LDL-C and TG was significantly reduced while HDL-C was increased after treatment with different concentrations of *M. martini* volatile oil. Meanwhile, a low AI value indicated significantly decreased possibility of atherosclerosis. Significantly reduced serum TC, TG, LDL-C level and AI in high fat diet rats further indicated *M. martini* volatile oil could prevent the occurrence of atherosclerosis by obviously improved lipid metabolism and reduced blood lipid. Besides, the lipid-lowering effects of *M. martini* volatile oil showed a dose dependent manner. Furthermore, *M. martini* volatile oil could also reduce fat pathological changes in liver and maintain normal morphology of liver cells, thereby prevent the occurrence of fatty liver.

The development of high blood lipids is always accompanied with cell membrane damages and of aminotransferases leakage, such as ALP, AST, ALT and GGT. In this study, *M. martini* volatile oil could substantially restore normal level of these enzymes in serum, and propose protective effects on hepatocytes by effectively reduced lipid level.

MDA was a final decomposition product of lipid per oxidation through attacking free radical to polyunsaturated fatty acids in biological membrane, which could reflect the rate and intensity of lipid peroxidation in tissue cells. As abnormally increased MDA was always accompanied with cell damage, the content of MDA was evaluated to reveal the antioxidant capacity of bodies in this study. As a result, different concentrations of *M. martini* volatile oil could significantly reduce the content of MDA in liver tissue.

In detection of antioxidant capacity, MDA is always mutually coordinated with SOD. SOD is necessary in the balance of oxidation and antioxidation, which could eliminate superoxide anion free radical, induce the formation of hydrogen peroxide through, thereby protect cell damage (Cohen and Hochstein 1964). In addition, CAT can decompose hydrogen peroxide into H_2O and O_2 and synergistic reaction of SOD and CAT can always reflect the ability of oxygen free radicals scavenging (Townsend *et al*., 2003). Besides, GSH was another important antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide. *In vivo*, GSH is a kind of enzyme system containing selenium with free radical scavenging and inhibition activities and its activity could reflect antioxidant capacity of the body. In this study, obvious antioxidant effects of *M. martini* volatile oil were observed.

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