Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Correspondence:

Hypolipidemic activity of low-cholesterol ovum oil of *Rana chensinensis* and phytosterol (stigmasterol) in rats*#

Chang-hui ZHAO, Chao ZHAO, Hai-qing YE, Chun-yu XI, Zheng-ping ZOU, Xin-hui ZHOU, Ping-ping HOU, Tie-hua ZHANG†‡

College of Food Science and Engineering, Jilin University, Changchun 130062, China

†E-mail: zhangth@jlu.edu.cn

https://doi.org/10.1631/jzus.B1900018

The ovum oil of forest frog has various health beneficial functions. In the current research, we evaluated the hypolipidemic effects of the low-cholesterol ovum oil from the forest frog and its combination with stigmasterol in rats.

The forest frog, *Rana chensinensis*, has been consumed as a precious tonic food for years as it is known to nourish the body and has many pharmacological effects (Bao et al., 2016). The ovum oil of *R. chensinensis* (OORC) is rich in unsaturated fatty acids (Jin et al., 2004) that can alleviate hyperlipidemia. Since forest frog oil (Hasma) is sold at high prices, the other parts of the frog are treated as by-products and often used to feed animals (Wang et al., 2015). OORC is an important part of the forest frog and is rich in proteins and unsaturated fatty acids that can improve the immune system and delay senescence (Ji et al., 2013). However, high intake of cholesterol has long been linked with health problems (Yu et al., 2013). In the current study, we used low-cholesterol OORC, which has reduced cholesterol levels but high unsaturated fatty acid levels (Zhang et al., 2019). As reported previously, plant sterols, when sometimes consumed with other nutrients, also effectively reduce fat absorption in the intestine and decrease the levels of total blood lipids and low-density lipoproteins with no negative effects on the levels of high-density lipoproteins (Chen et al., 2005). Therefore, we aimed to investigate the effects of low-cholesterol OORC and its combination with the phytosterol (stigmasterol) in improving lipid profiles, which may provide a theoretical basis for the development of functional foods that prevent hyperlipidemia.

After five weeks of feeding, Sprague-Dawley rats that were fed a high-fat diet had significantly higher body weights than rats that were fed a standard diet (P<0.05). Unexpectedly, the positive control group, in which rats were administered simvastatin, was not significantly different from high-fat-induced hypolipidemic rats with respect to weight. However, the other groups of rats treated with OORC, phytosterol (stigmasterol), or their combinations, had significantly lower body weights than the high-fat-induced hypolipidemic rats (P<0.05). Details are shown in Data S1.

Rats that were fed a high-fat diet, with or without any intervention, had moderately higher hepatic indexes, which indicated that the increased weights of the liver were probably caused by fat accumulation. However, only the group that was concurrently fed a low dose of OORC and phytosterol reached statistically significant levels (Table S1). No significant difference was observed between the splenic indexes. Hyperlipidemia is characterized by abnormal blood lipid profiles. Compared to those in the normal group, the total cholesterol (TC), triglycerides (TG), and

‡ Corresponding author

* Project supported by the Jilin Province Research and Industrialization with Key Technologies for High Value Processing and Safety Control of Agricultural Products (No. SF2017-6-4) and the Jilin Provincial Department of Education (No. JJKH20180172KJ), China

Electronic supplementary materials: The online version of this article (https://doi.org/10.1631/jzus.B1900018) contains supplementary materials, which are available to authorized users

ORCID: Chang-hui ZHAO, https://orcid.org/0000-0002-1882-7490

low-density lipoprotein cholesterol (LDL-C) levels in the sera of the hyperlipidemic group were found to be significantly increased (P<0.05), whereas the high-density lipoprotein cholesterol (HDL-C) level was found to be significantly decreased (P<0.05), indicating that the high-fat diet caused hyperlipidemia in rats (Fig. 1). Simvastatin, OORC, phytosterol, and the OORC-phytosterol combination improved their respective lipid profiles by reducing TG and LDL-C levels and increasing HDL-C levels (P<0.05). No significant difference was observed between the treatments of simvastatin, OORC, phytosterol, and the OORC-phytosterol combination with respect to blood lipid regulation. OORC and phytosterol also showed an effect similar to that of simvastatin with respect to hyperlipidemia prevention in rats.

Hyperlipidemia is caused by abnormal blood lipid metabolism that mainly manifests under high TC, TG, and LDL-C levels or low HDL-C levels in the blood (Bahmani et al., 2015). HDL-C transports cholesterol from the extrahepatic tissue to the liver for degradation, which is then distributed to the whole body in order to reduce cholesterol deposition. On the other hand, LDL-C transports the cholesterol synthesized in the liver to the whole body. Hyperlipidemia is a risk factor for cardiovascular diseases (Nelson, 2013). Accumulation of serum cholesterol in blood vessels can lead to heart disease, strokes, and cardiovascular diseases (Cholesterol Treatment Trialists' (CTT) Collaboration, 2015). Therefore, inhibition of cholesterol synthesis via drugs or diet therapy has been the primary strategy to reduce risk of cardiovascular diseases for

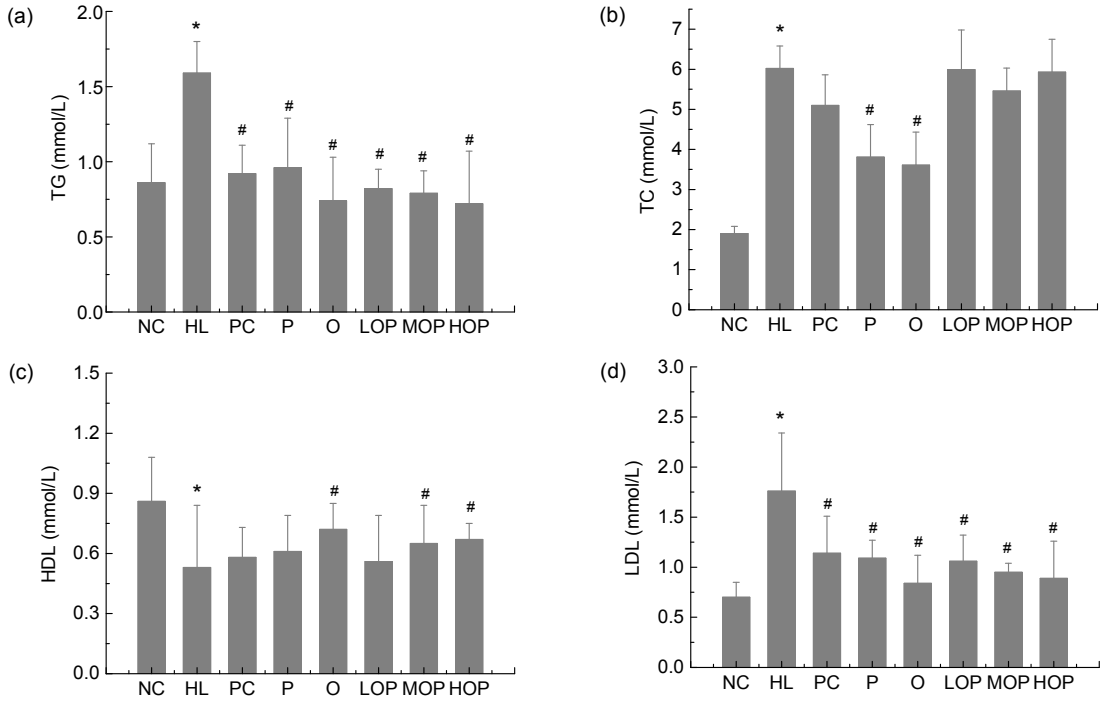

Fig. 1 Lipid profiles of rats over different treatments

Lipid profiles that included TG (a), TC (b), HDL-C (c), and LDL-C (d) were observed to improve after simvastatin, OORC, and phytosterol (stigmasterol) treatments. No significant difference was observed between the treatments of simvastatin, OORC, phytosterol, and OORC-phytosterol combination. Values represent mean±standard deviation (SD), with n=8. * represents significant difference between the hyperlipidemic and normal groups with respect to the corresponding index (P<0.05); # represents significant difference when compared to the hyperlipidemia model group with respect to the corresponding index (P<0.05). TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; NC, normal control group; HL, hyperlipidemic group; PC, positive simvastatin control group; P, phytosterol group; O, ovum oil of *R. chensinensis* (OORC) group; LOP, low dose of OORC-phytosterol group; MOP, middle dose of OORC-phytosterol group; HOP, high dose of OORC-phytosterol group

years. The most important finding of the current research was that low-cost OORC demonstrated effective cholesterol-lowering effects and tremendous potential as a functional food or as an adjunct medicine to prevent and/or treat hyperlipidemia.

In the normal group, liver cells were normally shaped and neatly arranged with no fat infiltration. In the hyperlipidemic group, many small vacuoles were observed in the liver cells. Furthermore, the OORC, phytosterol, and OORC-phytosterol groups showed improved liver phenotypes (since more normal hepatocytes were found in the liver) when compared to the hyperlipidemic group (Fig. 2).

Due to several side effects of the currently used lipid-lowering drugs, natural medicine has attracted the attention of the scientific community. Phytosterols are known to improve blood lipid profiles. As reported previously, unsaturated fatty acids either alone or combined with phytosterols can help remove cholesterol esters generated within the body by regulating lipoproteins (Park and Carr, 2013). Therefore, OORC can be used in combination with phytosterols to prevent hyperlipidemia, which is confirmed in our study.

In conclusion, OORC, phytosterol (stigmasterol), and a combination of both showed lipid-lowering functions in our study. Interestingly, the OORC-phytosterol and OORC groups showed no significant difference in their results. Furthermore, no synergistic effect between phytosterol and OORC was observed. Therefore, OORC and phytosterol (stigmasterol) show similar effects with respect to improving the lipid profile. Overall, OORC is a healthy food component that can be consumed by people looking to prevent hyperlipidemia.

Contributors

Chang-hui ZHAO, Hai-qing YE, Chun-yu XI, and Tie-hua ZHANG conceived and designed the experiments. Chao ZHAO, Zheng-ping ZOU, Xin-hui ZHOU, and Ping-ping HOU performed the experiments. Tie-hua ZHANG contributed reagents, materials, and analysis tools. Chang-hui ZHAO wrote the paper. All authors read and approved the final manuscript. Therefore, all authors have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Chang-hui ZHAO, Chao ZHAO, Hai-qing YE, Chun-yu XI, Zheng-ping ZOU, Xin-hui ZHOU, Ping-ping HOU, and Tie-hua ZHANG declare that they have no conflicts of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

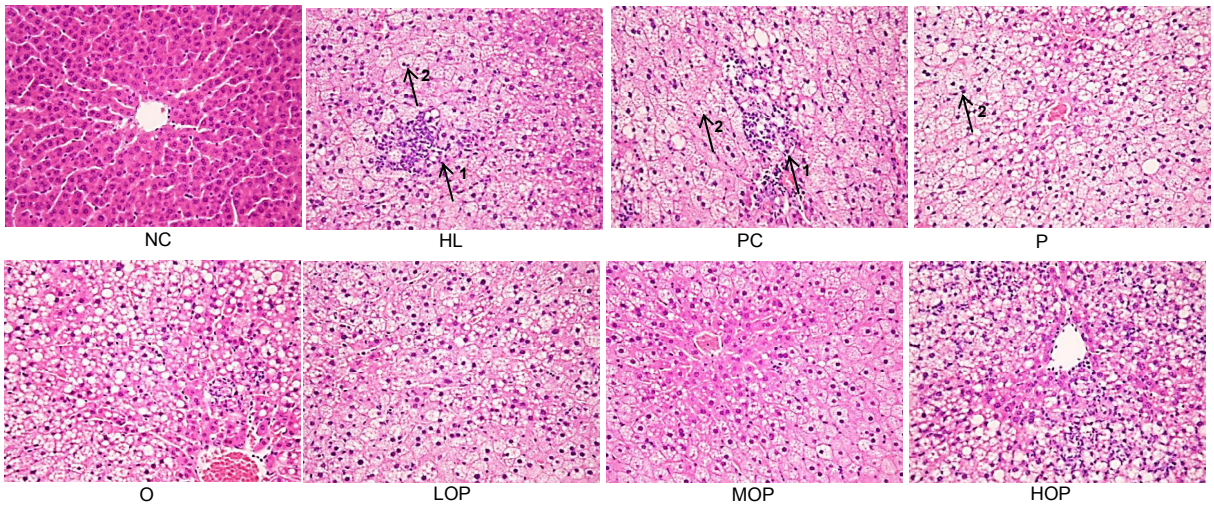

Fig. 2 Hematoxylin and eosin (H&E) staining of liver tissues in the different rat groups

The representative H&E-stained liver slice images are listed as follows: NC (normal control group), HL (hyperlipidemic group), PC (positive simvastatin control group), P (phytosterol group), O (ovum oil of *R. chensinensis* (OORC) group), LOP (low dose of OORC-phytosterol group), MOP (middle dose of OORC-phytosterol group), and HOP (high dose of OORC-phytosterol group). Different from the healthy, many small vacuoles were observed in the liver cells of the hyperlipidemic rats. Administration of OORC, phytosterol, or OORC-phytosterol improved liver phenotypes. Arrow 1, inflammatory cell infiltration; Arrow 2, liver cytoplasm mesh shape (small cavity), nuclear enrichment. 200× magnification

References

Bahmani M, Mirhoseini M, Shirzad H, et al., 2015. A review on promising natural agents effective on hyperlipidemia. *J Evid Based Complementary Altern Med*, 20(3):228-238. https://doi.org/10.1177/2156587214568457

Bao Y, Jiang W, Liu D, et al., 2016. Advances in pharmacological research of Chinese forest frog. *Mod Chin Med*, 18(8):1077-1080 (in Chinese). https://doi.org/10.13313/j.issn.1673-4890.2016.8.031

Chen JT, Wesley R, Shamburek RD, et al., 2005. Meta-analysis of natural therapies for hyperlipidemia: plant sterols and stanols versus policosanol. *Pharmacotherapy*, 25(2):171-183. https://doi.org/10.1592/phco.25.2.171.56942

Cholesterol Treatment Trialists' (CTT) Collaboration, 2015. Efficacy and safety of LDL-lowering therapy among men and women: meta-analysis of individual data from 174000 participants in 27 randomised trials. *Lancet*, 385(9976):1397-1405. https://doi.org/10.1016/S0140-6736(14)61368-4

Ji XF, Lin F, Qiu FP, 2013. Optimization on aqueous enzymatic extraction conditions of forest frog egg protein by response surface method. *J Changchun Univ Technol (Nat Sci Ed)*, 34(2):137-144 (in Chinese). https://doi.org/10.3969/j.issn.1674-1374.2013.02.005

Jin LL, Wang QY, Lou H, et al., 2004. The analysis and research of components of fatty acid in the ovum oil of *Rana chensinensis*. *Food Sci*, 25(10):232-234 (in Chinese). https://doi.org/10.3321/j.issn:1002-6630.2004.10.055

Nelson RH, 2013. Hyperlipidemia as a risk factor for cardiovascular disease. *Prim Care*, 40(1):195-211. https://doi.org/10.1016/j.pop.2012.11.003

Park Y, Carr TP, 2013. Unsaturated fatty acids and phytosterols regulate cholesterol transporter genes in Caco-2 and HepG2 cell lines. *Nutrit Res*, 33(2):154-161. https://doi.org/10.1016/j.nutres.2012.11.014

Wang ZY, Zhao YY, Su TT, et al., 2015. Characterization and antioxidant activity in vitro and in vivo of polysaccharide purified from *Rana chensinensis* skin. *Carbohydr Polym*, 126:17-22. https://doi.org/10.1016/j.carbpol.2015.03.031

Yu L, Morishima C, Ioannou GN, 2013. Dietary cholesterol intake is associated with progression of liver disease in patients with chronic hepatitis C: analysis of the hepatitis C antiviral long-term treatment against cirrhosis trial. *Clin Gastroenterol Hepatol*, 11(12):1661-1666. https://doi.org/10.1016/j.cgh.2013.05.018

Zhang T, Zhao C, Zhao C, et al., 2019. Optimization of conditions for cholesterol removal from *Rana chensinensis* ovum oil. *Curr Topics Nutraceut Res*, 17(2):135-139.

List of electronic supplementary materials

Data S1 Materials and methods

Table S1 Organ indices of the rats in different groups

中文概要

题　目：低胆固醇林蛙卵油和植物甾醇（豆甾醇）在大鼠中的降脂作用

目　的：探索林蛙副产物林蛙卵油的开发价值。

创新点：首次验证了低胆固醇林蛙卵油的减重降脂功能。

方　法：Sprague-Dawley 大鼠连续被灌胃 8 周后，分成 8 组：（1）对照组喂食标准饲料；（2）高脂组喂食 60%高脂饲料；(3)阳性对照组喂食 3.33 mg/(kg·d) 辛伐他汀；（4）OORC 组喂食 2 g/(kg·d)的低胆固醇林蛙卵油（OORC）；（5）植物甾醇组喂食 0.48 g/(kg·d)的豆甾醇；（6）低剂量组喂食 1 g/(kg·d)的低胆固醇 OORC 和 0.24 g/(kg·d)的豆甾醇；（7）中剂量组喂食 2 g/(kg·d)的低胆固醇 OORC 和 0.48 g/(kg·d)的豆甾醇；（8）高剂量组喂食 4 g/(kg·d)的低胆固醇 OORC 和 0.96 g/(kg·d)的豆甾醇。经过不同方法处理后，比较各组大鼠的体重、器官指数，以及血清中总胆固醇、甘油三酯、低密度脂蛋白和高密度脂蛋白浓度。同时，对动物肝脏进行组织切片染色并评价损伤情况。

结　论：低胆固醇林蛙卵油或者其与植物甾醇（如豆甾醇）联用可以预防高脂饮食诱导的增重、肝损伤和高血脂症状。

关键词：林蛙卵油；林蛙；肥胖；高血脂