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NOTE

Ellagic Acid from the Dried Fruits of *Canarium album* with Antihepatitis B Activity

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The anti-HBsAg/HBeAg activity of ellagic acid from the dried fruits of *Canarium album*, was tested by ELISA and the cytotoxicity of ellagic acid on HepG2215 cells was detected using MTT method. The results indicate that ellagic acid at 8 μg/mL inhibited replication of hepatitis B virus (HBV) in HepG2215 cells over 8 days, Production of hepatitis B virus surface antigen and hepatitis B virus e antigen was 19.3 and 43.2 % of controls without ellagic acid. The inhibition was ellagic acid dosage dependent. The anti-HBsAg/HBeAg activity is better than that of gallic acid, regarded as effective component of anti-HBsAg/HBeAg of *C. album*. And ellagic acid was nontoxic to HepG2215 cells at the tested dosages. Therefore, the ellagic acid is the effective component of anti-hepatitis B from the dried fruits of *C. album* and had potential to be an antihepatitis medicine.

Key Words: Ellagic acid, *Canarium album*, Hepatitis B.

Canarium album (Lour.) Raeusch or Chinese olive (Burseraceae) is widely distributed in southern China, The dried fruit is a traditional medicine material in China and possesses some pharmacological functions, such as antibacterium[1], antiinflammation and detoxification[2], antioxidation[3], antialcohol and hepatoprotective activities[4] and antihepatitis B activities[5]. Further investigations found that the gallic acid (GA) is effective component of anti-HBsAg/HBeAg[6,7]. Previous study has shown *C. album* is rich in phenolic compounds, which is responsible for its pharmacological characteristics[2]. Although some hepatoprotective compounds from *C. album*, including seven triterpenes[8], brevifolin, hyperin and ellagic acid[9] were reported, but all these compounds were isolated from the dried stem and leaf of *C. album*. Up to date, reports on the effective components from the dried fruit of *C. album* were very scarce.

Ellagic acid (EA) (2,3,7,8-tetrahydroxy[1]benzopyrano-[5,4,3-cde][1] benzopyran-5,10-dione) (Fig. 1) is a plant-derived polyphenol found in a wide variety of fruits and nuts, such as raspberries, strawberries, walnuts, grapes and black currants. Ellagic acid has a variety of biological activities including antioxidant[10], antiinflammatory[11], antifibrosis[12] and anticancer[13,14]. In this study, we have investigated the antihepatitis B activity of ellagic acid from the dried fruit of *C. album* and made the comparison between the activity of ellagic acid and gallic acid for further investigating effective component of anti-hepatitis B activities of *C. album* and finding new drug or pre-drug for many people infected with hepatitis B virus.

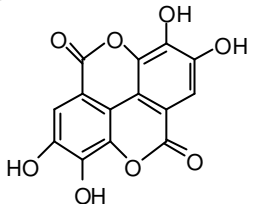

Fig. 1. Structures of ellagic acid

The plant material was collected in September 2006 from Jiangjin, Chongqing municipality and identified as *Canarium album* (Lour.) Raeusch by Professor Zhang Hanming, College of Pharmacy, Second Military Medical University.

Extraction and identification of ellagic acid: The dried fruits were chopped and extracted with 80 % EtOH three times under reflux and concentrated under vacuum to yield an EtOH extract. The residue was further suspended in water and partitioned with petroleum ether and EtOAc successively. The EtOAc extract was repeatedly subjected to column chromatography over silica gel with the gradient $CHCl_3$-CH_3OH (80:1; 30:1; 10:1;1:1; 0:1) to afford five fractions and the fraction 4 was purified over silica gel and Sephadex LH-20 with MeOH repeatedly to afford ellagic acid. Data of MS and NMR are as

follows: ESI-MS m/z: 303 $[M+H]^+$; ^1H NMR (DMSO-d_6) δ: 7.45 (2H, S); ^{13}C NMR (DMSO-d_6) δ: 158.7 (C-5,10), 148.4 (C-3,8), 139.8 (C-2,7), 136.4 (C-1a,6a), 111.7 (C-4a,9a), 110.3 (C-4,9), 107.3 (C-4b,9b), which are similar to the data of ellagic acid. The compound was dissolved in dimethyl sulfoxide (DMSO) for bioactivity assay, which had purity f 99.2 % by the external-standard HPLC method in our laboratory.

Cell line and antiviral assay: Cell line HepG2215 (HepG2 cell transferred with hepatitis B virus DNA) was obtained from Department of Microbiology, College of Basic Medical Sciences, Second Military Medical University. Cells were cultured in 96-well plates at 10^5 cells/well in MEM medium containing 10 % (v/v) fetal bovine serum, 100 units streptomycin/mL and 100 units penicillin/mL, 1 mM glutamine/mL and 200 μg Geneticin G418/mL. Every 3 days, the medium was replaced with fresh medium containing ellagic acid/gallic acid from 1 to 8 μg/mL (the control group contained no ellagic acid/gallic acid). The concentrations of fetal bovine serum and Geneticin G418 in the fresh medium were amended to 2 % (v/v) and 380 μg/mL, respectively. After 8 days, the cultured medium was replaced with PBS solution containing 0.5 mg MTT/mL. The cultured medium was used to detect hepatitis B virus surface antigen(HBsAg) and hepatitis B virus e antigen (HBeAg) using the radioimmunoassay kits (purchased from Institute of Atomic Energy of China, Bejing, China). After 4 h incubation, PBS solution was removed and 1 mL DMSO was added into each well. The absorbance at 490 nm was measured with a microplate reader and the results are shown in Table-1.

TABLE-1
INHIBITION EFFECTS OF ELLAGIC ACID ON HEPATITIS VIRUS ANTIGEN AND ITS CYTOTOXICITY TO HepG2215 CELLS

Sample	Dosage (μg/mL)	Inhibition ratio on HbsAg (%)	Inhibition ratio on HbeAg (%)	Cytotoxinic rate (%)
Ellagic acid	1	25.5	39.7	0
	2	37.8	45.2	0
	4	56.6	48.5	0
	8	80.7	56.8	8
Gallic acid	1	20.3	26.6	0
	2	29.6	32.5	0
	4	47.8	40.8	0
	8	68.5	47.7	11

Inhibition ratio (IR) = (antigen content of control-antigen content of ellagic acid/gallic acid group)/antigen content of control × 100 %
Cytotoxitic rate = (absorbance of control - absorbance of ellagic acid/gallic acid group)/absorbance of control × 100 %.

The results in Table-1 indicate that ellagic acid at 8 μg/mL inhibited replication of hepatitis B virus in HepG2215 cells over 8 days, Production of hepatitis B virus surface antigen and hepatitis B virus e antigen were 19.3 and 43.2 % of controls without ellagic acid. and the inhibition was ellagic acid dosage dependent. The anti-HBsAg/HBeAg activity is better than that of gallic acid, regarded as effective component of anti-HBsAg/HBeAg of *C. album*. What is more, ellagic acid was nontoxic to HepG2215 cells at the tested dosages. Which showed that it had potential to be an anti-hepatitis medicine, though further investigation on the mechanism is necessary.

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