

Full Length Research Paper

Areca nut oil with arecoline can enhance hypolipidemia in rats

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Both Areca nut oil (OL) and arecoline play some roles in moderating hypolipidemia but the pathways may be different. The authors hypothesize OL plus arecoline can lead to an improved hypolipidemia in animal model. In this study, the fed rats with series doses of OL alone or OL plus arecoline. The gaining weight, the level of total cholesterol (TC), triacylglyceride (TG), high density lipoprotein cholesterol (HDL-C) and arteriosclerosis index (AI) from the rats were then examined. The authors found low dose of OL (0.335 g·kg⁻¹) plus arecoline (3 mg·kg⁻¹) could significantly reduce the level of TC and AI, and increase the level of HDL-C compared to other groups. They conclude OL (0.335 g·kg⁻¹) plus arecoline (3 mg·kg⁻¹) can play a synergistic role in enhancing hypolipidemia in rats.

Key words: Areca nut oil, total cholesterol, triglyceride, high density lipoprotein cholesterol, arteriosclerosis index.

INTRODUCTION

The areca-nut contains 14% of fatty oil. The component of fatty acid in areca-nut is complicated, including lauric acid (19.5%), myristic acid (46.2%), palmitic acid (12.7%), tetradecenoic acid (7.2%), oleic acid (6.2%), linoleic acid (5.4%), octadecanoic acid (1.6%), capric acid (0.3%), dodecenoic acid (0.3%) (Huang et al., 2008). The fatty acid of areca-nut contains moderate level of both unsaturated fatty acid (linoleic acid) and saturated fatty acid (palmitic acid). The palmitic acid has shown inhibition on the spore of *Bacillus cereus* and can decrease the content of cholesterol in serum (Zheng et al., 2006), Palmitic acid and arecoline coexist in areca-nut oil.

Shan and Zhang (2004) found that arecoline can inhibit the expression of some key molecules that contribute to atherosclerosis in blood and vascular tissues thus prevent cardio-cerebral-vascular diseases. The areca tea has already been used to treat some cardio-cerebral-vascular diseases such as hyperlipaemia in elderlies (Liu, 2008). Ontengco et al. (1999) proposed that Areca nut oil (OL) contains the components with the function of anti-inflammatory and inhibition of growth of some microorganisms. Both arecoline and OL can be served as hypolipidemic agents but they may have different roles in lowering blood cholesterol. In this study, they compared the roles of OL alone to OL with arecoline in hypolipidemic functions. The authors purposely add arecoline into OL based on following reasons: (1) the arecoline and OL are taken at the same time when people are chewing areca nuts; (2) the arecoline has some toxicological effects, and it will promote the hypolipidemic effects when it is mixed with the OL. They found here low dose of OL (0.335 g·kg⁻¹) plus arecoline (3 mg·kg⁻¹) can moderate hyperlipaemia significantly. This study provides useful information for the

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Abbreviations: OL, areca nut oil; OB, OL plus arecoline; TC, total cholesterol; TG, triacylglyceride; HDL-C, high density lipoprotein cholesterol; AI, arteriosclerosis index.

development of healthier OL.

MATERIALS AND METHODS

Materials

OL is extracted and purified in the lab at Food Engineering College in Central South University of Forest and Technology by using supercritical CO₂ extraction technology and molecular distillation purified technology (Zhang et al., 2008) arecoline, products of Sigma (Cat# 300-08-3). Total Cholesterol (TC) kit and triglyceride (TG) kit are from Zhejiang Dongou Biological Engineering Co., Ltd (Zhejiang, China); High density lipoprotein cholesterol (HDL-C) kit is from Elikan biological technology Co., Ltd (Zhejiang, China). GF-2245-Automatic biochemical analyzer, Shandong Gaomi rainbow apparatuses Co., Ltd, Shandong China) is used for TC, TG and HDL-C analysis.

Laboratory animal : Sprague-Dawley (SD) male rats (100±20 grams) were purchased from Laboratory Animal Center in Hunan University of Traditional Chinese Medicine. Basal feed (containing corn flour 34%, bean cake powder 25%, flour 25%, bran 5%, fish meal 5%, yeast 1%, salt 1%, vegetable oil 1%, cod liver oil 1%, and VB21%) was purchased from Laboratory Animal Center in Hunan University of Traditional Chinese Medicine. High lipid diet (ingredient: basal feed 93.8%, cholesterol 1%, lard 5%, cholate 0.2%) was obtained from Laboratory Animal Center in Hunan University of Traditional Chinese Medicine, Hunan, China. The animal protocol was approved by Animal Research Committee at Central South University. Methods

Experimental environment

Temperature: 23 - 25°C, humidity: 55 - 65%.

Dose selection

A series dose of 0.335 g·kg⁻¹ (low group), 0.670 g·kg⁻¹ (media group) and 1.340 g·kg⁻¹ (high group) OL were set that are equivalent to 5, 10 and 20 times of recommended doses of body weight, respectively. The blank control and model control groups were set at the same time. The amount of filling stomach is calculated as 1.0 mL/100g. OB groups were set by adding 3 mg·kg⁻¹ arecoline into series above OL groups. The value of added arecoline is the mean value of arecoline residue after processing (Li, 2010).

Treatment of experimental animal

SD male rats were fed with basal feed for 3 - 4 days and divided into 8 groups according to their weights with 10 rats for each group. The blank control group was fed with basal feed throughout the experiments. The model control group was fed with high lipid diet. Each study group was fed with either OL or OB as the established doses. The blank control and model group were fed with same dose of vegetable oil once a day.

The rats were weighted every other day till 30 days. The rats were kept off of food (not water) for 14 h and blood was obtained from the eyes. The whole blood was centrifuged at 3000 rpm/min for 15 min to separate serum. The values of TC, TG and HDL-C were determined with GF-2245-Automatic biochemical analyzer. The atherogenic index (AI) was calculated as the following formula (IARC, 2004).

$$AI = \frac{LDL - C}{HDL - C} = \frac{TC - (HDL - C)}{HDL - C}$$

Data analysis

The experimental data were processed with Independent-Sample T Test which was completely random designed by SPSS13.0 software (SPSS, Chicago, USA). The data were expressed as mean ± SD (m ± s).

RESULTS AND ANALYSIS

The effects of areca nut oil on the gain of weight fed by various doses of OL and OB, the rats grew normally during the experimental procedures. The rats gained significantly more weight in model group than blank control group (Table 1 and Figure 1, ** p < 0.01). There are no significant differences of weight gaining in rats among each study group and model group (Table 1 and Figure 1, p > 0.05).

The effect of OL on TC concentration in rats

The rats were fed by OL or OB and serum TC concentrations were measured. The TC concentrations in OL media group and OB low group are significantly higher than that in model groups. The TC concentrations from rats' serum in medium dose of OL group decreases 21.75%, significantly lower than that in the model group (Table 1 and Figure 2, # p < 0.05).

The low and high dose OL groups also show the decrease of TC concentrations about 13.90% and 14.80, respectively, but there is no significance that was found (Table 1 and Figure 2, p > 0.05). The rats fed by the low dose of OB show 34.53% decrease of the TC which is significant lower than that in the model group (Table 1 and Figure 2, ## p < 0.01). The rats from medium and high doses of OB groups show some decrease of the TC value but no significance (Table 1 and Figure 2, p > 0.05).

The effect of OL on TG concentrations in rat

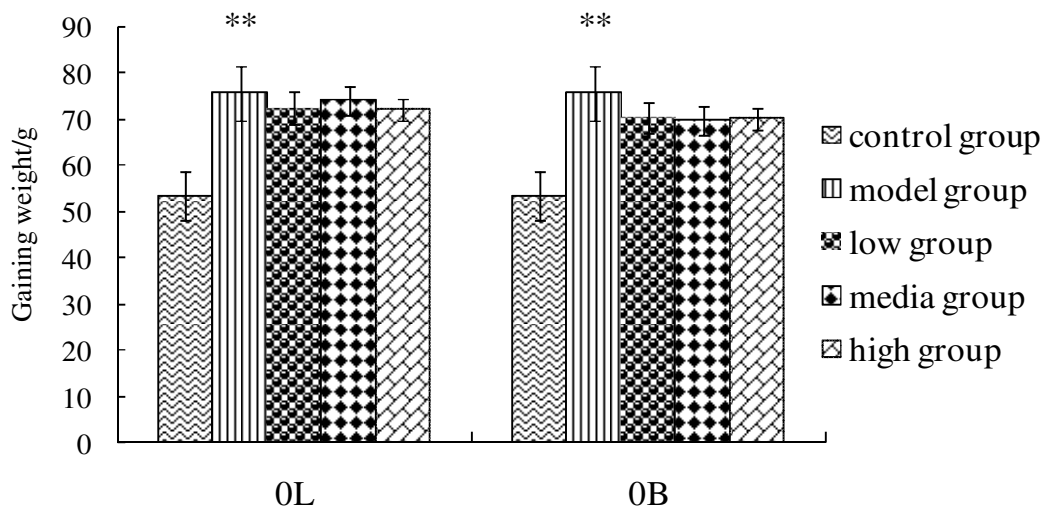
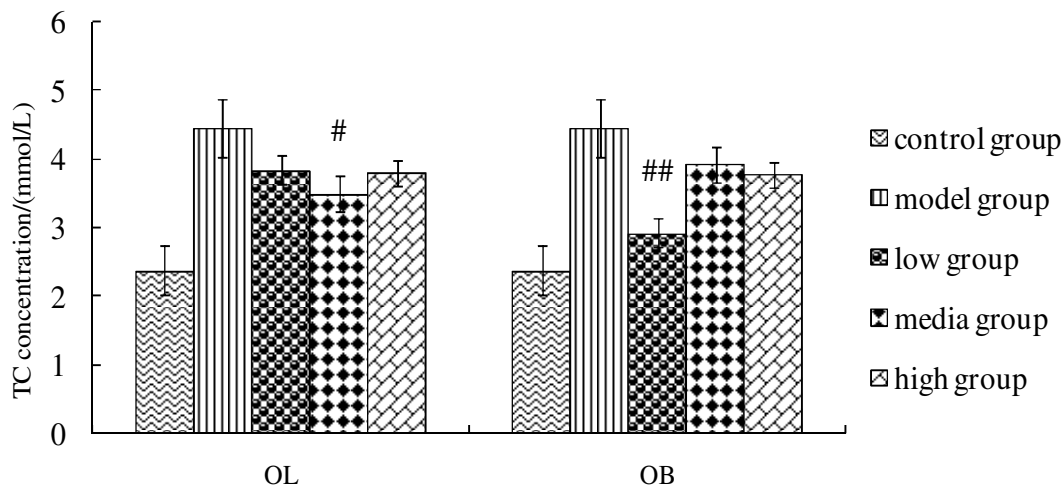
The rats were fed by OL or OB and serum TG concentrations were measured. TG concentrations show significant decrease in all low, media and high groups than model groups. The TG values in both OL and OB groups show no significant changes compared to control group. Compared to model group, the TG values decrease 40.56, 47.20 and 41.89%, respectively, from three groups treated by different doses of OL (Table 1 and Figure 3, # p < 0.05), and 47.06, 60.26 and 54.81%, respectively, from groups treated by different doses of OB (Table 1 and Figure 3, # p < 0.05).

The effect of OL on HDL-C concentrations in rats

The rats were fed by OL or OB and serum HDL-C concentrations were measured. Comparison to model

Table 1. Values of body weight, TC, TG, HDL-C and AI for each group.

Food	Group	Weight (g)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	AI
OL	Control	53.28±5.28	2.37±0.36	3.05±0.63	1.31±0.12	1.31±0.23
	Model	75.62±5.83	4.46±0.42	6.97±0.69	0.79±0.15	0.79±0.47
	Low	72.42±3.42	3.84±0.21	4.15±0.33	0.86±0.08	0.86±0.32
	Media	73.98±3.11	3.49±0.26	3.68±0.29	1.26±0.06	1.26±0.24
	High	72.06±2.34	3.8±0.19	4.05±0.22	0.92±0.04	0.92±0.31
OB	Control	53.28±5.66	2.37±0.33	3.05±0.59	1.31±0.14	1.31±0.26
	Model	75.62±4.93	4.46±0.37	6.97±0.62	0.79±0.17	0.79±0.39
	Low	70.21±2.89	2.92±0.29	3.69±0.36	2.13±0.07	2.13±0.51
	Media	69.74±2.99	3.93±0.17	2.77±0.22	1.16±0.03	1.16±0.18
	High	70.01±1.98	3.78±0.14	3.15±0.19	1.08±0.05	1.08±0.34

**Figure 1.** The effects of OL and OB on the gain of weight in rats.**Figure 2.** The effects OL and OB on TC concentrations in rats.

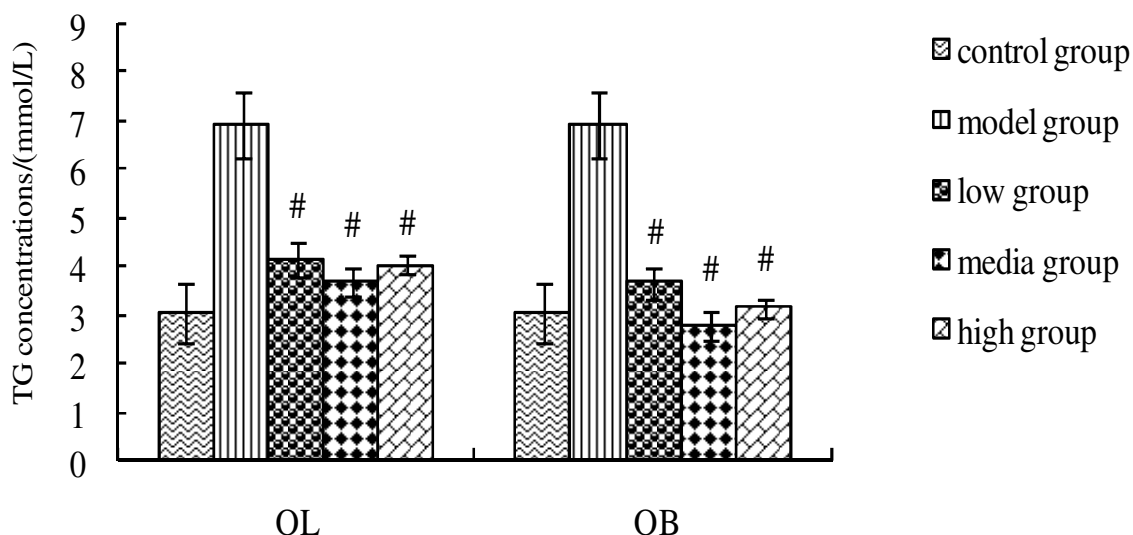


Figure 3. The effects of OL and OB on TG concentrations in rats.

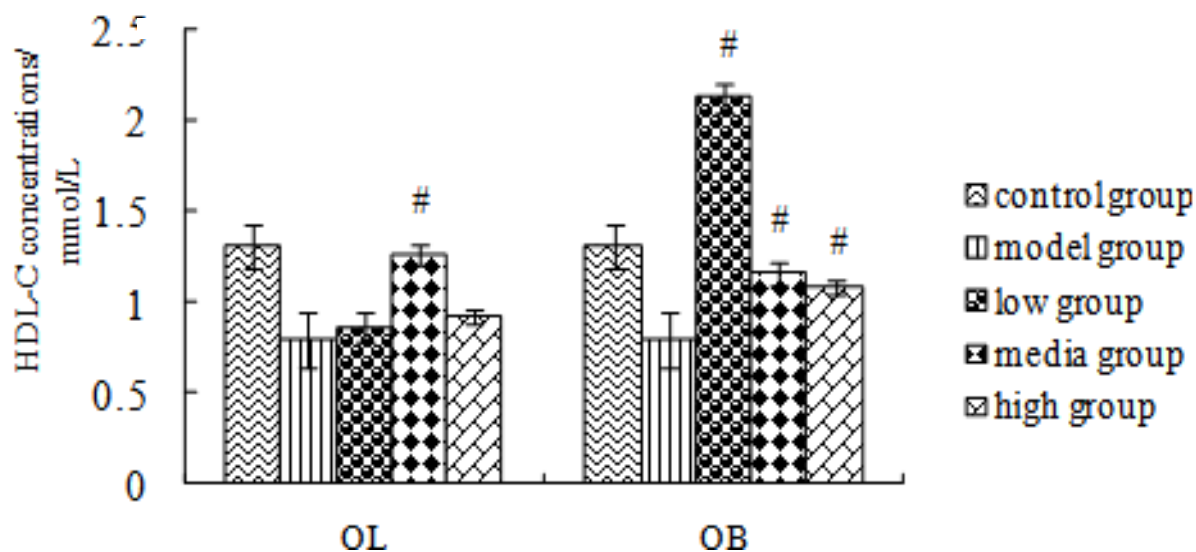


Figure 4. The effects of OL and OB on HDL-C concentrations in rats.

groups, rats in OL media group and OB low group show significant higher concentrations of HDL-C than that in model groups. The HDL-C values in different OL group's increase 8.86, 59.49, and 16.46%, respectively, compared to the model control group. The significance is found in the medium dose of OL (Table 1 and Figure 4, # $p < 0.05$). The HDL-C values in low dose OB group show 169.42% increase (Table 1 and Fig 4, # $p < 0.05$) compared to model group. The HDL-C values in media and high doses OB groups show 46.84 and 36.71% increases, respectively, compared to model group (Table 1 and Figure 4, #

$p < 0.05$), but slightly decrease compared to control group.

The effect of OL on the arteriosclerosis index (AI) of rats

Rats in OB low group show the lowest AI value among significantly lower than control group and model groups. Among OL groups, AI shows highest value in low dose group, and lowest value in media dose group. The AI values in OL groups are significantly higher than control

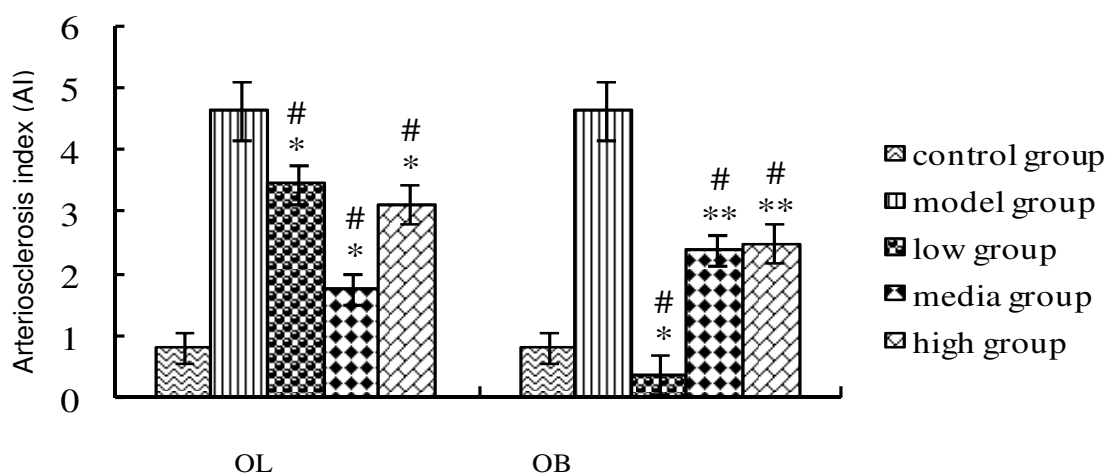


Figure 5. The effects of OL and OB on the arteriosclerosis index of rats.

group (* $p < 0.05$), and lower than model group (Table 1 and Figure 5, # $p < 0.05$). Increase of AI values among OB groups is correlated to the doses of OB in the diets. Low dose of OB significantly decrease AI value compared to both control group (Table 1 and Figure 5, * $p < 0.05$) and model group (Table 1 and Figure 5, # $p < 0.05$). The rats in medium and high doses of OL groups show significant lower AI value compared to model group (Table 1 and Figure 5, ** $p < 0.05$), but higher than control group (Table 1 and Figure 5, # $p < 0.01$).

DISCUSSION

Epidemiological studies have shown that the unbalanced proportion of edible oil and fat can cause hyperlipemia and arteriosclerosis (Li, 2010). Many works have suggested unsaturated fatty acids such as linoleic acid are able to decrease the cholesterol levels (Jiang, 2009). The vegetable oil and fat usually have one saturated or unsaturated fatty acid. For example, coconut oil contains 91% saturated fatty acid among which lauric acid accounts for nearly half of total fatty acid (45%) (Zou, 2002); Soybean oil contains 80% unsaturated fatty acid among which linoleic acid accounts for half (51%) (Xue, 2008). This study further confirmed that the OL can decrease the level of blood fat and prevent atherosclerosis in animal models. It also indicates that the OL plays a role in maintaining the balance of human fatty acid, protecting heart and prevention from angiocardopathy. (Shan and Zhang, 2004) has shown proper dose of arecoline that can promote the release of NO, increase the expression of eNOS protein and mRNA, decrease the level of IL-8 in blood, and inhibit the excess expression of CXCR-2v and MCP-1 mRNA which was acceptor of adhesion molecule g all groups, which are ICAM-1 and chemotactic factor IL-2006). However, a few studies showed that mixing the

Areca nut with tobacco and sirih by chewing can cause oral cancer, oropharyngeal cancers and oesophageal carcinoma (Yin, 2004; Rajendran, 1994a, b). Centro International Investigations Sobre Cancer (IARC), an organization belongs to WTO, issues a warning to the international societies: chewing Areca nut can cause cancer. They identify the Areca nut as a carcinogen in first grade based on epidemiological evidence (Hsing et al., 2005).

The good aspects in pharmacology of Areca nut are related with arecoline (Peng et al., 2005; Shieh et al., 2003a, b). The authors hypothesized that the pharmacologic and toxicologic functions of arecoline are related to the doses of OL. The residual quantity of arecoline after being processed was added into OL in this study. We found that OL with addition of arecoline was indeed better than OL alone in terms of moderating hyperlipaemia. Low dose of OL ($0.335 \text{ g}\cdot\text{kg}^{-1}$) plus arecoline ($3 \text{ mg}\cdot\text{kg}^{-1}$) can moderate hyperlipaemia significantly better than any other groups. The arecoline can improve brain blood flow, increased cardiac blood flow and reduce cholesterol level, while OL has the function of antioxidation both of them can dissolve into each other and absorbed by human body. They speculate, this is why OL and arecoline work together but the mechanism is unknown. Their next step is going to investigate the mechanism of synergistic actions from OL and arecoline.

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