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MASTER OF SCIENCE IN FOOD AND NUTRITION

Effects of Crude Rapeseed Glucosinolates and Genistein on Thyroid in Male Wistar Rat

글루코시놀레이트를 포함한 유채씨 추출물과 제니스테인이 흰 쥐의 갑상선에 미치는 영향 연구

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Abstract

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Glucosinolates (GLSs) and isoflavone, both indicated as goitrogenic, are important dietary factors in Asian diets. In this study, crude rapeseed GLSs and genistein were administered orally to male rats for 30 days, individually and simultaneously. To determine the effect on the thyroid, both the thyroid hormone (T_3, T_4 and TSH) levels in serum and the expression levels of genes related to the thyroid function (thyroglobulin (Tg), thyroid peroxidase (TPO) and thyrotropin receptor (TSHr)) were analyzed in thyroid tissue on days 3, 10, 20 and 30. Goitrin, the main breakdown product of the GLSs in rapeseed extract, was measured in the serum of treated groups for day 30. No treatment-related changes were observed in

body and organ weights. Goitrin was not detected in the serum. For thyroid hormone analysis, no statistically significant changes were observed except for a decrease in T_4 in the genistein group from day 3. Some slight fluctuations of thyroid hormones were found. Significant changes in mRNA expression levels were only observed on day 10, showing decreased Tg, TPO and TSHr expression levels compared to the control group. The present study has shown that both GLSs and genistein have a weak effect on the thyroid when treated with a dose that is similar to the intake of high consumers in humans. The fluctuations caused in thyroid hormone levels and gene expression levels are assumed to be a function of the pituitary–thyroid axis. Thus, it is difficult to observe the antithyroid effect where the individual intake levels of GLSs and isoflavone are relatively safe.

Key words: rapeseed extract, glucosinolates, genistein, thyroid

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List of Abbreviation

GLSs: glucosinolates

Tg: thyroglobulin

TPO: thyroid peroxidase

TSH: thyroid stimulating hormone

TSHr: thyrotropin receptor

T_3: triiodothyronine

T_4: thyroxine

Ⅰ. Introduction

Glucosinolates, β-thioglucoside N-hydroxysulfate derivatives, are known to be secondary plant metabolites that are present in important edible Brassica vegetables (Fahey *et al.*, 2001). After hydrolyzed and converted to their derivative products, which is caused by myrosinase during food processing and also by high temperatures or the activity of the intestinal microflora (Mawson *et al.*, 1993), GLSs become active molecules that have diversified biological effects.

It has been demonstrated that GLSs can be protective against cancers (Talalay and Fahey, 2001). However, on the other hand, GLSs have also been reported to have some antinutritional effects, such as growth depression (Tripathi and Mishra, 2007) and goitrogenic activity (Gaitan, 1990).

Rapeseeds contain three major GLSs, progoitrin and gluconapin, as well as sinigrin in lower concentrations. Among the GLSs in rapeseeds, progoitrin has been reported to exist at the highest concentrations and to contribute most to antithyroid activity through its breakdown product, goitrin (Greer, 1957). It has been suggested

that progoitrin intake can cause a decrease of thyroid hormone levels in plasma; as well as increased thyroid weights according to some animal studies (Vermorel, 1988, Burel *et al.*, 2001, Vermorel *et al.*, 1986).

Isoflavones are the major phytoestrogens present in soy foods, which have been consumed for centuries in Asian countries. Although it has been demonstrated that isoflavones have various potential health benefits, excessive intake would cause some adverse effects, especially thyroid toxicity (Xiao, 2008). The isoflavone-mediated inactivation of TPO has been recognized as a general phenomenon across mammalian species based on the studies of microsomal rat TPO (Chang and Doerge, 2000), porcine TPO (Divi *et al.*, 1997) and human TPO. It also has been suggested that soy protein diet can increase T_4, free T_4 and TSH levels in animals, whereas T_3 is generally not affected (Scholz-Ahrens *et al.*, 1990, Forsythe, 1995). According to Jurjevic *et al.* (2010), both genistein and daidzein, the major isoflavones present in soy foods, can increase serum TSH levels and reduce the level of thyroid hormones in orchidectomized middle-aged male rats.

GLSs and isoflavone, both indicated to be goitrogenic, are

important dietary factors in Asian diets. Despite their health benefits, many studies have been conducted to study their antithyroid effect respectively. However, the concentrations of GLSs used in earlier studies are much higher than those consumed by humans typically, making it difficult to estimate the effects in humans with a normal diet. Although both GLSs and isoflavone are dietary factors with goitrogenic activity, no studies have determined if there is an interrelationship between them. In the current study, we treated Wistar rats for 30 days with crude rapeseed GLSs and genistein, individually and simultaneously. Both thyroid hormones (T_3, T_4 and TSH) and the expression levels of genes related to the thyroid function (Tg, TPO and TSHr) were analyzed to determine their effects on the thyroid. The results of the groups treated with GLSs and genistein individually were also compared to those of the combined treatment groups.

Ⅱ. Literature Review

1. Glucosinolates in rapeseed and their antithyroid effect

Glucosinolates (GLSs), a large group of sulphur-containing secondary plant metabolites, occur in all the economically important varieties of *Brassica* (Tripathi and Mishra, 2007). The content and composition of GLSs vary due to many factors, such as plant species, agronomic practices, climatic conditions, and so forth. The primary GLSs in rapeseeds are progoitrin and gluconapin, as well as sinigrin in lower concentrations.

GLSs can be hydrolyzed and converted to their derivative products, including thiocyanate anions, organic isothiocyanates, organic nitriles, etc. This breakdown is mainly caused by myrosinase during food processing and also by high temperatures or the activity of the intestinal microflora (Mawson *et al.*, 1993). GLSs themselves are biologically inactive molecules, but their breakdown products are biologically active and are known for their diverse range of biological effects.

Cruciferous vegetables, which are the dietary sources of GLS, have been suggested to be protective against cancers by

epidemiological studies (Talalay and Fahey, 2001). Some antinutritional effects of GLSs, mainly on food intake, growth and thyroid function, were also observed in many animal studies (Table 1).

Table 1. Antinutritional effects of GLSs on animals (Adapted from Tripathi and Mishra, 2007)

Animal	TGls[1] (μmol g^{-1} diet)	Effect on animals	Reference
Monogastrics			
Rat	3.3-4.4	Reduced intake and growth	Vermorel *et al*. (1988)
	6.6	Poor gain, increase thyroid weight and changed thyroid morphology	Wallig *et al*. (2002)
	7.7	Depressed intake and growth	Vermorel *et al*. (1987)
Pigs	1.3-2.79	Reduced feed intake and growth	Bell *et al*. (1991)
	7.0	Severe growth depression	Mawson *et al*. (1994)
	9-10	Induced liver and thyroid hypertrophy	Bourdon and Aumaitre (1990)
	10	Induced iodine deficiency, Hypothyroidism, reduced bone and serum zinc content and alkaline phosphatase activity	Aumaitre *et al*. (1989)
Poultry	4.6	Reduced feed intake by 0.09 and gain by 0.12 levels.	Seškeviciene *et al*. (2004)
	7.6-15.3	Severe growth depression	Thomas *et al*. (1983)
	34.0	Severe growth depression	Pearson *et al*. (1983)
Rabbits	17.9-25.3	Severe growth depression and	CSWRI (2002)

Table 1. ***(Continued)***

		increased mortality	
Ruminants			
Cow	11.0	Induced iodine deficiency in cow	Laarveld *et al*. (1981c)
	11.7-24.3	Depressed feed intake and milk production in dairy cow	Waldern (1973)
	≥23.0	Reduced intake and milk production in cow	Ingalls and Sharma (1975)
	31.0	Thyroid disturbance and depressed fertility in cow	Ahlin *et al*. (1994)
Sheep	1.2-1.6	Reduced plasma levels of estradiol provoked reproductive disturbance	Mandiki *et al*. (2002)
	1.2-2.2	Weight loss during lactation in ewes	Mandiki *et al*. (2002)
	≥4.22	Induced iodine deficiency and influenced thyroid weight and histology in lambs	Derycke *et al*. (1999)
	15.0	Reduced growth in lambs	Thomas *et al*. (1984)
	17.5	No effect on intake but increased thyroid weight in lamb	Hill *et al*. (1990)
	33.0	Growth depression in lamb	Tripathi *et al*. (2004)
Fish	2.18	Reduced growth by 0.15 level	Glencross *et al*. (2004b)

Table 1. ***(Continued)***

	19.3	Severe growth depression and thyroid disturbances	Burel *et al*. (2000c)

[1]TGIs: total glucosinolates

Since the main GLSs in rapeseeds are progoitrin, gluconapin and sinigrin, the enzymatic hydrolysis of rapeseed GLSs primarily produces goitrin (5-vinyloxazolidine-2-thione) and thiocyanate ions. According to Greer and Deeney (1959), progoitrin would produce consistent antithyroid activity either with or without the simultaneous administration of myrosinase, which indicates that the hydrolysis of progoitrin occurs within the body in the absence of exogenous myrosinase.

In the studies conducted by Vermorel *et al.* (1986), rats were fed diets containing six individual GLSs over a period of 29 days. Progoitrin, when fed at 3 g kg^{-1} diet, increased liver and thyroid weights, but no depression on growth and plasma thyroid hormone levels were caused. The results of a human study (McMillan *et al.*, 1986) also showed that a daily intake of 150 g cooked Brussels sprouts (containing 69 mg of progoitrin without myrosinase) for 28 days had no effect on plasma T_3 or T_4 levels.

However, according to Vermorel (1988), plasma T_4 was reduced when rapeseed meal was introduced into the diet of the growing rats. A study (Burel *et al.*, 2001) in rainbow trout has also demonstrated that rapeseed meals could lead to a rapid decrease of

plasma T_3 and T_4 levels, and the main processes of action of the GLSs in rainbow trout were also indicated (Figure 1).

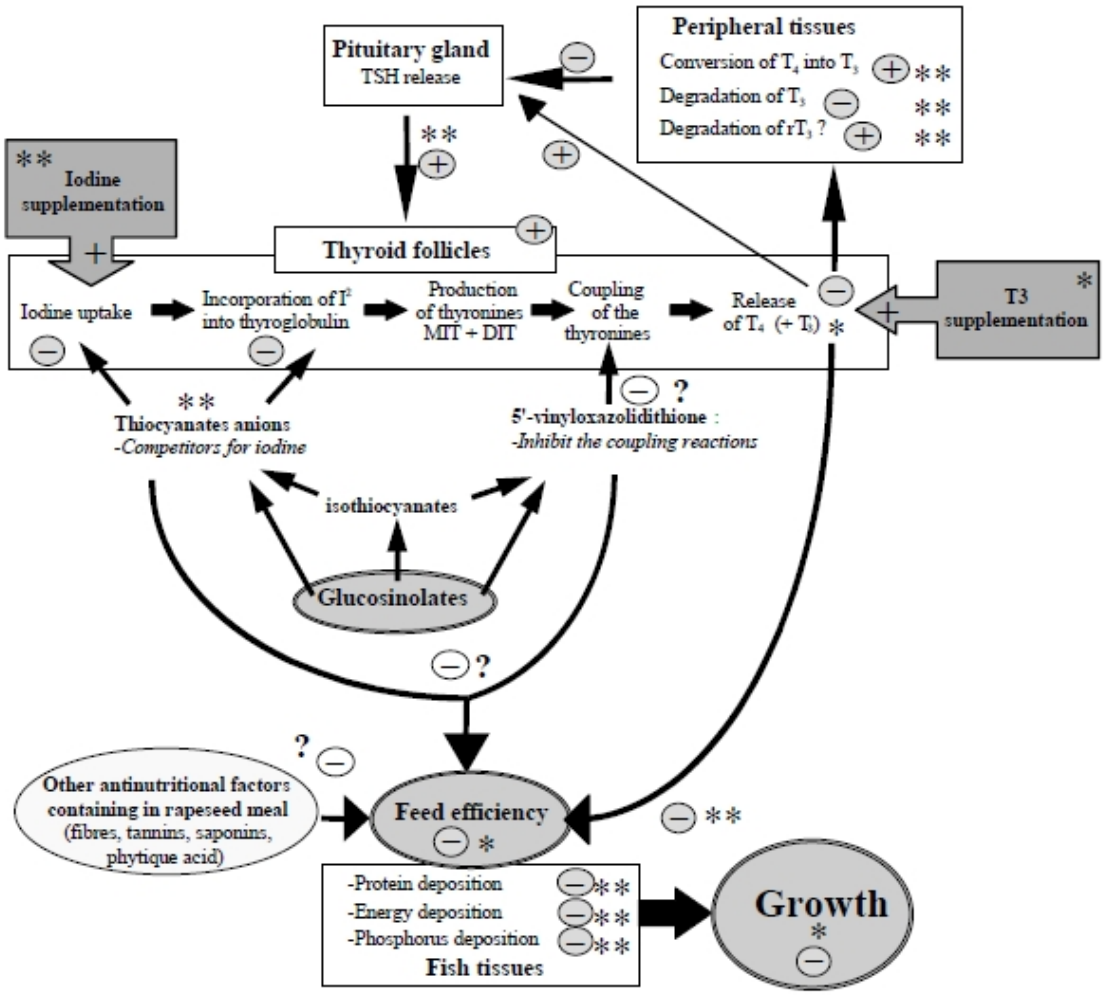

Figure 1. Main processes of action of the GLSs in rainbow trout. Two asterisks, original observations; one asterisks, observations made during this study that are not original; question mark, processes not yet clearly demonstrated in fish. (Burel *et al*., 2001)

2. Dietary exposure and goitrogenic activity of isoflavones

Isoflavones, the major phytoestrogens present in soy foods, have been widely studied for their potential health benefits, including cholesterol-lowering effect, decreasing risk for heart disease, protection against breast or prostate cancer, relief of menopausal symptoms, and so forth (Xiao, 2008). The main isoflavones in soybeans are genistin and daidzin, as well as small amounts of glycitein. Genistin and daidzin, both conjugated to sugars as glycosides, cannot be absorbed unless hydrolyzed and converted to their respective aglycone forms, genistein and daidzein. Among the isoflavones, the most biologically active form is genistein (Szkudelska and Nogowski, 2007).

Given that soy foods have been consumed for centuries in Asian countries and most traditional Asian soy foods contain high levels of aglycone isoflavones, the isoflavone intake levels of Asians are expected to be higher than those of non-Asians. According to Skibola and Smith (2000), the daily isoflavone intakes would range from 20 to 240 mg for Asians, and 1 to 9 mg in other populations. The dietary isoflavone intake of Korean population has been estimated as 14.88 mg/day, which was attributed to genistein (7.32 mg), daidzein (5.81 mg), and glycitein (1.75 mg) (Kim and Kwon,

2001).

Despite the various benefits, some adverse effects of isoflavones have also been observed (Xiao, 2008). One of them is the antithyroid effect, which means excessive isoflavone intake can be responsible for the development of goiter. Chang and Doerge (2000) have demonstrated that the long-term soy isoflavone consumption could reduce the TPO activity of male and female rats in a dose-dependent manner. The mechanisms for inhibition of TPO by genistein, shown in Figure 2, have been proposed by Doerge and Sheehan (2002). TPO is a heme-containing enzyme found in the apical membrane of thyroid follicular cells. It can catalyze the reactions required for thyroid hormone synthesis, including iodination of thyroglobulin tyrosyl residues and following oxidative coupling to yield T_4 and T_3. As shown in Figure 2, genistein could react with compound Ⅰ and produce a reactive isoflavone radical at the active site, which could combine with compound Ⅱ to form inactivated enzyme presumably through covalent modification of active site amino acid residues. Based on the studies of microsomal rat TPO, porcine TPO (Divi *et al.*, 1997) and human TPO, it has been suggested that the isoflavone-mediated inactivation of TPO is a general phenomenon across mammalian species.

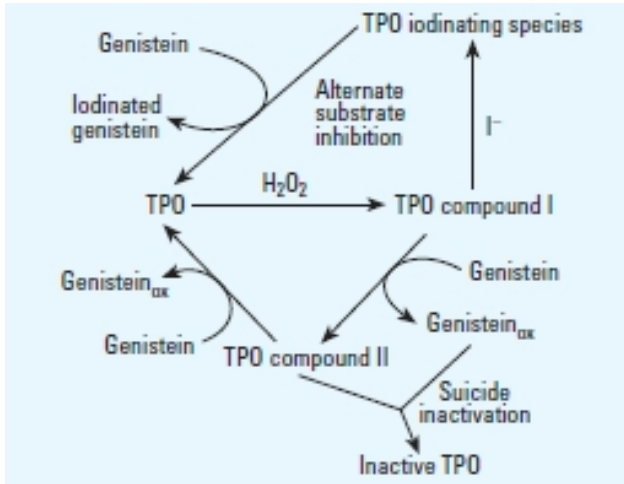

Figure 2. Mechanisms for inhibition of TPO by genistein (Doerge and Sheehan, 2002)

It has also been reported that a soy protein diet can increase T_4, free T_4 and TSH levels in animals, whereas T_3 is generally not affected (Scholz-Ahrens *et al.*, 1990, Forsythe, 1995). However, according to Dillingham *et al.* (2007), soy isoflavones in a protein matrix do not significantly influence circulating thyroid hormones in healthy young men.

3. Genes and hormones related to thyroid function

Tg, TPO and TSHr are thyroid-specific proteins and closely related to thyroid function. Tg is a dimeric glycoprotein which is synthesized in the thyroid follicular cell and stored in the follicular lumen. Through the iodination of tyrosyl residues in Tg, monoiodotyrosine (MIT) and diiodotyrosine (DIT) can be formed, followed by production of T_3 and T_4. Tg mRNA is very abundant in thyroid cells, and the steady-state mRNA levels are regulated by different hormones (Damante and Di, 1994). In dogs treated with T_4, the Tg mRNA expression levels depressed in the quiescent thyroids (Maenhaut *et al.*, 1992). TPO, as described before, can catalyze the reactions required for thyroid hormone synthesis, including iodination of Tg tyrosyl residues and following oxidative coupling to yield T_4 and T_3. TPO mRNA has been reported to be rapidly down-regulated by TSH (Gérard *et al.*, 1987). TSHr, as the largest glycoprotein hormone receptor, has been suggested in a variety of cell types. According to Huber *et al.* (1992), TSHr levels are positively regulated by TSH in normal cells. However, the hormonal regulation of TSHr mRNA levels seems to be very complex. The levels of TSHr mRNA in FRTL-5 cell have been reported to be down-regulated by TSH through cAMP-dependent mechanisms,

but TSH is also suggested to be able to up-regulate TSHr mRNA when basal levels of cAMP are lowered (Saji *et al.*, 1992).

The most important iodothyronine secreted by the thyroid gland is T_4, which has little, if any, biological activity (Hennemann and Visser, 1997). T_4, as a prohormone and a reservoir for the most active thyroid hormone T_3, can be converted to T_3 as required in the tissues by iodothyronine deiodinase. In human, about 80% of total plasma T_3 production is extrathyroidally converted to T_3, while 20% is secreted by the thyroid gland. TSH is known as a pituitary hormone that can stimulate the thyroid gland to produce T_4. Its production and release are controlled by thyroid hormone levels in the blood, via negative feedback of the pituitary-thyroid axis (Hill *et al.*, 1989).

According to Jurjevic *et al.* (2010), both genistein and daidzein can increase serum TSH levels and simultaneously reduce the level of thyroid hormones in orchidectomized middle-aged male rats. On the other hand, in the study conducted by Chang and Doerge (2000), no differences of T_3, T_4 and TSH levels in serum were observed in the normal rats treated with soy isoflavone, which suggested that even though substantial amounts of TPO activity are lost

concomitant to soy isoflavone consumption, the remaining enzymatic activity is sufficient to maintain thyroid homeostasis in the absence of additional perturbations.

Ⅲ. Materials and methods

1. Preparation of rapeseed extract and genistein suspension

Rapeseed extracts were separated from *Brassica napus* L. seeds (Asia Seed Co., Ltd., Seoul, Korea). The seeds were initially heated in a drying oven at 110℃ for 2 hr to deactivate myrosinase. They were then ground to powder using a mortar and pestle, followed by defatting with hexane five times. The residual solvent was removed by a rotary evaporator (Heidolph VV 2000; Heidolph, Kelheim, Germany) and the seeds were then extracted with 70% methanol (1:10 w/w) in a 75℃ shaking water bath (BS-21 Shaking & Heating bath; Jeio tech Co., Ltd., Daejeon, Korea) for 10 min. The extracts were filtered at a reduced pressure with filter paper (Cat No. 1541150, GE Healthcare Life Sciences, UK), and were concentrated to approximately 30% of the original volume by a rotary evaporator. The remaining water was further removed using a speed vacuum concentrator (ScanVac; LaboGene, Lynge, Denmark) at 2000 rpm, 15℃ overnight. The concentrates were then dried to a powder form by a freeze dryer (NB-504LSCIR; N-BIOTEK Inc., Bucheon, Korea) and stored at −80℃ until used.

The concentrations of GLSs in the rapeseed extracts were determined using the HPLC method. An amount of twenty milligrams of the powder was dissolved in 5 ml of distilled water and 200 μl of glucotropaeolin (4 mg/ml) was added as the internal standard. Ion-exchange chromatography was utilized to separate the GLSs according to the ISO norm (1992). DEAE-Sephadex A-25 resin was mixed with 2 M acetic acid until the liquid volume reached twice the sediment volume. The DEAE-Sephadex A-25 suspension was prepared two days before use and stored at 4℃. A DEAE-Sephadex column was prepared using a 5.75 inch glass Pasteur pipet (Corning Incorporated, New York, USA), and was then conditioned with 2 ml of 6 M imidazole formate and washed with 2 ml of deionized water. Two milliliters of the rapeseed extract were loaded, washed with 2 ml of 0.02 M sodium acetate buffer (pH 4.0), and desulfonated with 150 μl of sulfatase (80 units) overnight. Lastly, the GLSs were eluted with 2 ml of deionized water and filtered through a PTFE syringe filter (Dismic®-13HP, hydrophilic, 0.20 μm). A 20-μl aliquot of the elutes was injected into a HPLC (Dionex ultimate 3000 equipped with a UV detector; Dionex Co., Sunnyvale, CA, USA) for analysis. The UV detector was operated at a wavelength of 229 nm. A reverse phase Luna C18 (250x4.6 mm, 5 μm, 100 Å) column (Phenomenex, Torrance,

CA, USA) with a flow of 1.0 ml/min was used. Distilled water and acetonitrile were used as the mobile phases, which were filtered through PTFE membrane filters (hydrophilic, 0.5 μm, Millipore Co., Bedford, MA, USA) and were degassed before use. The gradient of the mobile phases was as follows: 100% water for 3min, 0 to 20% acetonitrile in water for 20min, and holding for 3 min, 20% to 100% acetonitrile in water for 10min, 100% to 0 acetonitrile in water for 5 min, and holding for 10 min. Progoitrin, the main GLS in the rapeseed extract, was eluted at approximately 11~12 min. Sinigrin, gluconapin and glucotropaeolin were eluted at approximately 12~13 min, 17min, 22~23 min, respectively. The concentrations of the GLSs were calculated according to the internal standard and the results are shown in Table 2. Before being administrated, the powder was dissolved in distilled water to create solutions with the target concentrations of the total GLSs.

Table 2. Concentrations of GLSs in the rapeseed extract powder

GLSs	Progoitrin	Sinigrin	Gluconapin	Total
Concentration (μmol/g)	184.84 ± 5.99	30.06 ± 0.96	173.44 ± 5.29	388.34 ± 12.24

Genistein (99+%, LC Laboratories, USA) was suspended in distilled water, water-bathed at 37℃ for 30 min, and was then mixed with a vortex (Vortex Genie 2; Scientific Industries, Inc., New York, USA) vigorously until the genistein particles were invisible before gavage.

Both the rapeseed extract solutions and the genistein suspensions were made every 10 days during the 30-day experiment period. The target concentrations (shown in Table 3) were calculated with the average body weights of the rats during the previous 10-day period, in order to ensure that the administered concentrations during the 30 days were equal to 25.8 μmol/kg/day for total GLSs and 8 mg/kg/day for genistein. After the rapeseed extracts being prepared as solutions, the concentrations of GLSs were ensured using the HPLC method described before.

Table 3. Target concentrations of total GLSs and genistein in the respective 10-day periods

Periods	Estimated average body weights (g)	Total GLSs (μmol/ml)	Genistein (mg/ml)
Day 0-9	230	5.93	1.84
Day 10-19	310	8.00	2.48
Day 20-29	350	9.03	2.80

2. Animals experiment and drug administration

Four-week-old male Wistar rats were purchased from Orientbio Inc. (Seoul, Korea) and housed at the Animal Center for Pharmaceutical Research at Seoul National University (Seoul, Korea). Following their arrival, all animals underwent a physical examination for clinical signs of ill health and then were given 2 weeks for acclimatization. Three animals were housed in a cage and the animal room was environmentally controlled (22±2℃, 12-h light/dark cycle, 50±5% relative humidity). Food and tap water were provided *ad libitum.* The diet used in this study was isoflavone-free AIN-76A rodent purified diet (Dyets, Inc., Bethlehem, PA, USA). The composition of this diet is shown in Table 4. Body weight was measured every 5 days through the experimental period.

Table 4. Composition of AIN-76A rodent purified diet

Ingredient	g/kg
Casein	200.00
DL-Methionine	3.00
Corn starch	150.00
Sucrose	500.00
Cellulose	50.00
Corn oil	50.00
Salt mix, AIN-76	35.00
Vitamin mix, AIN-76A	10.00
Choline bitartrate	2.00

The rats were randomly divided into four groups of three animals: (1) control groups, which were gavaged daily with distilled water; (2) rapeseed extract groups, which were gavaged with crude rapeseed GLSs about 25.8 μmol/kg/day; (3) genistein groups, which were gavaged with a genistein suspension about 8 mg/kg/day, and (4) rapeseed extract & genistein groups, in which animals were treated with crude rapeseed GLSs and genistein simultaneously at the same concentrations used in groups (2) and (3). The gavage volume in all groups was 1 ml per day. The animals were sacrificed on the first day of the study, and on days 3, 10, 20 and 30. Rats were anesthetized by carbon dioxide inhalation following an overnight fast. Blood was collected from the postcava into serum collection tubes (Becton, Dickinson and Company, New Jersey, USA), and major organs including pituitary, thyroid, liver, adrenal and kidney were removed. The organ samples were washed with 0.9% NaCl, weighed, and then frozen in liquid nitrogen before storage at −80℃.

All animal procedures in this study were carried out in accordance with the Institutional Animal Care and Use Committee of Seoul National University (Approval No. SNU−140429−9).

3. Measurement of serum thyroid hormones

Blood collected from the postcava was allowed to clot in serum collection tubes at 4℃ for 1 hr, and centrifuge at 3000 x g, 4℃ for 25 min (Combi 514R; Hanil Science Medical, Daejeon, Korea). Serum was then collected and stored at −80℃ until the analysis.

Total triiodothyronine(T_3) and total thyroxine(T_4) were measured with ELISA kits (Calbiotech, Spring Valley, CA, USA) according to the manufacture's protocols. For T_3 analysis, 25 ㎕ of serum samples or standards, 100 ㎕ of working enzyme conjugate solution were added sequentially to the microwells coated with T_3 polyclonal antibody, and the mixture was incubated for 60 min at room temperature with shaking. Then the liquid was removed and the wells were washed with 300 ㎕ of 1X wash buffer for three times. The wells were covered and incubated at room temperature for 15 min after adding 100 ㎕ of TMB substrate solution. 50 ㎕ of stop solution was added to each well and gently mixed for 15−20 sec to stop the reaction. Lastly, the absorbance was read on a Microplate reader (SpectraMax 190; Molecular Devices, Sunnyvale, CA, USA) at 450 nm. The protocol of T_4 analysis was similar with that of T_3, except that the sample volume was 10 ㎕.

Thyroid stimulating hormone (TSH) was also measured using an ELISA kit (Demeditec Diagnostics GmbH, Lise-Meitner-Str.2, Germany). The calibrators from 2.5 ng/ml to 40 ng/ml should be prepared first by 2-fold dilution. Then 25 ㎕ of each calibrator or sample were added to the wells coated with a monoclonal TSH antibody. 200 ㎕ of enzyme-labeled anti-rat TSH antibody was added and the mixture was incubated for 18-20 hr at 4℃. After incubation, the contents of the wells were discarded and the wells were washed four times with 300 ㎕ of buffered wash solution. The wash solution was removed as much as possible and 200 ㎕ of TMB/Substrate solution was added with incubating for 30 min in the dark. Finally, 50 ㎕ of stop solution was added and the optical density at 450 nm was read using a Microplate reader.

The standard curves of T_3, T_4 and TSH were constructed through the concentrations and the absorbance results of their respective standards or calibrators. Then the sample concentrations can be calculated according to the standard curves.

4. Analysis of goitrin in serum

The serum of the rapeseed extract group and the rapeseed extract & genistein group from day 30 was used for the goitrin analysis. Goitrin, the breakdown product of progoitrin, was measured using the EI-GC-MS method. Phenethyl isothiocyanate (Sigma-Aldrich, St. Louis, MO, USA) was used as the internal standard and was created to a level of 2 mM with dichloromethane. One microliter of 2 mM phenethyl isothiocyanate was added to 750 μl of the serum, followed by extraction with 750 μl of dichloromethane and dehydration with $MgSO_4$. An amount of five hundred microliters of the extract was then concentrated to 50 μl with nitrogen and analyzed by electron ionization gas chromatography mass spectrometry. A Trace^{TM} 1310 Gas Chromatograph (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with an ISQ GC-MS (Thermo Fisher Scientific Inc., Waltham, MA, USA) and DB-5MS (30 m x 0.25 mm I.D., 0.5 μm df) column (Agilent Technologies, Palo Alto, CA, USA) was used. The carrier gas was helium with a flow rate of 1.0 ml/min, and the injection volume was 1 μl. The temperatures of the MS transfer line, ion source and injection port were all maintained at 250℃. The GC oven was programmed from 50℃ (5min) to 280℃ (10min) at a rate of 4℃/min. Goitrin (m/z 129) and phenethyl isothiocyanate

(m/z 91) were eluted at approximately 34.43 min and 6.00 min, respectively. DL-goitrin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as a standard.

5. RNA extraction and cDNA synthesis

Total RNA was extracted from thyroids using the RNeasy Mini kit (Qiagen Korea Ltd., Seoul, Korea) according to the manufacture's suggestions. A frozen single lobe of thyroids (5~14 mg) was placed into a tube with 350 ㎕ of buffer RLT and homogenized by the TissueLyser Ⅱ (Qiagen Korea Ltd., Seoul, Korea) at 25 Hz for 1 min. The lysate was centrifuged at full speed (21,055 x g) for 3 min (Smart R17; Hanil Science industrial Co., Ltd., Seoul, Korea), and the supernatant was then carefully removed to a new microcentrifuge tube. One volume of 70% ethanol was added to the supernatant and mixed immediately by pipetting. The mixture (up to 700 ㎕) was transferred to an RNeasy spin column placed in a 2 ml collection tube, and centrifuged at 8000 x g for 15 sec. The flow-through was discarded. Then 700 ㎕ of buffer RW1 was added to the RNeasy spin column, followed by centrifuging for 15 sec at 8000 x g to wash the spin column membrane. After discarding the flow-through, 500 ㎕ of buffer RPE was added and centrifuged at 8000 x g for 15 sec. Another 500 ㎕ of buffer RPE was added after removing the flow-through, and then centrifuged for 2 min at 8000 x g. In order to dry the membrane, the spin column was placed in a new 2 ml collection tube, and centrifuged at full speed for 1 min. The spin column was then placed in a new 1.5 ml

collection tube. To elute the RNA, 50 μl of RNase-free water was added directly to the spin column membrane and centrifuged at 8000 x g for 1 min. Another 30 μl of RNase-free water was added and centrifuged at the same condition. The concentrations of the RNA solutions were quantified by a spectrophotometer (Optizen 2120UV, Mecasys Co., Ltd, Daejeon, Korea), and the quality of the RNA samples was assessed by electrophoresis (Mupid-2plus System, Takara Korea Biomedical Inc., Seoul, Korea) using 1% denaturing agarose gels. The extracted RNA was stored at -80℃ until the use for cDNA synthesis.

The cDNA was synthesized from 1.5 μg of total RNA using Oligo(dT)$_{12-18}$ primers and SuperScript™ Ⅱ Reverse Transcriptase (Invitrogen™, Life Technologies Korea, Seoul, Korea) according to the manufacturer's suggested procedure. The condition for reverse transcription was 42℃ for 50 min and 70℃ for 15 min. The cDNA was stored at -20℃ until quantification.

6. Quantification of the gene expression

In order to examine the effects of crude rapeseed GLSs and genistein on the thyroid, the mRNA expression levels of thyroid peroxidase (TPO), thyroglobulin (Tg) and thyrotropin receptor (TSHr), which were closely related to thyroid functions, were quantified using the StepOne Real-time PCR System (Applied Biosystems, CA, USA) with the SYBR Green qPCR Mix (Doctor protein, Seoul, Korea).

The PCR reaction for each sample was carried out with a volume of 20 μl containing 1 μl of synthesized cDNA, 10 μl of qPCR Mix with SYBR Green, 0.4 μl of a 10 μM forward primer, 0.4 μl of a 10 μM reverse primer, and 8.2 μl of distilled water. The reaction mixtures were denatured for 5 min at 95℃, and then subjected to 40 cycles of two-step PCR consisting of a denaturation step at 95℃ for 30 sec and an annealing/extension step at 60℃ for 1 min. After the PCR reactions, melting curve analyses were performed at 95℃ for 15 sec, at 60℃ for 1 min and at 95℃ for 15 sec to assess the dissociation characteristics of the double-stranded DNA products during heating. The specificity of the PCR was further verified by electrophoresis using 3% agarose gels. Triplicate reactions were performed for each sample and cycle threshold (Ct) values were

analyzed by StepOne™ Software version 2.1 (Applied Biosystems, CA, USA).

The house-keeping gene β-actin was used as an internal reference to normalize the results. The relative expression levels of mRNA were calculated using the comparative Ct method (△△Ct) and the resultant data were presented as the fold change relative to the time-matched control groups. The specific oligonucleotide sequences of the primers used in the current study are shown in Table 5.

Table 5. The oligonucleotide sequences of the primers used for quantification

Gene	Forward primer	Reverse primer
β-actin	AAGAGCTATGAGCTGCCTGACG	TGGATGCCACAGGATTCCATACCC
Tg	TCCAGACTCTGAAGGATGCAGATG	ACTTCCAAGTCCTCCTCTCCAC
TPO	AGTCAGGAGAGTGGGATTTCACC	GGATCCATAAGCCGGTTCCTGTTC
TSHr	TTGTCTGCTCCTGCTATGTGAA	TCTTGGCAATCTTGGTGTCTTT

7. Statistical analysis

SPSS Ver. 21 (IBM Corp., Armonk, NY, USA) software was used for the statistical analysis. Data were assessed with a one-way ANOVA followed by Tuckey's HSD test. Differences were considered statistically significant at $p < 0.05$.

Ⅳ. Results

1. Body and organ weights

No animals died during the experimental period and no treatment-related clinical signs were found. The data of body weights are given in Appendix 1 and the body weight curves of rats in different groups are shown in Figure 3. Compared to the control groups, there were no significant changes of body weights in the treatment groups. However, a slight decrease in the genistein groups up to day 15 and a slight increase in the rapeseed extract & genistein groups from day 20 to day 30 were observed.

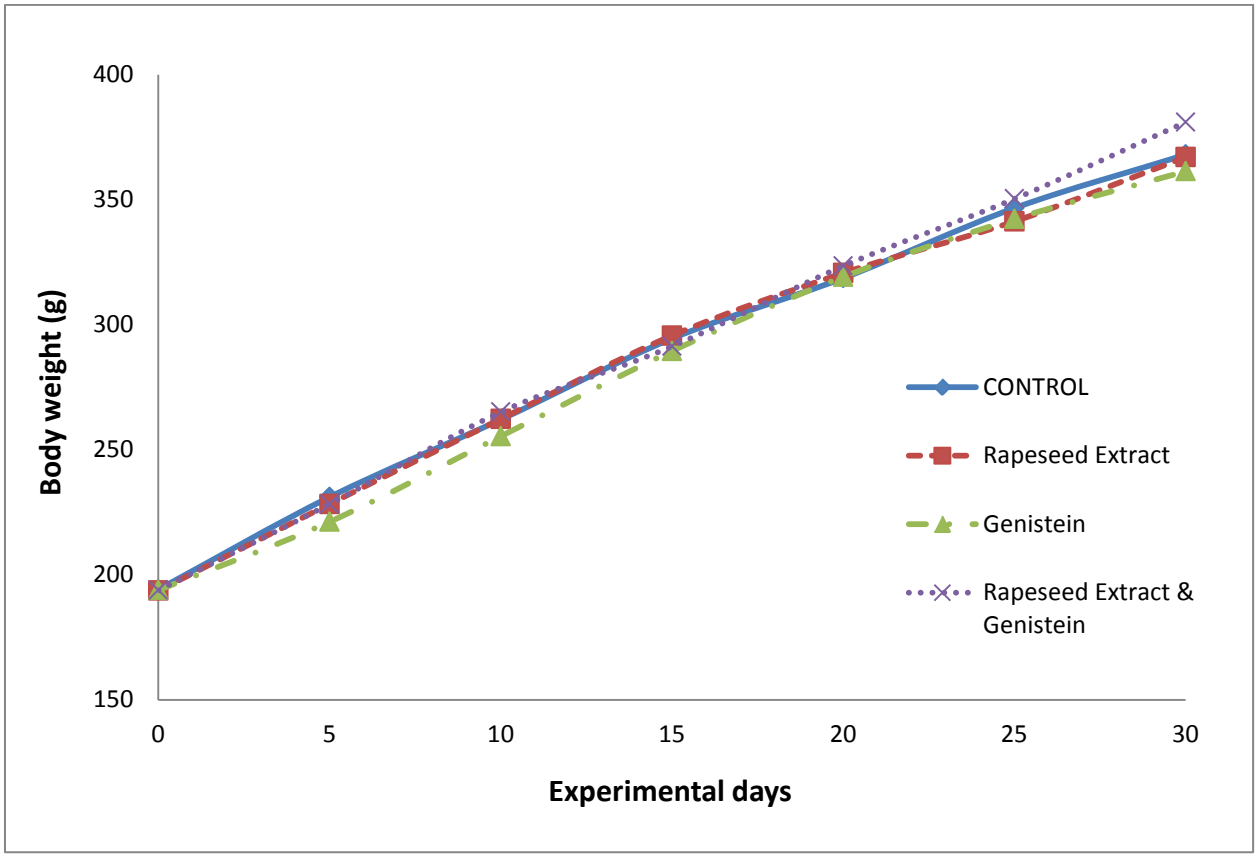

Figure 3. Body weight curves in the 30-day experimental period

No significant changes were found in both the absolute and relative organ weights of pituitary, thyroid, liver, adrenal and kidney, except for the decrease of absolute liver weight in the rapeseed extract group at day 3 and relative pituitary weight in the rapeseed extract & genistein group at day 30 as compared to the control group. The absolute and relative organ weights are shown in Tables 6~7.

Table 6. Absolute organ weights (pituitary, thyroid and adrenals: mg; liver, kidneys: g)

	Control	Rapeseed extract	Genistein	Rapeseed extract & genistein
Day 0				
Pituitary	6.17 ± 1.25	--	--	--
Thyroid	11.53 ± 1.70	--	--	--
Liver	7.12 ± 0.60	--	--	--
Adrenals	49.40 ± 6.95	--	--	--
Kidneys	1.73 ± 0.16	--	--	--
Day 3				
Pituitary	7.90 ± 0.70	7.80 ± 1.59	7.73 ± 0.75	7.77 ± 0.21
Thyroid	14.30 ± 1.23	12.43 ± 2.02	11.43 ± 0.49	13.73 ± 3.12
Liver	8.50 ± 0.06	7.44 ± 0.33*	8.22 ± 0.33	7.83 ± 0.46
Adrenals	42.30 ± 5.26	40.17 ± 1.62	34.57 ± 5.18	43.10 ± 13.46
Kidneys	2.10 ± 0.10	1.98 ± 0.14	1.98 ± 0.11	2.02 ± 0.02
Day 10				
Pituitary	8.63 ± 0.78	8.80 ± 0.75	7.90 ± 1.56	8.27 ± 0.55
Thyroid	15.33 ± 0.90	15.73 ± 5.80	13.33 ± 1.45	14.73 ± 2.23
Liver	9.58 ± 1.32	9.19 ± 0.65	8.00 ± 0.87	9.21 ± 1.03
Adrenals	49.70 ± 10.17	56.90 ± 10.60	47.80 ± 11.25	49.20 ± 4.45
Kidneys	2.17 ± 0.11	2.37 ± 0.13	2.06 ± 0.38	2.22 ± 0.22
Day 20				
Pituitary	8.97 ± 0.45	10.03 ± 0.55	10.33 ± 2.15	9.50 ± 0.53
Thyroid	15.40 ± 1.71	20.63 ± 4.22	18.30 ± 4.37	18.20 ± 4.95

Table 6. ***(Continued)***

Liver	9.95 ± 0.21	10.57 ± 1.23	10.44 ± 1.73	10.15 ± 0.70
Adrenals	54.53 ± 12.25	49.47 ± 4.21	60.17 ± 7.31	48.03 ± 6.55
Kidneys	2.45 ± 0.16	2.65 ± 0.22	2.75 ± 0.42	2.61 ± 0.32
Day 30				
Pituitary	10.80 ± 0.89	9.93 ± 0.65	9.17 ± 0.67	9.10 ± 0.56
Thyroid	19.97 ± 5.92	21.33 ± 4.74	17.47 ± 0.70	22.37 ± 4.19
Liver	11.39 ± 0.43	11.97 ± 1.75	10.63 ± 1.08	11.54 ± 0.59
Adrenals	55.47 ± 9.72	57.57 ± 10.80	48.20 ± 6.46	65.73 ± 6.96
Kidneys	2.85 ± 0.22	2.73 ± 0.16	2.74 ± 0.14	3.39 ± 0.48

* Significant differences in comparison with the control ($p < 0.05$).

Table 7. Relative organ weights (pituitary, thyroid and adrenals: mg/100g body weight; liver, kidneys: g/100g body weight)

	Control	Rapeseed extract	Genistein	Rapeseed extract & genistein
Day 0				
Pituitary	3.35 ± 0.57	--	--	--
Thyroid	6.32 ± 1.30	--	--	--
Liver	3.82 ± 0.26	--	--	--
Adrenals	26.95 ± 4.28	--	--	--
Kidneys	0.94 ± 0.03	--	--	--
Day 3				
Pituitary	3.77 ± 0.29	3.96 ± 0.75	3.73 ± 0.27	3.84 ± 0.14
Thyroid	6.81 ± 0.37	6.31 ± 0.91	5.53 ± 0.23	6.75 ± 1.31
Liver	4.06 ± 0.16	3.78 ± 0.09	3.97 ± 0.13	3.87 ± 0.11
Adrenals	20.20 ± 2.70	20.43 ± 1.08	16.70 ± 2.42	21.28 ± 6.38
Kidneys	1.00 ± 0.01	1.01 ± 0.06	0.96 ± 0.06	1.00 ± 0.05
Day 10				
Pituitary	3.38 ± 0.16	3.48 ± 0.03	3.38 ± 0.37	3.28 ± 0.35
Thyroid	6.00 ± 0.17	6.16 ± 1.86	5.73 ± 0.34	5.83 ± 0.87
Liver	3.74 ± 0.36	3.64 ± 0.18	3.43 ± 0.16	3.63 ± 0.31
Adrenals	19.38 ± 3.26	22.37 ± 2.59	20.39 ± 2.99	19.42 ± 0.98
Kidneys	0.85 ± 0.01	0.94 ± 0.07	0.88 ± 0.05	0.88 ± 0.09
Day 20				
Pituitary	2.97 ± 0.20	3.19 ± 0.17	3.33 ± 0.47	3.01 ± 0.11

Table 7. ***(Continued)***

Thyroid	5.12 ± 0.78	6.53 ± 1.02	5.88 ± 1.06	5.74 ± 1.43
Liver	3.30 ± 0.09	3.35 ± 0.19	3.36 ± 0.30	3.22 ± 0.12
Adrenals	18.05 ± 3.78	15.71 ± 0.39	19.46 ± 1.45	15.17 ± 1.16
Kidneys	0.81 ± 0.02	0.84 ± 0.03	0.89 ± 0.05	0.83 ± 0.16
Day 30				
Pituitary	3.02 ± 0.23	2.86 ± 0.22	2.65 ± 0.20	2.52 ± 0.01*
Thyroid	5.54 ± 1.36	6.14 ± 1.38	5.04 ± 0.24	6.25 ± 1.52
Liver	3.19 ± 0.15	3.44 ± 0.45	3.07 ± 0.33	3.20 ± 0.11
Adrenals	15.44 ± 1.76	16.57 ± 3.18	13.91 ± 1.78	18.24 ± 2.23
Kidneys	0.80 ± 0.06	0.79 ± 0.04	0.79 ± 0.04	0.93 ± 0.08

* Significant differences in comparison with the control ($p < 0.05$).

2. Goitrin in rat serum

Goitrin in the serum of rapeseed extract group and rapeseed extract & genistein group from day 30 was measured using EI-GC-MS method. However, no peak of goitrin was detected, suggesting that the goitrin level in serum was very low, if any goitrin exists at all.

3. Thyroid hormones analysis

Though no statistically significant changes of T_3, T_4 and TSH were observed except for a decrease of T_4 in the genistein group from day 3 (Tables 8~10), slight fluctuations of the thyroid hormones were noted.

In general, TSH showed more changes than T_3 and T_4 during the experimental period. In the rapeseed extract groups, a slight decrease of TSH was found between day 3 to day 20, while an apparent increase (about 33%) was observed on the last day. In the rats treated with genistein, although T_4 decreased significantly on day 3, the change was attenuated after that day, and T_4 started to show a slight increase on day 10. TSH increased with T_4 on day 10 and showed highest level on day 30. In contrast, the T_3 level was generally not affected during the 30-day period. In the rapeseed extract & genistein groups, T_4 levels fluctuated during the experimental period, while an increase was observed in both T_3 and TSH levels. On day 30, TSH clearly increased (about 66%) and was higher than that of the groups treated with rapeseed extract and genistein individually.

Table 8. Serum T_3 levels

Day	Control	Rapeseed extract		Genistein		Rapeseed extract & genistein	
		Value (ng/ml)	Ratio to the control group (%)	Value (ng/ml)	Ratio to the control group (%)	Value (ng/ml)	Ratio to the control group (%)
0	0.99 ± 0.04	--	--	--	--	--	--
3	0.95 ± 0.18	0.95 ± 0.16	100	1.08 ± 0.18	114	1.02 ± 0.10	107
10	1.10 ± 0.10	0.95 ± 0.11	86	0.92 ± 0.11	84	1.15 ± 0.28	105
20	0.84 ± 0.06	0.79 ± 0.03	94	0.83 ± 0.10	99	0.79 ± 0.06	94
30	0.86 ± 0.03	0.92 ± 0.22	107	0.84 ± 0.21	98	0.97 ± 0.05	113

Table 9. Serum T_4 levels

Day	Control	Rapeseed extract		Genistein		Rapeseed extract & genistein	
		Value (μg/dl)	Ratio to the control group (%)	Value (μg/dl)	Ratio to the control group (%)	Value (μg/dl)	Ratio to the control group (%)
0	6.30 ± 0.27	--	--	--	--	--	--
3	6.36 ± 0.50	5.48 ± 0.37	86	5.18 ± 0.46*	81	7.09 ± 0.45	111
10	4.92 ± 0.12	4.81 ± 0.39	98	5.35 ± 0.90	109	4.55 ± 0.69	92
20	5.76 ± 0.08	5.76 ± 0.56	100	5.69 ± 1.39	99	6.32 ± 0.15	110
30	5.33 ± 0.41	5.87 ± 0.84	110	5.74 ± 0.47	108	5.00 ± 0.99	94

* Significant differences in comparison with the control ($p < 0.05$).

Table 10. Serum TSH levels

Day	Control	Rapeseed extract		Genistein		Rapeseed extract & genistein	
		Value (ng/ml)	Ratio to the control group (%)	Value (ng/ml)	Ratio to the control group (%)	Value (ng/ml)	Ratio to the control group (%)
0	1.40 ± 0.29	--	--	--	--	--	--
3	2.15 ± 0.68	1.91 ± 0.34	89	1.91 ± 0.33	89	1.84 ± 0.21	86
10	1.84 ± 0.60	1.77 ± 0.38	96	2.05 ± 0.07	111	2.72 ± 0.75	148
20	1.51 ± 0.30	1.09 ± 0.30	72	1.52 ± 0.50	101	1.62 ± 0.37	107
30	1.65 ± 0.35	2.20 ± 0.61	133	2.30 ± 0.39	139	2.74 ± 1.00	166

4. Expression of genes involved in thyroid function

Significant changes of mRNA expression levels were only observed on day 10. As shown in Table 11, when compared to the control group, the mRNA expression levels of Tg , TPO and TSHr all showed decreases in the genistein group on day 10. In the group treated with rapeseed extract & genistein, both the Tg and TPO expression levels showed a significant decrease. In the rapeseed extract group, the only significant decrease was observed in the Tg expression level.

On day 30, although not statistically significant, the mRNA expression levels of both Tg and TSHr showed an increase in all treated groups, with a higher level in the rapeseed extract & genistein group. However, no change was observed in the TPO mRNA expression level.

Table 11. The mRNA expression levels of thyroid function related genes

Day	Gene	Control	Rapeseed extract	Genistein	Rapeseed extract & genistein
3	Tg	1.00 ± 0.04	0.98 ± 0.12	1.33 ± 0.18	1.05 ± 0.15
	TPO	1.00 ± 0.03	0.72 ± 0.08	1.18 ± 0.33	0.78 ± 0.22
	TSHr	1.00 ± 0.11	1.15 ± 0.30	1.47 ± 0.39	0.92 ± 0.31
10	Tg	1.03 ± 0.29	0.51 ± 0.04*	0.12 ± 0.07*	0.43 ± 0.06*
	TPO	1.03 ± 0.33	0.69 ± 0.15	0.34 ± 0.26*	0.41 ± 0.09*
	TSHr	1.03 ± 0.31	1.00 ± 0.14	0.39 ± 0.21*	0.51 ± 0.12
20	Tg	1.05 ± 0.40	0.72 ± 0.13	1.31 ± 0.12	1.25 ± 0.16
	TPO	1.02 ± 0.27	0.77 ± 0.14	0.91 ± 0.07	1.12 ± 0.07
	TSHr	1.01 ± 0.15	1.02 ± 0.17	1.17 ± 0.23	1.09 ± 0.03
30	Tg	1.01 ± 0.15	1.23 ± 0.35	1.14 ± 0.20	1.37 ± 0.82
	TPO	1.01 ± 0.18	1.08 ± 0.06	1.03 ± 0.16	1.06 ± 0.19
	TSHr	1.02 ± 0.25	1.10 ± 0.09	1.35 ± 0.12	1.55 ± 0.90

* Significant differences in comparison with the control ($p < 0.05$).

Ⅴ. Discussion

In this study, it was found that crude rapeseed GLSs and genistein had only a weak effect on rat thyroids, which can be attributed to the concentrations used here. For genistein, according to Chang and Doerge (2000), rats fed a 100-ppm genistein diet, which is equal to an intake of genistein of 8 mg/kg/day, had genistein serum levels similar to those found in adults on typical Asian diets (0.1~1.2 μM) (Adlercreutz *et al.*, 1994) or soy nutritional supplements (0.5~0.9 μM) (Doerge *et al.*, 2000). Therefore, the concentration of genistein given to the rats was set to 8 mg/kg/day to match the serum levels in Asian adults. Unlike genistein, no toxicokinetic data of GLSs has been reported to suggest a relationship between animals and humans. Therefore the concentration of GLSs was determined based on the daily intake of high consumers in humans. The vegetables and processed foods that contribute most to the Korean dietary intake of GLSs were reported by Han and Kwon (2009), and their thiocyanate contents were also measured. With the food consumption data from the Korean National Nutrition Survey (2008~2011), the daily intake level of the total GLSs in the 99^{th} percentile of high consumers was calculated to be 154.79 μmol/day/person (Table 12). As a result, the GLS intake of

Koreans can be estimated to be 2.58 μmol/kg/day after dividing the average body weight of adults, 60 kg. The treated concentration of GLSs was then determined to be 25.8 μmol/kg/day, which was ten times the daily intake of humans, so as to determine the effect in the rat with a safety margin. Because the treated concentration of genistein, 8 mg/kg/day, is approximately 16 times as much as the intake of Asian adults (Messina *et al.*, 2006), the determined concentration of GLSs is also assumed to be at a level similar to that of genistein.

Table 12. The estimated daily GLSs intake of Korean adults (99th high consumers)

Vegetable	Thiocyanate contents[a] (μmol/g fresh weight)	Daily intake[b] (g fresh weight/day)	GLSs daily intake (μmol/day/person)
Radish	0.04	198.98	7.96
Leaves of radish	0.64	133.85	85.66
Dried radish	0.57	3.73	2.13
Chinese cabbage	0.10	176.65	17.67
*Kimchi**	0.05	362.76	18.14
Broccoli	0.24	19.44	4.67
Cabbage	0.15	114.30	17.15
Shepherd's purse	0.51	2.76	1.41
Total			154.79

*Made with Chinese cabbage.

[a] Han and Kwon (2009)

[b] National Food & Nutrition Statistics 2011: based on 2008~2011 Korea National Health and Nutrition Examination Survey.

In the results of body and organ weights, no significant change was observed except for the decrease of absolute liver weight in the rapeseed extract group at day 3 and relative pituitary weight in the rapeseed extract & genistein group at day 30 when compared to the control group. The decrease of absolute liver weight was not treat-related and can be ignored because no significant change was found in the relative liver weight. For the relative pituitary weight in the rapeseed extract & genistein group at day 30, the decrease should be attributed to the higher body weights compared to the control group.

Goitrin was not detected in the serum of both rapeseed extract group and rapeseed extract & genistein group from day 30, which had highest exposure to GLSs or progoitrin in the current study. According to a recent study (Choi *et al.*, 2014), when administered with progoitrin at a single dose of 250 μmol/kg body weight, a high level of goitrin was detected in the serum of the rats after 5 hours. Compared to this reference, the concentration used in the present study was much lower. Therefore, it is possible that most of the goitrin converted from the administered progoitrin was excreted from the body.

Although most of changes observed in thyroid hormones and mRNA expression levels are not statistically significant, some trends can be found among them. In general, TSH showed more changes than T_3 and T_4 in the experimental period. In the rapeseed extract groups, a slight decrease of TSH was found from day 3 to day 20, while an apparent increase (about 33%) was observed on the last day. In the rats treated with genistein, although T_4 decreased significantly on day 3, the change was then attenuated with the increase of TSH. In the rapeseed extract & genistein groups, an increase was also observed in TSH levels. TSH, which can stimulate the thyroid to produce T_4, is controlled by thyroid hormone levels in the blood via negative feedback of the pituitary–thyroid axis (Hill *et al.*, 1989). Therefore, the relatively steady states of T_3 and T_4 should be attributed to the changes in the TSH level.

For the mRNA expression levels of thyroid function related genes, significant changes were only observed on day 10. Among them, the depression of the TPO expression level was consistent with the isoflavone–mediated inactivation of TPO reported in former studies. However, the reduced TSHr expression level is confusing, as the TSH level in serum showed a slight increase on day 10.

The expression level of TSHr increased starting on day 20, consistent with the pattern of TSH. On day 30, although not statistically significant, the mRNA expression levels of both Tg and TSHr showed an increase in all treated groups, with a higher level in the rapeseed extract & genistein group. However, no change was observed in the TPO mRNA expression levels, suggesting a symptom of recovery to the normal level.

Compared to the groups treated with rapeseed extract and genistein individually, no synergistic effect was observed in the combined treatment groups. Goitrin has been reported to enlarge the thyroid, decrease iodine uptake by the gland, and decrease T_4 synthesis (Vanderpas, 2006). On the other hand, it has been demonstrated that isoflavone exerts its antithyroid effect through the inactivation of TPO, and thyroid homeostasis can be maintained if the remaining enzymatic activity is sufficient (Chang and Doerge, 2000). In the current study, only slight fluctuations, which can be relieved by the body, were observed in the treated groups because the intake levels of both the GLSs and genistein were relatively safe. In addition, the different mechanisms of GLSs and genistein make it difficult to determine if an interrelationship exists between them.

The present study has shown that both GLSs and genistein have a weak effect on the thyroid when treated with a dose that is similar to the intake level of high consumers in humans. It was also found that fluctuations in thyroid hormone levels and gene expression levels can be relieved by the body itself, primarily through the pituitary–thyroid axis. Thus, when the treated concentrations of GLSs and genistein are similar to the dietary exposure levels of human, close observation intervals and a long treated time are essential to determine the presence of any antithyroid effect and related interrelationship of GLSs and genistein.

Ⅵ. Summary

GLSs and isoflavone, both indicated to be goitrogenic, are important dietary factors in Asian diets. In the present study, Wistar rats were treated for 30 days with crude rapeseed GLSs and genistein, individually and simultaneously. Both thyroid hormones (T_3, T_4 and TSH) and the expression levels of genes related to thyroid function (Tg, TPO and TSHr) were analyzed to determine the effects on the thyroid. Goitrin, the main breakdown product of the GLSs in rapeseed extract, was measured in the serum of treated groups at day 30.

In the results of body and organ weights, no treat-related change was observed. For the serum levels of goitrin, no peak was detected in the serum of both rapeseed extract group and rapeseed extract & genistein group from day 30.

Although most of changes observed in thyroid hormones and mRNA expression levels are not statistically significant, some fluctuations can be found. In the 30-day experimental period, TSH showed more changes than T_3 and T_4. In the rapeseed extract groups, TSH slightly decreased from day 3 to day 20, and clearly

increased (about 33%) on day 30. In the rats treated with genistein, although T_4 decreased significantly on day 3, the change was then attenuated with the increase of TSH. In the rapeseed extract & genistein groups, increase was also observed in TSH levels. Since TSH is controlled by thyroid hormone levels in the blood via negative feedback of the pituitary-thyroid axis (Hill *et al.*, 1989), and can stimulate thyroid to produce T_4, it can be estimated that the relatively steady state of T_3 and T_4 is the result of the TSH changes.

For the mRNA expression levels of thyroid function related genes, Tg, TPO and TSHr were all depressed significantly on day 10. The depression of TPO expression level was consistent with the isoflavone-mediated inactivation of TPO reported in former studies, and the expression level of Tg also showed the same pattern with T_3. The expression level of TSHr increased starting on day 20, consistent with the pattern of TSH. On day 30, no change was observed in the TPO mRNA expression levels, suggesting a symptom of recovery to the normal level.

For the groups treated with both rapeseed extract and genistein, no synergistic effect was observed. In this study, only slight

fluctuations, which can be relieved by the body itself, were observed in the treated groups because the intake levels of both GLSs and genistein were relatively low. In addition, the different mechanisms of GLSs and genistein also make it difficult to see the interrelationship between them.

The present study has shown that both GLSs and genistein have a weak effect on the thyroid when treated with a dose that is similar to the intake level of high consumers in humans. It was also found that the fluctuations in the thyroid hormone levels and gene expression levels were supposed to be a function of the pituitary–thyroid axis. Thus, when the treated concentrations of GLSs and genistein are similar to the dietary exposure levels of human, close observation intervals and a long treated time are essential to determine the antithyroid effect and related interrelationship of GLSs and genistein.

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Appendix

Appendix 1. Body weights (g)

Day	Control	Rapeseed extract	Genistein	Rapeseed extract & genistein
0	194.1 ± 9.7	193.7 ± 10.0	194.0 ± 11.5	193.8 ± 10.9
5	231.1 ± 10.8	228.3 ± 12.8	221.2 ± 17.6	228.3 ± 11.8
10	262.1 ± 10.0	262.3 ± 15.7	255.4 ± 20.6	265.1 ± 13.5
15	294.5 ± 13.1	295.7 ± 16.1	289.5 ± 18.9	291.3 ± 16.6
20	318.7 ± 13.6	320.7 ±17.0	319.2 ± 21.0	323.5 ± 21.4
25	346.7 ± 18.6	341.3 ± 5.0	342.3 ± 5.0	350.3 ± 21.5
30	368.0 ± 21.6	367.0 ± 6.2	361.3 ± 2.9	381.0 ± 20.1

국문 초록

글루코시놀레이트 (glucosinolate)와 이소플라본 (isoflavone)은 아시아 식이에 있는 중요한 식이요인으로서 갑상선에 부정적인 영향을 미치는 것으로 알려져 있다. 글루코시늘레이트의 반갑상선 영향에 관한 연구를 살펴보면 대부분이 명확한 효과를 보기 위해 높은 농도로 투여하였다. 따라서 실제 식생활에 섭취되는 농도에서 갑상선에 영향을 미칠 수 있는지를 예측하는데 어려움이 있다. 그리고 글루코시놀레이트와 이소플라본이 갑상선에 대한 동반효과에 관련된 연구는 미비한 실정이다. 본 연구에서는 글루코시놀레이트를 포함한 유채씨 추출물과 이소플라본의 일종인 제니스테인 (genistein)을 수컷 랫드에게 30일 동안 경구투여 하였으며, 갑상선에 미치는 영향을 확인하기 위해 혈청 중의 갑상선 호르몬 (T_3, T_4, TSH) 농도와 갑상선 기능과 관련된 유전자 (Tg, TPO, TSHr)의 발현 수준을 3일, 10일, 20일, 30일에 각각 측정하였다. Goitrin은 유채씨 추출물에서 농도가 가장 높은 글루코시놀레이트의 대사체로서 30일의 유채씨 추출물 처리그룹과 병용처리그룹의 혈청을 이용하여 EI-GC-MS 방법으로 측정하였다. 처리물질로 인한 체중 변화와 장기 무게 변화가 나타나지 않았다. Goitrin은 혈청에서 검출되지 않았다. 갑상선 호르몬의 혈청 농도는 유의적인 변화가 거의 없었지만 경미한 변동 추세가 나타났다. 10일의 제니스테인 처리그룹에서 T_4가 유의적으로 감소하였다. 유전자의 mRNA 발현수준은 10일에만 유의적인 차이가 나타났고 모두가 대조군보다 떨어졌다. 본 연구를 통해 사람 중의 상위섭취군의 섭취량과 비슷한 정도로 투여했을 때 글루코시놀레이트와 제니스테인은 갑상선에 약한 영향만 미칠 수 있는 것을 알 수 있다. 갑상선 호르몬 및

관련 유전자 발현 수준에 일어나는 변동은 뇌하수체-갑상선 축을 통해 몸 자체로 경감할 수 있다. 따라서 인체 섭취량과 비슷한 양을 투여할 때 글루코시놀레이트와 제니스테인의 반갑상선 작용 및 관련된 상호관계를 확인하기 위해 보다 더 짧은 관찰 간격과 긴 실험기간이 필요하다고 사료된다.

주요어: 유채씨 추출물, 글루코시놀레이트, 제니스테인, 갑상선

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