การศึกษาฤทธิ์ของสารสกัดพิกัดนวโกฐต่อการแสดงออกของยีนแอลดีแอลรีเซปเตอร์และยีนเอชเอ็มจีโคเอ รีดักเตส

Effect of Navakot Extract Studies on Expression of LDL-Recepteor (*LDL-R*) and HMG-CoA Reductase Genes (*HMGR*)

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บทคัดย่อ

คณะผู้วิจัยได้ทำการทดสอบความเป็นพิษของสารสกัดจากสมุนไพรในพิกัดนวโกฐ (NVKE) โดยใช้เซลล์มะเร็งตับ HepG2 พบว่า NVKE มีค่า IC50 = 9.12 มก./มล. (เทียบกับสารมาตรฐาน Simvastatin IC50 = 10.54 มก./มล.) จากนั้นจึงทำการศึกษาฤทธิ์ลดไขมันของ NVKE โดยใช้วิธี quantitative real-time PCR (qRT-PCR) พบว่า NVKE สามารถกระตุ้นการสร้างยีนแอลดีแอล รีเซปเตอร์ 1.21 เท่า และฤทธิ์ยับยั้งการแสดงออกของยีนเอชเอ็มจีโคเอรีดักเตส -0.91 เท่า

ABSTRACT

We have studied the cytotoxicity of ethanol-extracts of Navakot extract (NVKE) in human hepatocellular carcinoma cell line (HepG2). The results showed that the NVKE exhibited cytotoxicity to HepG2 cells (IC50 = 9.12 mg/ml) nearly equivalent level of Simvastatin (IC50 = 10.54 mg/ml). Furthermore, we have studied the lipid lowering effect of NVKE using quantitative real-time PCR (qRT-PCR). It is found that, NVKE could enhance synthesis of LDL-Receptor (*LDL-R*) gene (fold amplification = 1.21) but inhibit HMG-CoA reductase gene (*HMGR*) (fold amplification = -0.91).

คำสำคัญ: สารสกัดพิกัดนวโกฐ, แอลดีแอลรีเซปเตอร์, เอชเอ็มจีโคเอ รีดักเตส

Keywords: *Navakot extracts*, *LDL-R*, HMG-CoA *reductase*

INTRODUCTION

Hypercholesterolemia is a risk factor for development of cardiovascular disease (CVD). The condition is rapidly becoming more prevalent in developing countries, leading to a global increase in CVD. In addition, CVD is one of the leading causes of death in Thailand. Cholesterol biosynthesis is one of the most intensively studied biochemical pathways due to its well-known relevance to human health and disease. HMG-CoA reductase (*HMGR*), a highly conserved, membrane-bound enzyme, catalyzes a rate-limiting step in cholesterol biosynthesis and is the primary target of hypocholesterolemic drug therapy (Notarnicola M, 2010). *HMGR* is regulated at the levels of transcription, translation, post-translational modification and degradation (Goldstein JL, 1990). Consequently, inhibition of this enzyme results in decreased synthesis of cholesterol and other products downstream of mevalonate. LDL-Receptor (*LDL-R*) is a cell surface transmembrane protein that mediates the uptake and lysosomal degradation of plasma LDL cholesterol (LDL-C). *LDL-R* is ubiquitously expressed and is a key receptor for maintaining cholesterol homeostasis in mammals. *LDL-R*-mediated endocytosis which is essential for lipoprotein and lipid metabolism provides much of our understanding of lipoprotein clearance. Impaired *LDL-R* function by genetic mutations results in a condition with extremely elevated serum LDL levels and early onset atherosclerosis known as familial hypercholesterolemia (Hobbs HH, 1992). The use of herbs have been researched and tested for their positive effects on the cardiovascular system. Moreover, the pharmacological properties of Thai herbs have been well documented. Ya-Hom Navakot is a Thai medicinal plant recipe which originated a long time ago from the Thai wisdom. It is recommended to use in primary health care in Thailand and is included in the Thai traditional household drug. The Ministry of Health has listed Ya-Hom Navakot in the Lists of Herbal Medicinal Products A.D.2006. Ya-Hom Navakot is composed of many herbal medicines including nine most important herbs: *Angelica dahurica* Benth., *Atractylodes lancea* (Thung.) DC, *Ligusticum sinense* Oliv. Cv. Chuanxiong, *Angelica sinensis* (Oliv.) Diels, *Artemisia annua* L., *Saussurea lappa* C.B.Clarke, *Picrorhiza kurrooa* Royle ex Benth., *Terminalia chebula* Retz., *Anacyclus pyrethrum* (L.) Lagasca. Various modes of action of Ya-Hom Navakot are anti-dizziness, anti-nausea, anti-vomiting, and improving blood circulation etc.

This study aims to determine the effect of ethanol-extracts of Navakot extract (NVKE) on the expression of the genes encoding *LDL-R*, HMG-CoA reductase which are the genes involving in LDL metabolism at the transcriptional level in the human hepatocellular carcinoma cell by using quantitative real-time PCR (qRT-PCR).

MATERIALS AND METHODS

1. Preparation of NVKE extract

Nine herbs, the core component of Ya-Hom Navakot, were dried and equally mixed before grinding into powder. Ethanol extracts of Ya-Hom Navakot recipe were prepared using the method from Department of Pharmacognosy, Faculty of Pharmacy, Silpakorn University.

2. Cell culture conditions

The human hepatocellular carcinoma (HepG2) cell line has maintained most liver-specific cell functions and serves as a useful model system for studies of human hepatic lipid synthesis and lipoprotein synthesis and secretion. These characteristics allow this cell line to serve as a viable substitute for the human hepatocyte in investigations of the co-ordinate regulation of the expression of genes involved in cholesterol biosynthesis and receptor-mediated uptake of LDL (Molowa DT, 1989). HepG2 cells were provided by the American Type Culture Collection (ATCC).The culture protocol was followed as the method recommended by ATCC. To elucidate the effect of NVKE on the expression of *LDL-R*, HMG-CoA reductase genes, HepG2 cells were plated at a

density of 1×10^6 cells/ml in a T25 culture flask. When the cells were approximately 80% confluent, they were cultured in standard medium containing 1 mg/ml of NVKE (test medium) for 24 hrs. 1 mg/ml of Simvastatin (a cholesterol-lowering drug) was used as a positive control. As a negative control, cells were only cultured in DMEM complete medium.

3. Cytotoxicity assessment of NVKE using MTT assay

The MTT3-(4, 5 di-methylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was modified from Mossmann (Mossmann, 1983).

4. RNA Extraction and Quantitation

The cell pellet was used for RNA extraction using Trizol reagent (Mol. Res. Center Inc., Cincinnati, Ohio, USA), following the manufacturer's instruction. Concentration of the extracted RNA was measured using a nanophotometer (NanoPhotometer™ (version2.0), Implen GmbH, Munich, Germany).

5. cDNA synthesis

First-strand complementary DNA was synthesized by priming with oligo$(dT)_{15}$ primer (Promega Mannheim, Germany). Reverse transcription was performed by using ImProm-II™ Reverse Transcriptase (Promega Mannheim, Germany).

6. Study of the effect of NVKE on gene expression using qRT-PCR

The RT-PCR was carried out by the method modified from Notarnicola M, 2010 (Agilent Mx3005P QPCR Systems (real-time thermal cycler)) using the following parameters: one cycle of 95°C for 10 min, 1 cycle; at 95°C for 30 min, specific annealing temperature, 61°C for 45 s (depending on product length) and 72°C for 1 min for 40 cycles and a further melting curve step for 1 cycle. *GAPDH* gene, a housekeeping gene, was used as an internal control. Expression of *LDL-R* and HMG-CoA reductase were calculated by fold amplification=$2^{-\Delta\Delta Ct}$.

7. Statistical Analysis

All experiments were done in triplicates. Experimental values will be expressed as mean ± standard deviation (S.D). Statistical significance of this mean will be assessed by Student's t-test, with a value of *p<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

The results showed that NVKE could enhance synthesis of *LDL-R* gene (fold amplification=1.21, P=0.01) when compared to control. However, Simvastatin was found to up-regulate *LDL-R* gene twice more than NVKE (fold amplification=2.83, P<0.01) (Fig.1). Furthermore, NVKE was shown to down-regulate the synthesis of HMG-CoA reductase gene (fold amplification= -0.91) (P<0.01). This inhibition effect of NVKE was more than simvastatin (fold amplification= 0.99, P=0.159) when compared to control (Fig.2).

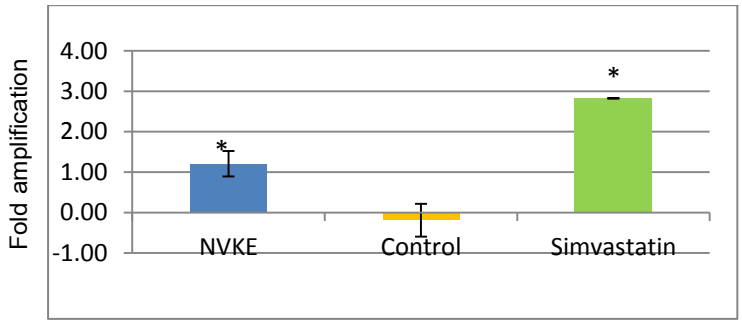

Figure 1 Effect of 1mg/ml NVKE on expression of the gene encoding *LDL-R* in HepG2 cells. Bars represent mean ± SD of triplicate experiments. * P-value < 0.05.

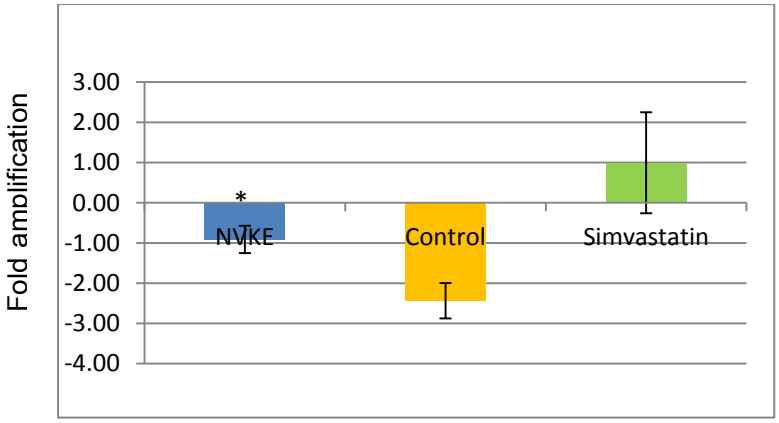

Figure 2 Effect of 1mg/ml NVKE on expression of the gene encoding HMG-CoA reductase in HepG2 cells. Bars represent mean ± SD of triplicate experiments. *P-value <0.05.

CONCLUSION

Ya-Hom Navakot has been shown to have several modes of action on improving the blood circulation. However, scientific research data that supports previously known mode of action of Ya-Hom Navakot is very limited. This study, NVKE extracts has been found to have lipid lowering effects by enhancing *LDL-R* gene and inhibiting HMG-CoA reductase gene expressions.

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