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Brief Communication

Inhibitory Effects of Curcuminoids on 17β-hydroxysteroid Dehydrogenase Type 1 Activity in Animal Livers

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17-β-hydroxysteroid dehydrogenase type 1 (17β-HSD type 1) mediates the reaction of 17β-estradiol (E2) production from estrone (E1). Inhibitory effects of curcuminoids on 17β-HSD type 1 activity were investigated to find a lead compound for treating estrogen-dependent diseases including breast cancer. Among curcuminoids, demethoxycurcumin showed potent inhibitory effect (IC_{50} = 2.7 μM) on mouse 17β-HSD type 1. Curcuminoids also displayed their inhibitory effects on the production of 17α-estradiol which is a carcinogenic metabolite produced by the enzyme. Bisdemethoxycurcumin (IC_{50} = 1.3 μM) showed potent inhibitory effect on the 17α-estradiol production by chicken 17β-HSD type 1. Curcuminoids did not inhibit ERE transcriptional activity with and without E2. Taken together, curcuminoids can be used for treating and preventing E2-dependent diseases via inhibition on 17β-HSD type 1 activity.

Key Words: Curcuminoids, 17β-hydroxysteroid dehydrogenase type 1, 17β-estradiol, 17α-estradiol, Demethoxycurcumin

Breast cancer is the most common malignant disease in Korea woman (Yoo et al., 2002; Jung and Lee, 2009; Park et al., 2011) and several causative factors in dietary issues include a change to a westernized life style increasing body mass index, changes in dietary fiber leading to altered gut flora, changing lipid intakes and dilution of a range of chemo-protective, largely phytonutrient factors (Hyman, 2007; Jung and Lee, 2009). Other major risk factors of breast cancer in Korean woman are early menarche, late menopause, late full-term pregnancy (FTP), and low numbers of FTP (Yoo et al., 2002; Park et al., 2011). In relation to the epidemiological approach to incidence of breast cancers, recent molecular pharmacological studies demonstrate that 17β-estradiol (E2) develops and progresses estrogen-dependent diseases including breast cancer, endometriosis, and endometrial hyperplasia (Travis and Key, 2003; Saloniemi et al., 2010). Therefore, endocrine therapy on breast cancer is based to deprive tumor cells of estrogen stimulation, especially inhibiting ovarian estrogen synthesis in premenopausal women (Lønning et al., 2011).

E2 biosynthetic pathway has been well documented and the final step is the reduction of the weakly active estrone (E1) (Fig. 1) catalyzed by 17β-hydroxysteroid dehydrogenases (17β-HSDs). 17β-HSDs mediate stereospecific oxido-reduction reactions at position 17 of steroid hormones and they are divided into NADPH dependent (17β-HSD

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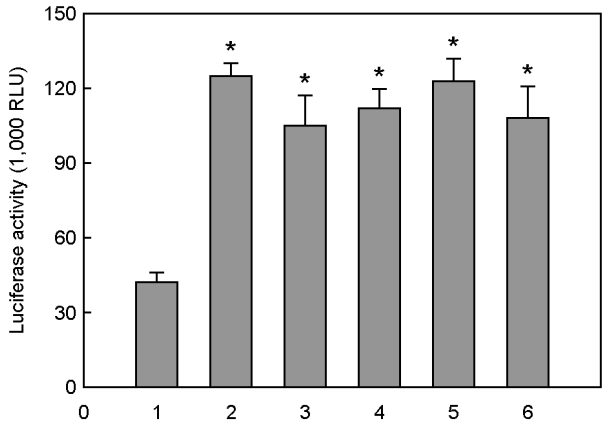

Fig. 1. Effect of curcuminoids on ERE transcriptional activity in CV-1 cells. CV-1 cells were transiently transfected with plasmid indicated as below; EREII-LUC, 250 ng; ER expression vector, 100 ng; 500 ng of each coactivator expression vector (GRIP1 and CARM1) with 20 nM E2 with or without 1 μM curcuminoids. Lane 1, ER only; lane 2, ERE transcriptional system with E2; lane 3, ERE transcriptional system with E2 and curcumin; lane 4, ERE transcriptional system with E2 and demetoxycurcumin; lane 5, ERE transcriptional system with E2 and bisdemethoxycurcumin; lane 6, ERE transcriptional system with E2 and tetrahydroxycurcumin. An asterisk (*) indicates the level of significance with a *P* value at 0.05 compared to control (without E2).

types 1, 3, 5, 7 and 12) and NAD^+ dependent (17β-HSD types 2, 4, 6, 8, 10, 11, and 14). Recently, many studies on developing inhibitors on 17β-HSD type 1 enzyme have showed a promising strategy to control estrogen receptors-positive post-menopausal breast cancer (Aka et al., 2009; Aka et al., 2010).

On the other hand, 17α-estradiol has been produced during E2 formation and it is a potent estrogen and carcinogen in developmental stage (Hajek et al., 1997). Suppression on 17α-estradiol production is for preventing occurrence of cancers. However, no information has been reported for developing inhibitors in relation to suppression on 17α-estradiol. Therefore, a biological assay method should be established for determining 17α-estradiol formation and finding inhibitors on it.

Herein, curcuminoids isolated from *Curcuma longa* L. (Fig. 1) were used for determining their inhibitory activities on 17β-HSD type 1 presented in mouse, rat, and chicken liver cytosols as an assay method using rat liver preparations developed previously (Kruchten et al., 2009a). In relation to determine inhibitory properties of curcuminoids on 17α-estradiol production, only chicken liver preparation was used in this study. Antiestrogenic activities on esterogen receptor (ER) of curcuminoids were also determined using CV-1 cells in this study (Lee et al., 2002).

17-β-estradiol (E2), estrone (E1), NADPH, phenylmethanesulfonyl fluoride (PMSF), and EDTA were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Dithiothreitol (DTT) was purchased from Aldrich Chemical Co. (Milwaukee, WI). All purchased chemicals were of the highest grade commercially available.

Curcumin, demethoxycurcumin, bisdemethoxycurcumin, and tetrahydrocurcumin were isolated according to the protocols previously reported (Park et al., 2005). The dried rhizomes (2 kg) of *Curcuma longa* were purchased from a medicinal herb shop, Kyungdong Market (Seoul, South Korea). The rhizomes finely powered, extracted with methanol (3 L × 2) at room temperature. After filtering of the methanol extracts, the filtrates were pooled. The combined filtrate was concentrated under vaccum at 35℃ to yield about 10% of the weight of the dried rhizomes. The methanol extract (10 g) was fractionated using a column (5.5 × 70 cm) packed with silica gel (70~230 mesh, Merck, Darmstadt, Germany). The fractions were eluated with CH_2Cl_2/methanol (50:1 --> 0:100) in a stepwise fashion. The fractions were pooled into five major fractions on the basis of their TLC profiles. The fraction, M2 (2.91 g), was further separated by column chromatography (4.5 × 65 cm) on silica gel (180 g) using n-hexane/ethylacetate (2:1 --> 3:2 --> 4:3), yielding orange crystals of curcumin (380 mg, Rf = 0.5), orange crystals of demethoxycurcumin (79 mg, Rf = 0.35), orange crystals of bisdemethoxycurcumin (71 mg, Rf = 0.25), and white crystals of tetrahydrocurcumin (38 mg, Rf = 0.6). TLC was performed on precoatedsilica gel 60 F254 and RP-18 F254s plates, which were developed with CH_2Cl_2 (20:1). Structural determination of the isolated compounds was conducted by comparison of their physical and spectral data with those reported in the literature.

All preparations were followed by the method reported previously (Lee et al., 2001) and carried out at 4℃. One gram of animal liver was homogenized in a glass homo-

genizer with 15 mL of 100 mM phosphate buffer, pH 7.4, containing 0.4 mM PMSF, 0.1 mM DTT and 1 mM EDTA. The resultant homogenates were filtered through 4 layers of cheesecloth. The homogenates were centrifuged at 12,100 g at 4℃ for 20 min using an Eppendorf centrifuge 5417 R. Supernatants were reserved as crude enzyme extracts.

Crude enzyme extracts were transferred to 15 mL polycarbonate ultracentrifuge tubes and centrifuges at 100,000 g at 4℃ for 1 h, including acceleration time, in a Beckman L8-M ultracentrifuge using a Ti 70 rotor. The supernatant was reserved as the cytosolic fraction and kept for further inhibition study of 17β-HSD type 1 enzyme.

Biosynthesis of E2 from E1 was studied using an incubation mixture (250 μL final volume) consisting of 92 mM sodium phosphate buffer, pH 7.4, 0.5 mM NADPH, and 2 mg mL^{-1} protein of the animal liver cytosol preparation. After a preincubation period of 10 min at 37℃, E1 (10 mL of a 1,000 ppm solution in DMSO) was added as substrate to the reaction mixture. After 1 h of incubation, reactions were stopped by adding 1 mL of ice-cold methanol. This mixture was centrifuged at 12,100 g for 10 min at room temperature. The supernatant was analyzed by a reversed-phase Supelcosil LC-18 column (250 × 4.6 mm) equipped with a fluorescence detector. The mobile phase was a mixture of water/acetonitrile/methanol (60:20:20). E2 was detected at the retention time of 10.4 min and 17α-estradiol was detected at the retention time of 11.9 min under the excitation wavelength of 280 nm and the emission wavelength of 312 nm.

The IC_{50} value (expressed as micromolar) is the concentration at which 50% inhibition of E2 or 17α-estradiol formation was reached as calculated from the dose-response curve.

To determine transcriptional activity of curcuminoids on estrogen receptor α (ERα), mammalian expression vector encoding pHE0 for human ERα and the luciferase-expressing reporter genes were described previously (Lee et al., 2002). CV-1 cells were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum. Approximately 20 h before transfection, 10^5 cells were seeded into each well of six-well dishes. The cells in each well were transiently transfected with plasmid indicated as below; EREII-LUC, 250 ng; ER expression vector, 100 ng; 500 ng of each coactivator expression vector (GRIP1 and CARM1) using Super Fect Transfection Reagent. After transfection, the cells were grown in medium supplemented with 5% charcoal-stripped fetal bovine serum for 40 h before harvest; where indicated, the medium was supplemented with 20 nM E2 and 1 μM curcuminoids during the last 30 h of growth. Luciferase assays were performed with the Promega Luciferase Assay kit. The results shown are representative of at least three independent experiments.

Curcuminoids contain a β-diketonemoiety with various pharmacological properties as antioxidant activity (Kolodziejczyk et al., 2011), anti-inflammatory activity (Schaffer et al., 2011), anticarcinogenic activity (Park and Conteas, 2010; Singh and Singh, 2011), and antialzheimer activity (Yanagisawa et al., 2011). In our laboratory, serial experiments for determining pharmacological properties of curcuminoids have showed inhibitory effects on aflatoxin B1 biotransformation to aflatoxin B1-8,9-epoxide (Lee et al., 2001), inhibition on sortase A activity and *Staphylococcus aureus* cell adhesion to fibronetin (Park et al., 2005), and inhibition on β-amyloid fibril formation (Kim et al., 2005).

To determine inhibitory effects on E2 formation by 17β-HSD type 1, curcuminoids were tested. The effects of curcuminoids on E2 formation in mouse liver are shown in Table 1. Among the tested curcuminoids, demethoxycurcumin (IC_{50} = 2.7 μM) exhibited the most potent inhibitory effect on E2 formation. Tetrahydrocurcumin (IC_{50} = 7.4 μM) and bisdemethoxycurcumin (IC_{50} = 8.6 μM) showed strong inhibition of E2 formation, whereas curcumin (IC_{50} = 22.4 μM) showed weak inhibitory effect on the biotransformation. Demethoxycurcumin showed about 8.3 times stronger inhibitory effect on E2 formation than curcumin. The inhibitory pattern of curcuminoids on E2 formation by rat liver cytosol was similar to the results using mouse liver cytosol. However, there was almost two times difference between mouse liver and rat liver cytosols (Table 1). Interestingly, the activity of 17β-HSD type 1 enzyme in mouse liver cytosol (15.6 nmoles E2 produced min^{-1} $proteins^{-1}$) was much higher than that of the enzyme in rat liver cytosol (1.4 nmoles E2 produced min^{-1} $proteins^{-1}$),

Table 1. IC_{50} values for inhibition of biotransformation of estrone to an estrogen 17β-estradiol (E2) by curcumioids using animal liver cytosols

Compounds	IC_{50} values (μM)		
	Mouse liver cytosols[1)]	Rat liver cytosols[2)]	Chicken liver cytosols[3)]
Curcumin	22.4	47.4	32.6
Demethoxycurcumin	2.7	7.6	42.6
Bisdemethoxycurcumin	8.6	20.1	> 100
Tetrahydrocurcumin	7.4	14.7	> 100

[1)] The biotransformation reaction was mediated by 17β-hydroxysteroid dehydrogenase (17β-HSD) type 1 enzyme and the enzyme activity was 15.6 nmoles E2 produced min^{-1} $proteins^{-1}$.
[2)] The biotransformation reaction was mediated by 17β-HSD type 1 enzyme and the enzyme activity was 1.4 nmoles E2 produced min^{-1} $proteins^{-1}$.
[3)] The biotransformation reaction was mediated by 17β-HSD type 1 enzyme and the enzyme activity was 14.2 nmoles E2 produced min^{-1} $proteins^{-1}$.

whereas the inhibitory effect of curcuminoids was generally found in mouse 17β-HSD type 1 enzyme. As shown in Table 1, demethoxycurcumin (IC_{50} = 7.6 μM) was the most potent inhibitory compound on E2 formation in the reaction mixtures including rat liver cytosol. Tetrahydrocurcumin (IC_{50} = 14.7 μM) and bisdemethoxycurcumin (IC_{50} = 20.1 μM) showed strong inhibition of E2 formation, whereas curcumin (IC_{50} = 47.4 μM) showed less inhibition of the biotransformation. Using chicken liver cytosol, the inhibition by curcuminoids on E2 formation was less effective as bisdemethoxycurcumin and tetrahydrocurcumin did not possess any inhibitory activities on the enzyme, 17β-HSD type 1. Curcumin (IC_{50} = 32.6 μM) and demethoxycurcumin (IC_{50} = 42.6 μM) showed inhibitory effects on E2 production.

A series of analogs of curcumin was used to determine the structural features necessary to possess inhibitory effects on E2 formation in this study. Bisdemethoxycurcumin lacking methoxy groups on both of the aromatic rings increased inhibitory activity, whereas demethoxycurcumin lacking a methoxy group on one of the aromatic rings possessed less inhibitory activity (Table 1) against mouse and rat liver cytosol preparations. On the other hand, bisdemethoxycurcumin did not show an inhibitory effect on the 17β-HSD type 1 in chicken liver cytosol. Therefore, the inhibitory effect of curcuminoids differs from the animal species tested because the active site of 17β-HSD type 1 enzyme may be different between birds and mammals. These findings demonstrate that the animal model for finding inhibitors of 17β-HSD type 1 should be carefully selected with the consideration on the structural similarity in relation to human originated 17β-HSD type 1 enzyme. 17β-HSD type 1 enzymes from mammals may be much closer to that of humans than that of birds.

Table 2. IC_{50} values for inhibition of biotransformation of estrone to a non-estrogen 17α-estradiol by curcumioids using chicken liver cytosol

Compounds	IC_{50} values (μM)
Curcumin	2.7
Demethoxycurcumin	> 20
Bisdemethoxycurcumin	1.3
Tetrahydrocurcumin	14.2

[1)] The biotransformation reaction was mediated by 17β-HSD type 1 enzyme in chicken liver cytosols and the enzyme activity was 7.0 nmoles of 17β-estradiol produced min-1 proteins-1.

Inhibitory effects of curcuminoids on 17α-estradiol formation were also determined in the study. To determine inhibitory effects on 17α-estradiol formation, only chicken liver cytosol was used because mouse and rat liver cytosols did not produce 17α-estradiol in the reaction mixtures. Bisdemethoxycurcumin (IC_{50} = 1.3 μM) was the most potent compound with different amounts of protein in chicken liver cytosol (Table 2). Curcumin (IC_{50} = 2.7 μM) and tetrahydrocurcumin (IC_{50} = 14.2 μM) showed potent inhibitory effects on 17α-estradiol formation. It was first report and worth while for doing further studies using other 17α-estradiol production system.

Furthermore, we examined the ERα transcriptional

activity induced by curcminoids using an estrogen responsive element (ERE) based luciferase reporter gene assay. To test transcriptional activation of ERα by curcuminoids, expression vectors were transfected into CV-1 cells along with expression vectors for glutamate receptor interacting protein 1 (GRIP1) and co-activator-associated arginine methyltransferase 1 (CARM1) and a luciferase reporter gene containing curcuminoids for the ERα. Coexpression of GRIP1 and CARM1 with estrogen receptor enhanced reporter gene expression (Fig. 1, lane 2) when E2 was present. However, the level of gene expression was not significantly changed when curcuminoids were treated into the expression system (Fig. 1). The results from the CV-1 cells treated with curcuminoids showed no difference in ERE transcriptional activity when compared to control. These results showed that curcuminoids exhibited no anti-estrogenic activity using ERα transcriptional expression.

As getting reasonable data using mammalian liver preparations, a report has introduced to use rat liver preparations for determining selective inhibitors on biotransformation of E2 from E1 (Kruchten et al., 2009a). The report demonstrates that an appropriate animal experiment should be established for further preclinical evaluation of inhibitors on E2 biosynthesis and two assays has been developed to determine E2 activation and inactivation as well as the identification of E2-formation in using rat liver preparations (Kruchten et al., 2009a). Using mammalian liver preparations, a β-diketone structure of curcuminoids linking two phenyl groups with the intermediate double bonds and a methoxy group on a phenyl ring should be necessary to inhibit 17β-HSD type 1 activity.

In recent, curcumin derivatives inhibit testicular 17β-hydroxysteroid dehydrogenase 3 (17β-HSD type 3), an enzyme catalyzing the final step in testosterone biosynthesis in Leydig cells (Hu et al., 2010). 17β-HSD type 3 is localized in the microsomal fraction of the Leydig cells of the testis and has become a major target in the treatment of hormone-dependent prostate diseases. These findings are significantly different from our findings dealing with 17β-HSD type 1 activity. Therefore, curcuminoids inhibit 17β-HSD type 1 activity, referring to use them for treating E2-dependent diseases.

As diet can modulate sex hormone biosynthesis, receptor activity, and sex hormone metabolism, Hyman (2007) has suggested that the influence of diet includes the hypothalamic-pituitary-adrenal axis and thyroid function and certain phytonutrients in the diet may enhance specific pathways of estrogen metabolism and detoxification as isoflavones, essential fatty acids, and indole-3-carbinol. On the other hand, certain phytonutrients are not easily absorbed with their low bioavailability. Anand et al. (2007) showed that curcumin was safe even at high doses (12 g/day) in humans but exhibited poor bioavailability in Phase I clinical trials. Major reasons contributing to the low plasma and tissue levels of curcumin appear to be due to poor absorption, rapid metabolism, and rapid systemic elimination. Anand et al. (2007) demonstrated that the use of structural analogues of curcumin was the best tool to improve the bioavailability of curcuminwith a rapid absorption in the tested methods. Enhanced bioavailability of curcuminoidsis the key to use this promising natural product for treatment of human diseases.

Further studies will be performed to evaluate the inhibitory effects on rat liver 17β-HSD type 1 *in vivo* developed previously (Kruchten et al., 2009b) with their bioavailability.

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REFERENCES

Aka JA, Mazumdar M, Chen CQ, Poirier D, Lin SX. 17beta-hydroxysteroid dehydrogenase type 1 stimulates breast cancer by dihydrotestosterone inactivation in addition to estradiol production. Mol Endocrinol. 2010. 24: 832-845.

Aka JA, Mazumdar M, Lin SX. Reductive 17beta-hydroxysteroid dehydrogenases in the sulfatase pathway: critical in the cell proliferation of breast cancer. Mol Cell Endocrinol. 2009. 301: 183-191.

Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. Mol Pharmaceutics. 2007. 4: 807-818.

Hajek RA, Robertson AD, Johnston DA, Van NT, Tcholakian RK, Wagner LA, Conti CJ, Meistrich ML, Contreras N, Edwards

CL, Jones LA. During development, 17alpha-estradiol is a potent estrogen and carcinogen. Environ Health Perspect. 1997. 105 (Suppl. 3): 577-581.

Hu GX, Liang G, Chu Y, Li X, Lian QQ, Lin H, He Y, Huang Y, Hardy DO, Ge RS. Curcumin derivatives inhibit testicular 17beta-hydroxysteroid dehydrogenase 3. Bioorg Med Chem Lett. 2010. 20: 2549-2951.

Hyman MA. The life cycles of woman: restoring balance. Altern Ther Health Med. 2007. 13: 10-16.

Jung D, Lee SM. BMI and breast cancer in Korean woman: A meta-analysis. Asian Nursing Res. 2009. 3: 31-40.

Kim H, Park BS, Lee GK, Choi CY, Jang SS, Kim YH, Lee SE. Effects of naturally occurring compounds on fibril formation and oxidative stress of beta-amyloid. J Agric Food Chem. 2005. 53: 8537-8541.

Kolodziejczyk J, Olas B, Saluki-Juszczak J, Wachowicz B. Antioxidative properties of curcumin in the protection of blood platelets against oxidative stress *in vitro*. Platelets. 2011. 22: 270-276.

Kruchten P, Werth R, Marchais-Oberwinkler S, Bey E, Ziegler E, Oster A, Frotscher M, Hartmann RW. Development of biological assays for the identification of selective inhibitors of estradiol formation from estrone in rat liver preparations. C R Chim. 2009a. 12: 1110-1116.

Kruchten P, Werth R, Marchais-Oberwinkler S, Frotscher M, Hartmann RW. Development of a biological screening system for the evaluation of highly active and selective 17β-HSD1-inhibitors as potential therapeutic agents. Mol Cell Endocrinol. 2009b. 301: 154-157.

Lee SE, Campbell BC, Molyneux RJ, Hasegawa S, Lee HS. Inhibitory effects of naturally occurring compounds on aflatoxin B1 biotransformation. J Agric Food Chem. 2001. 49: 5171-5177.

Lee YH, Koh SS, Zhang X, Cheng X, Stallcup MR. Synergy among nuclear recptorcoactivator: selective requirement for protein methyltransferase and acetyltransferase activities. Mol Cell Biol. 2002. 22: 3621-3632.

Lønning PE, Haynes BP, Straume AH, Dunbier A, Helle H, Knappskog S, Dowsett M. Recent data on intratumor estrogens in breast cancer. Steroids. 2011. 76: 786-791.

Park BS, Kim JG, Kim MR, Lee SE, Takeoka GR, Oh KB, Kim JH. *Curcuma longa* L. constituents inhibit sortase A and *Staphylococcus aureuscell* adhesion to fibronectin. J Agric Food Chem. 2005. 53: 9005-9009.

Park J, Conteas IN. Anti-carcinogenic properties of curcumin on colorectal cancer. World J Gastrointest Oncol. 2010. 2: 169 -176.

Park SK, Kim Y, Kang D, Jung EJ, Yoo KY. Risk factors and control strategies for the rapidly rising rate of breast cancer in Korea. J Breast Cancer. 2011. 14: 79-87.

Saloniemi T, Jarvensivu R, Koskimies P, Jokela H, Lamminen T, Ghaem-Maghami S, Dina R, Damdimopoulou P, Makela S, Perheentupa A, Kujari J, Brosens J, Poutanen M. Novel hydroxysteroid (17beta) dehydrogenase 1 inhibitors reverse estrogen-induced endometrial hyperplasia in transgenic mice. Am J Pathol. 2010. 176: 1443-1451.

Schaffer M, Schaffer PM, Zidan J, Bar Sela G. Curcumin as a functional food in the control of cancer and inflammation. Curr Opin Clin Nutr Metab Care. 2011. 14: 588-597.

Singh M, Singh N. Curcumin counteracts the proliferative effect of estradiol and induces apoptosis in cervical cancer cells. Mol Cell Biochem. 2011. 347: 1-11.

Travis RC, Key TJ. Oestrogen exposure and breast cancer risk. Breast Cancer Res. 2003. 5: 239-247.

Yanagisawa D, Taguchi H, Yamamoto A, Shirai N, Hirao K, Tooyama I. Curcuminoid binds to amyloid-β1-42 oligomer and fibril. J Alzheimers Dis. 2011. 24(Suppl. 2): 33-42.

Yoo KY, Kang D, Park SK, Kim SU, Shin A, Yoon H, Ahn SH, Noh DY, Choe KJ. Epidemiology of breast cancer in Korea: occurrence, high-risk groups, and prevention. J Korean Med Sci. 2002. 17: 1-6.