A Thesis for the Degree of Master of Science

Gomisin N, a major lignan in *Schisandra chinensis*, increases production of testosterone and 17β-estradiol via stimulating steroidogenic enzymes

고미신 N의 스테로이드 합성 효소 자극을 통한 테스토스테론 및 17베타-에스트라디올 생합성 촉진 효능

February, 2018

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ABSTRACT

Schisandra chinensis, which is called Omija in Korean, has been widely used as herbal medicine for many symptoms including sexual enhancement in East Asia. There are many studies that have focused on the biological activities of lignans in *Schisandra chinensis* such as gomisin N, gomisin A, and schizandrin (schisandrol A).

Estrogen is a primary sex hormone in females. Estrogen plays important roles in reproductive and non-reproductive organs. When estrogen level decreases as women experience menopause, the risk for cardiovascular disease, depression, osteoporosis etc. increases. Therefore, maintaining physiological estrogen levels is beneficial to women with low estrogen level.

As society becomes more and more aged, an interest in menopause is increasing. Although the hormone replacement therapy (HRT) is representative treatment for relieving symptoms of menopause, a demand for natural compounds to be used on menopausal symptoms

increases because of the side effects of long term HRT.

For these reasons, the effect of *Schisandra chinensis* on relieving menopausal symptoms and ovarian failure has been already reported. However, the active compound and mechanisms need to be determined.

We aimed to identify the active compound of *Schisandra chinensis*. In addition, we focused on the effects of its active compound on steroidogenesis.

In this study, we used NCI-H295R cell line derived from human adrenal glands which is a model for studying steroidogenesis pathway. Among the three major lignans, we found that gomisin N promoted the production of testosterone and 17β-estradiol. To consider the effect on steroidogenesis and an abundance of composition in *Schisandra chinensis*, Gomisin N was chosen as an active compound. Gomisin N also has effects on steroidogenic enzymes. It increased the protein and gene expression levels of CYP11A1 and CYP17A1. Therefore, gomisin N may have promoted the production of testosterone and 17β-estradiol by increasing the amount of substrates such as pregnenolone and

androstenedione. In addition, gomisin N tended to increase the concentration of testosterone and 17β-estradiol in mouse antral follicles and 40 μM of gomisin N was the most effective in inducing steroid synthesis.

Keywords:

Gomisin N; *Schisandra chinensis*; 17β-estradiol; Testosterone; Steroidogenesis; Perimenopause; CYP11A1; CYP17A1;

Student ID: 2016-20485

CONTENTS

Ⅰ. INTRODUCTION

In East Asia, *Schisandra chinensis,* which is called Omija in Korean, has been widely used as materials for traditional prescriptions for many purposes [1]. It is also mentioned in Dongui Bogam as prescriptions for enhancing sexual function [2]. Many studies have shown that the compounds such as lignans, polysaccharides, and organic acids can be isolated by extracting *Schisandra chinensis* [3-5]. Among the compounds, many investigators have focused on lignans. The representative lignans in *Schisandra chinensis* are known as gomisin N, gomisin A, schizandrin (schisandrol A) and etc. [6] Biological activities of lignans such as anti-cancer, [7] antioxidant [8], non-alcoholic fatty-liver disease [9], and anti-wrinkle [10-12] have been reported. However, it is still unclear if the lignans, bioactive compounds in *Schisandra chinensis,* are related to sex hormones or reproductive function even though *Schisandra chinensis* had been used as prescriptions for sexual enhancement in the past.

Estrogen is a sex hormone which is necessary for female reproduction. There are three different forms of estrogens, estrone (E1), estradiol (E2), and estriol (E3) whose active form is mostly E2. E2 is produced in gonadal and extra gonadal organs, including the ovaries, adrenal glands, brain, adipose tissue, skin, and pancreas [13-15]. In the ovary, the gonadal organ, there are two types of cells related to steroidogenesis. Theca cells produce testosterone, and granulosa cells synthesize 17β-estradiol. In adrenal gland, the representative extra gonadal organ, adrenocortical cells in human produce E2 [16-18]. Adrenal glands express key enzymes for steroidogenesis such as CYP11A1 and CYP17A1. Steroidogenic activity in adrenal gland is stimulated by SF-1 expression [19].

As society becomes more and more aged, the interest in menopause is increasing. When estrogen level decreases as women experience menopause, the risk for irregular menstrual cycle, cardiovascular disease, depression, osteoporosis, and etc. increases [20-22]. Therefore, estrogen plays important roles in both reproductive and non-reproductive organs. A number of women over 45 experience the early stage of menopause.

Many studies have shown that the circulating E2 level declines during the perimenopause. Moreover, over half of women in the stage of the perimenopause start to experience symptoms such as headache, joint achesr stiffness, back pain, night sweats, hot flushes and difficulty concentrating [23,24]. Therefore, maintaining physiological estrogen levels is beneficial to women's health. Eventually, increasing levels of estradiol in both adrenal glands and ovaries may help to maintain the levels of estrogen at the beginning of the ovarian dysfunction such as perimenopause.

Recent studies have shown that the demands for natural compounds to prevent or treat a number of medical conditions, including menopausal symptoms, sexual dysfunction, cancer, cardiovascular disease, neuronal injury, inflammation, and depression are increasing [25-29]. It means that various functional materials from nature need to be studied and produced more. In order to manage the menopausal symptoms, hormone replacement therapy (HRT) with estrogen or estrogen–progesterone is considered a common treatment. HRT is effective to relieve the related

symptoms such as vasomotor problems [30-32]. However, many studies have mentioned a caution on a long-term use of hormone therapy because it can increase the risk of heart disease, ovarian cancer, and breast malignancy [33-35]. Therefore, the efforts to find functional materials from nature for menopausal symptoms has been accompanied. Likewise, the effects of *Schisandra chinensis* on relieving the menopausal symptoms [36] and ovarian failure [37] have been reported. However, the active compounds and mechanisms are to be determined.

The aim of this study was to determine the relationship between steroidogenesis and *Schisandra chinensis.* In addition, we focused on identifying the active compound of *Schisandra chinens* which is effective to use to promote biosynthesis of 17β-estradiol and the mechanisms of how the active compound works scientifically.

Ⅱ. MATERIALS AND METHODS

1. Chemicals

Gomisin N, gomisin A and schisandrin (schisandrol A) were purchased from ALB Technology (New York, USA). Schisandrin A, schisandrin B, schisandrin C were purchased from Corescience (Hubei, China).

2. Animals

Animal study was performed in accordance with recommendations in *Guide for the Care and Use of Laboratory Animals of the National Institutes of Health*. Animal handling was done in accordance with the protocols approved by the University of Illinois at Urbana Champaign's Institutional Animal Care and Use Committee (IACUC). At 25-27 postnatal days of wild type C57BL/6 female mice were used.

3. Cell culture

The NCI-H295R human adrenocortical cell line (CRL-2128) was purchased from the American Type Culture Collection (ATCC, Virginia, USA). Cells were cultured in DMEM/F-12 medium (1:1 mixture of

Dulbecco's-modified Eagle's and Ham's F-12 media containing L-glutamine and 15mM HEPES (Welgene, Gyeongsan, South Korea), supplemented with 2.5% Nu-Serum, 1% insulin/transferrin/selenium premix (Corning, New York, USA) and 0.1% Penicillin-Streptomycin (Corning, New York, USA). The serum-free media contained only 0.1% Penicillin-Streptomycin in DMEM/F-12. Cells were maintained in 75 cm^2 flasks at 37 ˚C in an atmosphere of 5% CO_2. For RNA, protein extraction and media collection, 3.0×10^6 cells were plated in a 6 cm^2 cell culture dish. After subculturing for 48 h, cells were treated with dimethyl sulfoxide (DMSO) (vehicle), gomisin N at the doses indicated, or 20 μM of gomisin N, gomisin A, schisandrin (schisandrol A), schisandrin A, schisandrin B, and schisandrin C in serum-free media. RNA extraction was performed after 12 h of sample treatment. Protein extraction was done after 24 h of sample treatment. Media were collected after 48 h of sample treatment and immediately frozen in liquid nitrogen.

4. Antral follicle culture

Female mice were euthanized on postnatal days (PND) 25-27 and their ovaries were collected. Antral follicles about 250-400μm were isolated mechanically from the ovaries using fine watchmaker forceps. Isolated antral follicles placed individually in wells of a 96-well culture plate [38], and covered with supplemented α-minimum essential media (α-MEM). Supplemented α-MEM was prepared with 1% ITS (10 ng/ml insulin, 5.5 ng/ml transferrin, 5.5 ng/ml selenium), 100 U/ml penicillin, 100 mg/ml streptomycin, 5 IU/ml human recombinant follicle stimulating hormone (FSH; Sigma, Missouri, USA), 5% fetal bovine serum (ATCC, Virginia, USA). 2 - 4 mice were used per experiment, approximately 20 - 40 antral follicles were isolated from each mouse. Each experiment contained a minimum of 6-8 follicles per group. Antral follicles were treated with dimethyl sulfoxide (DMSO) (vehicle), gomisin N at the doses indicated in supplemented media and cultured for 96 hours in an incubator with 5% CO_2 at 37 ℃. After culture, media were collected and frozen immediately in liquid nitrogen.

5. Hormone measurement

The concentrations of testosterone and 17β-estradiol in the media were measured by enzyme-linked immunosorbent assays ELISA (DRG International, Germany). The samples were run in triplicates and had intra- and inter-assay coefficients of variability were below 10%.

6. Real-time quantitative PCR

Cells were harvested with RNAiso Plus (Takara Bio Inc., Shiga, Japan). RNA was quantified using NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). After reverse transcription with oligo-dT primers using a PrimeScriptTM 1st strand cDNA synthesis Kit (Takara Bio Inc.), Real-time quantitative RT-PCR was performed using IQ SYBR (Bio-Rad Laboratories). 2 μl of cDNA in triplicate with β-actin as internal control. Before PCR amplification, the primers were denatured at 95 ℃ for 3 min. Amplification was made up of 44 cycles at 95 ℃ for 10 seconds, 60 ℃ for 30 seconds, and 72 ℃ for 30 seconds. PCR was performed by CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories).

cDNA was probed by the following primer: CYP11A1 forward (5'-GAG ATG GCA CGC AAC CTG AAG -3'); CYP11A1 reverse (5'-CTT AGT GTC TCC TTG ATG CTG GC -3'); CYP17A1 forward (5'-GGC ACC AAG ACT ACA GTG ATT G -3'); CYP17A1 reverse (5'-AGA GTC AGC GAA GGC GAT AC -3'); CYP19 forward (5'- AGG TGC TAT TGG TCA TCT GCT C -3'); CYP19 reverse (5'- TGG TGG AAT CGG GTC TTT ATG G -3'); 3β-HSD2 forward (5'- TGC CAG TCT TCA TCT ACA CCA G -3'); 3β-HSD2 reverse (5'- TTC CAG AGG CTC TTC TTC GTG -3'); 17β-HSD forward (5'- TTC ATG GAG AAG GTG TTG G -3'); 17β-HSD reverse (5'- AAG ACT TGC TTG CTG TGG -3'); β-actin forward (5'- TCC TCA CCC TGA AGT ACC CCA T –3'); β-actin reverse (5'- AGC CAC ACG CAG CTC ATT GTA -3')

7. Western blotting

After removing media, cells were lysed with lysis buffer containing 10 mM Tris (pH 7.5), 150 mM NaCl, 5 mM ethylene diamine tetra acetic acid (EDTA), 1 % Triton X-100, 1 mM dithiothreitol (DTT), 0.1 mM

phenylmethylsulfonyl fluoride (PMSF), 10 % glycerol and protease inhibitor cocktail tablet. The protein concentration was measured using a protein assay reagent kits as described by the manufacturer. 60 μg of protein lysates were separated electrophoretically using a 10 % SDS-polyacrylamide gel and transferred onto an Immobilon P membrane (MERK Millipore). The membrane was blocked in 5 % fat-free milk for 1 h, and then incubated with the specific primary antibody at 4 ℃ overnight. Protein bands were visualized using a chemiluminescence detection kit (GE healthcare, London, UK) after hybridization with the HRP-conjugated secondary antibody (Life technologies, Waltham, MA).

8. Cell viability assay

The H295R cells were cultured in the 96 well plates at a density of 4.0×10^4 cells/well and incubated in DMEM/F-12 supplemented with 2.5% Nu-Serum, 1% insulin/transferrin/selenium premix and 0.1% Penicillin-Streptomycin at 37℃ in a 5% CO_2 atmosphere. Cells were starved in serum-free DMEM/F-12 for 24 h. After sample treatment, the cells were incubated for 48 h at 37℃ in a 5% CO_2. After addition of 3 mL of MTT

solution in 30 mL of serum free DMEM/F-12, cells were incubated for 2 h. The medium was removed and formazan crystals were dissolved by the addition of dimethyl sulfoxide (DMSO). The absorbance at 570 nm was then measured using a microplate reader.

9. Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics ver. 23.0 (IBM, Armonk, NY, USA). The data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD and expressed as mean ± standard error of the mean (SEM).

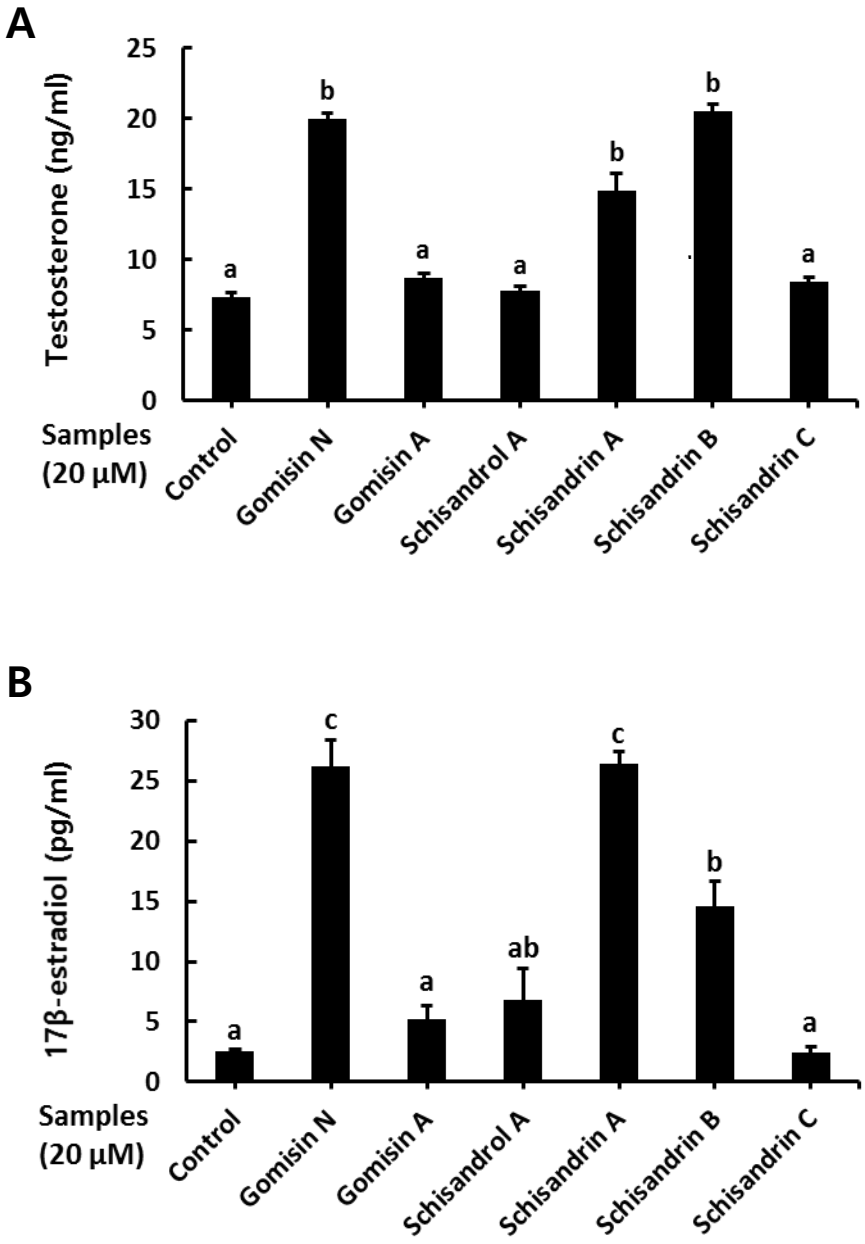
Ⅲ. RESULTS

1. Gomisin N promoted production of testosterone and 17β-estradiol in H295R cells among the major lignans in *Schisandra chinensis*

To identify which compound in *Schisandra chinensis* has an effect on promoting production of testosterone and 17β-estradiol in H295R cells, total six compounds (gomisin N, gomisin A, schisandrol A, schisandrin A, schisandrin B, and schisandrin C) were treated at 20 μM. Among the six compounds, gomisin N, schisandrin A, and schisandrin B promoted production of both testosterone and 17β-estradiol (fig. 1A and 1B). Especially, gomisin N and schisandrin A were effective in promoting 17β-estradiol synthesis. However, not all of compounds are abundant in *Schisandra chinensis.* Schisandrol A, gomisin N, and gomisin A are known as representative lignans. Schisandrin A, B and C has less portion in *Schisandra chinensis* than schisandrol A, gomisin N, and gomisin A. Therefore, among these three major lignans, gomisin N was the best compound which promote testosterone and 17β-estradiol. Considering

two conditions; function and abundance of compound, we chose gomisin N as an active compound to carry out this project further.

Figure 1


A
Testosterone (ng/ml)
25
20
15
10
5
0
a
b
a
a
b
b
a
Samples
(20 μM)
Control
Gomisin N
Gomisin A
Schisandrol A
Schisandrin A
Schisandrin B
Schisandrin C
B
17β-estradiol (pg/ml)
30
25
20
15
10
5
0
a
c
a
ab
c
b
a
Samples
(20 μM)
Control
Gomisin N
Gomisin A
Schisandrol A
Schisandrin A
Schisandrin B
Schisandrin C

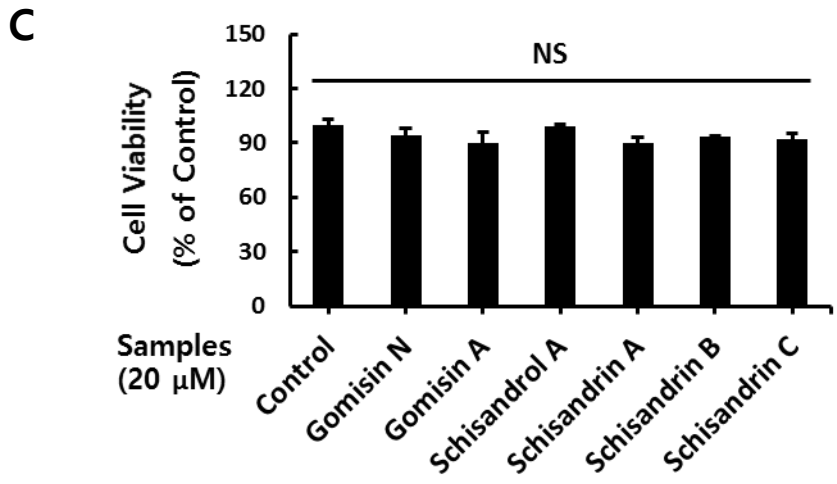


Figure 1. Effects of six indicator components in *Schisandra chinensis* on production of testosterone and 17β-estradiol in H295R cells.

All indicator compounds from S*chisandra chinensis* were used at 20 μM. After culture of H295R cells with six different components for 48 h, enzyme-linked immunosorbent assays to measure testosterone and 17β-estradiol were performed. **A.** Concentration of testosterone was expressed with the unit of ng/ml (n=5). **B.** Concentration of 17β-estradiol was expressed with the unit of pg/ml (n=5). **C.** Cell viability was evaluated using MTT assay. The graph represents the means ± SEM from five

separate experiments. Mean values with letters (a-c) within a graph are significantly different from each other at $p<0.05$. NC indicates non significance.

2. Gomisin N in the range of 5 μM to 20 μM increased testosterone and 17β-estradiol H295R cells

Due to gomisin N was selected as the active compound which increase both testosterone and 17β-estradiol, experiments to determine to what extent it will be effective were needed. Therefore, gomisin N was treated with three different concentrations; 5, 10, 20 μM. Gomisin N increased testosterone production significantly at 5 μM and high concentration. In addition, it showed greatest significance at 20 μM treated group (Fig. 2A). Gomisin N also promote biosynthesis of 17β-estradiol at 5 μM and high concentration. However, all doses showed same significance (Fig. 2B). None of concentration of gomisin N treatment has an effect on cell viability, even at 40 μM (Fig. 2C).

Figure 2

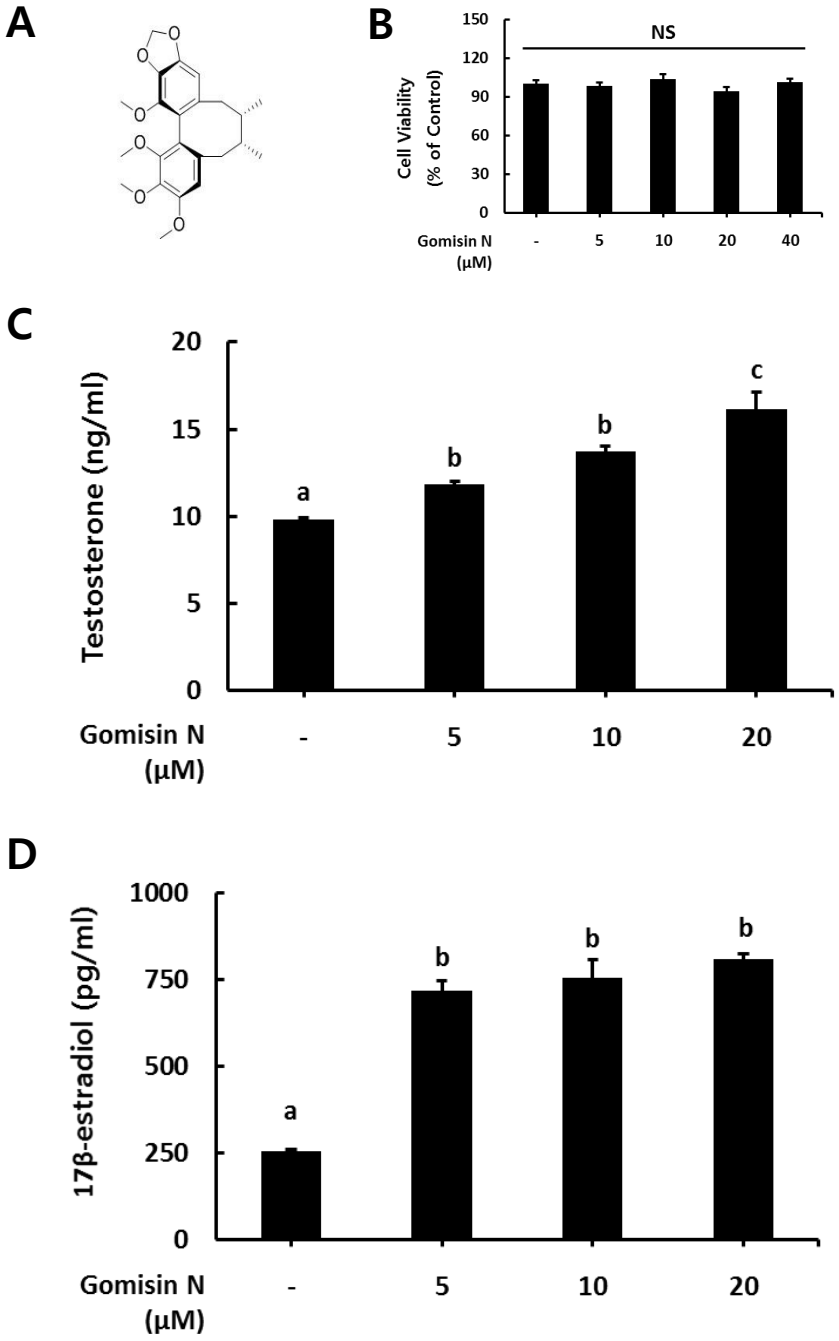

A
B
NS
Cell Viability (% of Control)
150
120
90
60
30
0
Gomisin N (μM)
-
5
10
20
40
C
Testosterone (ng/ml)
20
15
10
5
0
a
b
b
c
Gomisin N (μM)
-
5
10
20
D
17β-estradiol (pg/ml)
1000
750
500
250
0
a
b
b
b
Gomisin N (μM)
-
5
10
20

Figure 2. Effects of gomisin N on production of testosterone and 17β-estradiol in H295R cells.

5 - 20 μM of gomisin N were treated. After culture of H295R cells with gomisin N for 48 hours, enzyme-linked immunosorbent assays to measure testosterone and 17β-estradiol concentration were performed. **A.** The structure of gomisin N **B.** Concentration of testosterone was expressed with the unit of ng/ml (n=5). **C.** Concentration of 17β-estradiol was expressed with the unit of pg/ml (n=5). **D.** Cell viability was evaluated using MTT assay. All graphs represent the means ± SEM from five separate experiments. Mean values with letters (a-c) within a graph are significantly different from each other at $p<0.05$. NC indicates non significance.

3. Gomisin N increased protein and mRNA levels of CYP11A1 in H295R cells

To investigate how gomisin N increased production of testosterone and 17β-estradiol, an effect of gomisin N on the expression of enzymes which related to steroidogenesis was studied. CYP11A1 plays an important role at the beginning of biosynthesis of testosterone and 17β-estradiol to convert cholesterol to pregnenolone. Gomisin N increased protein expression of CYP11A1 significantly at 20 μM (Fig. 3A). It also increased mRNA levels of *CYP11A1* at 5 μM and high concentration. (Fig. 3B)

Figure 3

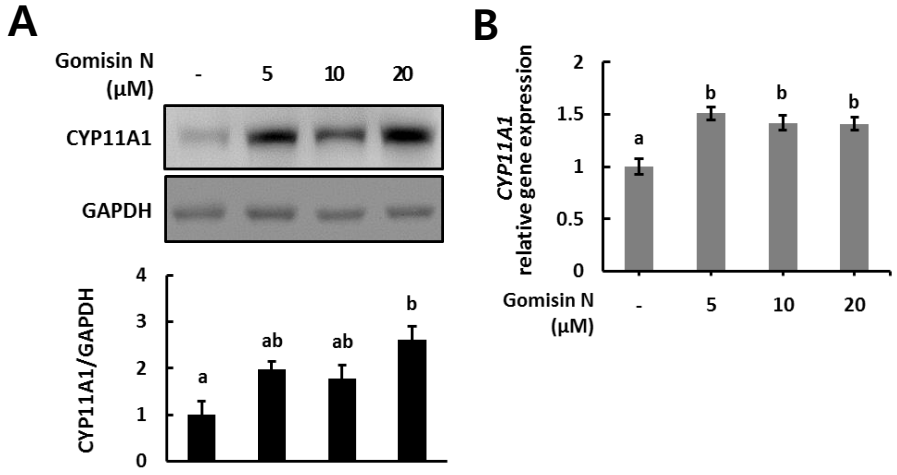


Figure 3. Effects of gomisin N on protein and mRNA levels of CYP11A1 in H295R cells

A. Protein levels of CYP11A1 were determined by western blot as described in the Materials and Methods. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. (n=5). **B.** mRNA levels of *CYP11A1* was analyzed by real-time quantitative PCR described in the Materials and Methods. Data (n=3) represented the mean values ± SEM. Letters (a, b) within a graph are significantly different from each other at $p<0.05$.

4. Gomisin N increased protein and mRNA levels of CYP17A1 in H295R cells

CYP17A1 plays a role at the three points to reach androstenedione from cholestrol. Gomisin N increased protein levels of CYP17A1 significantly at 10 - 20 μM (Fig. 4A). It increased mRNA levels of *CYP17A1* at least 5 μM as *CYP11A1* mRNA expression (Fig. 4B).

Figure 4

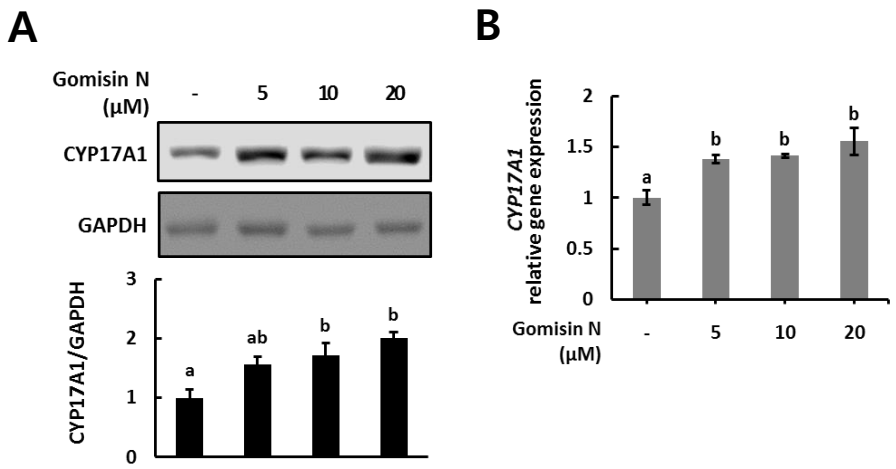


Figure 4. Effects of Gomisin N increased protein and mRNA levels of CYP17A1 in H295R cells

A. Protein levels of CYP17A1 were determined by western blotting as described in the Materials and Methods. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. (n=5). **B.** mRNA levels of *CYP17A1* was analyzed by real-time quantitative PCR described in the Materials and Methods. Data (n=3) represented the mean values ± SEM. Letters (a, b) within a graph are significantly different from each other at $p<0.05$.

5. Gomisin N increased only CYP11A1 and CYP17A1 in H295R cells

To biosynthesize 17β-estradiol and its substrate, testosterone, there are more enzymes are necessary such as 3β-HSD, 17β-HSD, and CYP19A1. Therefore, western blotting for rest of enzymes are performed. However, gomisin N did not have any effect on protein levels of those enzymes (Fig. 5A). It showed that gomisin N only induced the changes of proteins and mRNA levels of *CYP11A1* and *CYP17A1*. As steroidogenesis occurs as the pathway (Fig. 5B), gomisin N increased the substrates that could lead to increase production of testosterone and 17β-estradiol.

Figure 5

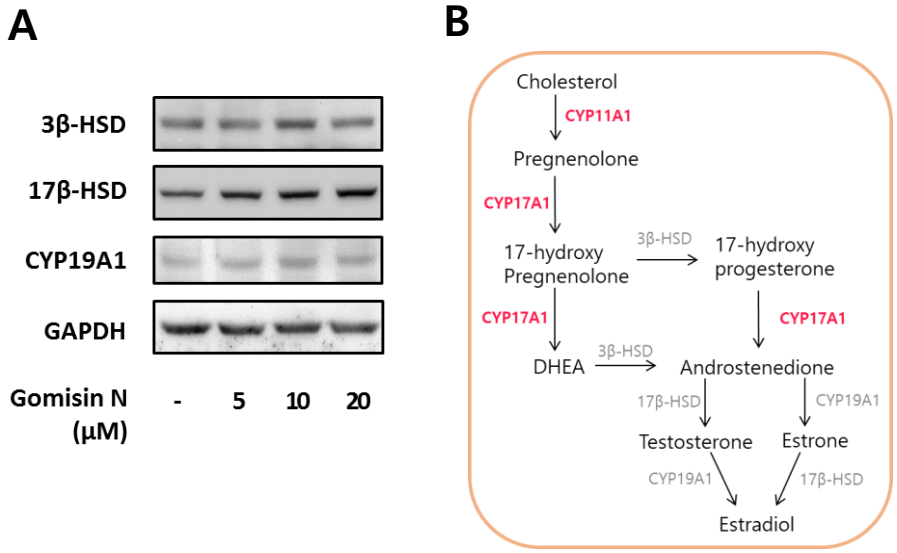


Figure 5. Gomisin N increased CYP11A1 and CYP17A1 in H295R cells.

A. Protein levels of 3β-HSD, 17β-HSD, CYP19A1 were determined by western blot as described in the Materials and Methods. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. Data (n=3) represented the mean values ± SEM. **B.** Steroidogenesis pathway related to biosynthesis of testosterone and 17β-estradiol

6. Gomisin N promoted biosynthesis of testosterone and 17β-estradiol in mouse antral follicles.

As gomisin N promoted biosynthesis of testosterone and 17β-estradiol in H295R cell line, gomisin N predicted as a compound that has an effect on ovaries also. To determine the effect of gomisin N on ovary, we used mouse antral follicles. As expected, the production of testosterone and 17β-estradiol was increased by gomisin N. Testosterone increased in dose dependent, and 17β-estradiol tended to increase and showed significance at 40 μM.

Figure 6

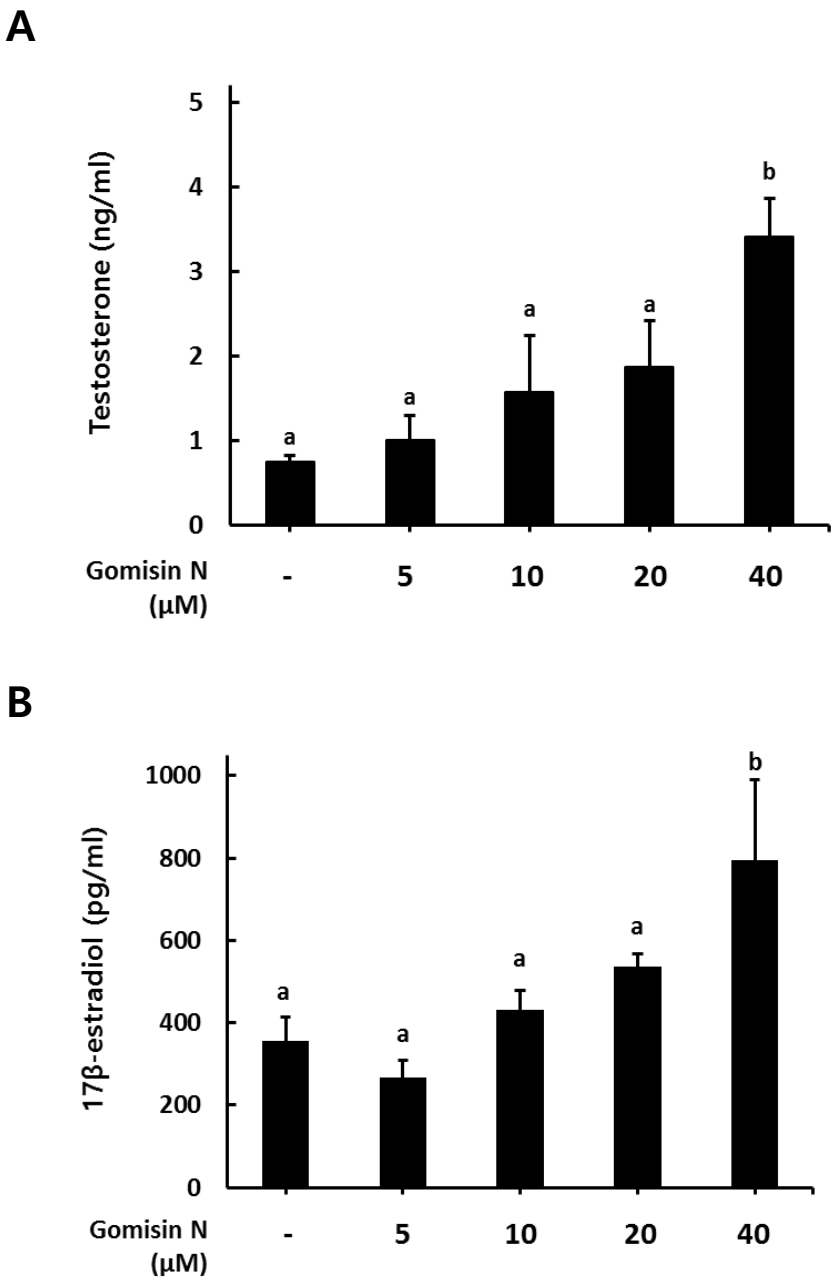

A
Testosterone (ng/ml)
0
1
2
3
4
5
a
a
a
a
b
Gomisin N (μM)
-
5
10
20
40
B
17β-estradiol (pg/ml)
0
200
400
600
800
1000
a
a
a
a
b
Gomisin N (μM)
-
5
10
20
40

Figure 6. Effects of gomisin N on biosynthesis of testosterone and 17β-estradiol in mouse antral follicles.

Gomisin N was treated 5 - 40 μM to mouse antral follicles. After culture of mouse antral follicles with gomisin N for 96 hours, enzyme-linked immunosorbent assays to measure testosterone and 17β-estradiol concentration were performed. **A.** Concentration of testosterone was expressed with the unit of ng/ml. (n=5). **B.** Concentration of 17β-estradiol was expressed with the unit of pg/ml (n=5). All graphs represent the means ± SEM from five separate experiments. Mean values with letters (a, b) within a graph are significantly different from each other at $p<0.05$.

Ⅳ. DISCUSSION

This current study has discovered that gomisin N was the best compound which promoted production of testosterone and 17β-estradiol in H295R cells among the three major lignans in *Schisandra chinensis*. To consider the effect on steroidogenesis and the content in *Schisandra chinensis*, gomisin N was chosen as an active compound to further carry out this project.

To identify the effects of gomisin N on adrenal steroidogenesis, NCI-H295R human adrenocortical cell line was used in that it is widely used as a model when it comes to studying steroidogenesis [39]. Gomisin N in the range of 5 μM to 20 μM increased the production of testosterone and 17β-estradiol in H295R cells. Gomisin N had effects on the expressions of steroidogenic enzymes. It increased both protein and gene expression levels of CYP11A1 and CYP17A1. Therefore, gomisin N may have promoted the production of testosterone and 17β-estradiol by increasing the amount of substrates such as pregnenolone and

androstenedione. As the effects of gomisin N on adrenal steroidogenesis, it was needed to determine whether gomisin N enhances ovarian steroidogenesis. Mouse antral follicles were used to determine the effects of gomisin N on ovarian steroidogenesis. As expected, it also showed an increase of the production of testosterone and 17β-estradiol at 40 μM in mouse antral follicles. As a result, gomisin N promoted the production of 17β–estradiol and its substrate, testosterone by increasing the expression of steroidogenic enzymes such as CYP11A1 and CYP17A1.

As the demand for natural products to prevent from various diseases is growing [25-29], many products for alleviating menopausal symptoms have been produced. *Schisandra chinensis* had been widely used as a prescription for sexual function improvement [2] in the past. Likewise, it is still used as functional materials for developing health functional food for women who are experiencing menopause. However, due to lack of scientific basis, we aimed to identify the effective components of *Schisandra chinensis* which promote the biosynthesis of 17β-estradiol and its mechanisms.

Consequently, our current study discovered the effects of active compound in *Schisandra chinensis* which promoted steroidogenesis. However, it is still unclear which factors are related to stimulating the expressions of CYP11A1 and CYP17A1. SF-1 is a key regulator for steroidogenesis in adrenal glands and ovaries and associated with the steroidogenic genes such as StAR, HSD3B, CYP11A1, and CYP17 [40]. Due to the increased gene expression levels of CYP11A1 and CYP17A1, gomisin N may have an effect on the expression or the activity of SF-1. To identify specific mechanisms additionally, target study is needed to be done. In addition, *in vivo* studies and clinical studies are necessary in order to apply the current knowledge to therapeutic application for easing menopausal symptoms in human.

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국문 초록

에스트로겐은 여성의 생식에 있어 중요한 호르몬이다. 난소에서는 난포의 발달과 배란에 역할을 하며 다른 기관에서도 생식 이외의 다양한 기능을 한다. 따라서 폐경기에 에스트로겐이 감소함에 따라 심혈관 질환, 우울증, 골다공증 등의 질병의 위험이 증가하며, 체내 에스트로겐 수치를 유지하는 것은 여성의 건강을 유지하는 데 중요하다.

최근 연구에 따르면 많은 사람이 다양한 질병에 따른 증상을 예방하거나 치료하기 위해 천연물을 섭취하고 있다. 특히, 동아시아에서는 오미자를 예로부터 생식기능 향상을 위한 처방을 비롯하여 다양한 목적으로 섭취해왔다.

또한, 오미자의 갱년기 증상의 완화와 난소 기능에 미치는 영향에 관한 연구와 같이 오미자가 여성건강에 미치는 영향에 관한 선행연구가 존재하지만, 오미자의 유효 성분과 그 메커니즘은 아직 알려지지 않았다.

따라서 본 연구의 목적은 스테로이드 생성 경로 연구에 널

리 사용되는 모델인 사람 부신 세포에서 유래한 NCI-H295R 세포주와 쥐 난소에서 유래한 난포를 이용하여 오미자의 유효성분을 찾고 그 유효성분이 스테로이드 생성 경로 및 17 베타 - 에스트라디올의 생합성에 미치는 영향과 그 메커니즘을 과학적으로 명확히 하는 것이다.

본 연구를 통해, 오미자의 주요 리그난 중 고미신 N이 스테로이드 생합성 촉진 효능이 뛰어난 것으로 밝혀졌다. 또한, 오미자 지표성분들의 구성 비율과 효능을 모두 고려하여 고미신 N을 유효성분으로 선정하였다. 고미신 N은 스테로이드 생성 효소의 단백질 및 유전자 발현을 증가시키면서 스테로이드 생합성 경로를 촉진하는 것으로 나타났다. 특히, CYP11A1과 CYP17A1의 증가로 스테로이드 생성에 관여하는 pregnenolone, androstenedione과 같은 기질의 양을 증가시켜 테스토스테론과 17 베타 - 에스트라디올의 생산을 촉진하는 것으로 생각된다.

결론적으로, 오미자의 주요 리그난인 고미신 N이 H295R 세포주와 쥐 난포에서 모두 테스토스테론 및 17베타 - 에스

트라디올의 농도를 증가시킨다는 것을 밝혀냄으로써 고미신 N이 여성의 갱년기와 같이 17베타 – 에스트라디올의 농도가 감소하는 시기에 스테로이드 생합성을 촉진하여 17베타 – 에스트라디올의 농도를 증가하는 데 도움을 줄 수 있는 기능성 식품 소재로 사용될 가치가 있을 것으로 사료된다.