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Effects of *Aloe vera* on the Regulation of Thyroxine Release in FRTL-5 Thyroid Cells

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Abstract: To determine whether *Aloe vera* (AV), a traditional herbal medicine, could maintain homeostasis, we investigated its ability to regulate the production of hormones, particularly thyroid hormones. T_4 ELISA assay with (6H media) and without thyrotropin (TSH) (5H media) showed that AV functions to maintain endocrine homeostasis. To understand the mechanisms by which AV regulates thyroxine release, we confirmed the protein expression of key upstream factors of thyroid hormone synthesis, such as phosphorylated extracellular signal-regulated kinase (ERK), phosphorylated cAMP response element binding protein (CREB), and phosphorylated protein kinase B (p-Akt). Although the protein expression of phosphorylated ERK and CREB were decreased in 6H media, they were increased and unchanged, respectively, in 5H media. However, protein expression of phosphorylated Akt was not changed by AV in both 6H and 5H media. Furthermore, inhibition of protein kinase A (PKA) by H89 eliminated AV-induced phosphorylation of ERK in 5H media. However, the inhibition of protein kinase C (PKC) by GF 109203X did not eliminate AV-induced ERK phosphorylation in 5H media. These results suggested that AV has TSH-like activity and could regulate thyroxine release through the TPO and PKA pathways. AV may regulate thyroid function to maintain a constant level of thyroid hormones in the body, and could be a candidate for thyroid disease therapies.

Keywords: *Aloe vera* (AV); FRTL-5 cells; homeostasis; thyroid hormones

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1. Introduction

Thyroid hormone actions are essential for the normal growth and development of vertebrates [1]. Their production and release are controlled by a feedback loop system that involves the hypothalamus, anterior pituitary gland, and thyroid. The hypothalamus secretes thyrotropin-releasing hormone (TRH), which stimulates the pituitary gland to produce thyroid-stimulating hormone (TSH). Thyroid-stimulating hormone stimulates the production of thyroid hormones via the thyroid gland, and this is regulated by a negative feedback loop to prevent the release of both TRH and TSH when the levels of thyroid hormones increase. This system allows the body to maintain a constant level of thyroid hormones in the body, and regulates the mechanism to prevent both hypo- and hyperthyroidism. Hypo- and hyperthyroidism indicate insufficient or excessive thyroid hormone production, respectively.

Herbal medicines provide natural, effective, and safe remedies in clinical practice, and natural sources of medicine are often considered a good way to maintain health. Herbal medicines are increasingly used in the world as they have fewer side effects than modern medicines, and are known for their various beneficial effects and a broad range of uses in treating various diseases. They consist of several herbs and ingredients that can regulate the body's balance and homeostasis holistically [2]. For example, the improvement of glucose homeostasis and serum lipid levels in type 2 diabetes by *Nigella sativa* has been

demonstrated using meta-analysis [3]. Effects of Chinese herbal formulas and herb-derived bioactive compounds on allergic asthma caused by an imbalance of immune regulation have been proven in several experimental models [4]. The Fuzi-Lizhong pill increased ghrelin release and improved chronic hypothermia in rats with hypothyroidism and indigestion [5]. However, there have been no reports on whether herbal medicines regulate the feedback system of thyroid hormones.

In this study, we found that *Aloe vera* (AV), a traditional herbal medicine, regulates the feedback system of thyroid hormones in FRTL-5 thyroid cells. In Ungok's illustrated guide medicinal materials, *Aloe vera* Linne (*A. barbadensis* Miller, *A. ferox* Miller, *A. africana* Miller, and *A. spicata* Baker), known as Nohoe, belonged to the family Liliaceae that originated in Republic of Korea, China, and South Africa [6]. Of the herbs of the Tangaek section of Donguibogam, a Korean medical book, it was first mentioned and was known to contain several bioactive components, such as vitamins, minerals, sugars, and enzymes [7]. Due to these components, AV has several nutritional and therapeutic properties and beneficial effects, including anti-inflammatory, anti-oxidative, anti-bacterial effects, and regulation of intestinal function, have been widely reported. Therefore, it is used to treat constipation, skin disease, infections, diabetes, and gastrointestinal disorders, among several other diseases [8–11]. For example, AV metabolites exhibit anti-inflammatory effects by reducing the production of pro-inflammatory cytokines in lipopolysaccharide (LPS)-induced septic mice, LPS-activated RAW264.7 macrophages, and murine peritoneal macrophages [12]. AV extract showed protective effects on pancreatic islets by restoring pancreatic islet mass and improving diabetes in rats with streptozotocin (STZ)-induced diabetes [13].

This study aimed to evaluate the ability of AV to maintain homeostasis, similar to TSH. We examined the role of AV in regulating the cellular export of thyroid hormones, and its cellular mechanism in FRTL-5 thyroid cells.

2. Materials and Methods

2.1. Material Purchase and Extraction

AV was purchased from a commercial supplier in Deajeon, Republic of Korea. We identified it using a genetic method with GenBank sequences before starting the study. To prepare AV water extract, it was extracted by refluxing with 1000 mL of water for 2 h at 100 °C. The extraction process was repeated twice, and the combined extracts were filtered, evaporated at 50 °C, and lyophilized. The authenticated sample was stored at the Korea Institute of Oriental Medicine until use.

2.2. Cell Culture and Treatment

FRTL-5 rat thyroid cells were purchased from the American Type Culture Collection (ATCC® CRL-8305, Rockville, MD, USA). Cells were cultured in Coon's F-12 medium (Merck KGaA, Darmstadt, Germany) supplemented with 5% fetal bovine serum (FBS; Hyclone, Inc., South Logan, UT, USA), 100 U/mL penicillin, 100 mg/mL streptomycin (Hyclone, Inc.), 2 mM glutamine (Hyclone, Inc.), and six hormones (6H): TSH (10 mU/mL; Sigma-Aldrich, St. Louis, MO, USA), insulin (10 μg/mL; Sigma-Aldrich), hydrocortisone (10 nM; Sigma-Aldrich), transferrin (5 μg/mL; Sigma-Aldrich), somatostatin (10 ng/mL; Sigma-Aldrich), and glycyl-L-hystidyl-L-lysine (10 ng/mL; Angene Chemical, London, UK). The cells were grown at 37 °C in an atmosphere of 5% CO_2. Before to AV treatment, FRTL-5 cells were grown in TSH-depleted 5H medium for 24 h. AV (10 μg/mL) was added to the cells in both 5H and 6H culture media for 10 min. Consequently, 1 nM NaI was added, and the cells were incubated for another 3 h. For the inhibitor treatment, 10 μM PKA inhibitor (H89; Merck KGaA) and 5 μM PKC inhibitor (GF 109203X; Tocris Bioscience, Bristol, UK) were added to the cells for 30 min before AV treatment.

2.3. Thyroxine Levels

Thyroxine levels expressed in the FRTL-5 culture media were tested using an enzyme-linked immunosorbent assay (ELISA) kit (Alpha Diagnostic Intl. Inc., San Antonio, TX,

USA). We experimented according to the manufacturer's instructions. To measure thyroxine levels, FRTL-5 cells were seeded at a density of 1.5×10^5 cells per well in a 96-well plate. After sample treatment, the cell supernatants were harvested and centrifuged to remove debris immediately. The centrifuged supernatant was measured using an ELISA kit at 450 nm on an ELISA reader (Molecular Devices Corporation, San Jose, CA, USA). Levels expressed in the FRTL-5 culture media were tested using an enzyme-linked immunosorbent assay (ELISA) kit (Alpha Diagnostic Intl. Inc., San Antonio, TX, USA).

2.4. Cell Proliferation

To evaluate cell proliferation, we used the EZ-CYTOX kit (DoGen Bio Co., Ltd., Seoul, Republic of Korea). EZ-CYTOX measures the number of viable cells using enzyme-based methods. The production of formazan, which is a water-soluble tetrazolium salt (WST) generated by dehydrogenase from active cells, is related to the number of active cells. To measure cell proliferation, FRTL-5 cells were seeded at a density of 2×10^4 cells per well in a 96-well plate. The cells were then incubated for 24 h, at 37 °C, and 5% CO_2. After treatment, 10% of the total volume of the WST solution was added to each well and incubated for 4 h. Finally, the absorbance was measured at 450 nm using a micro plate reader.

2.5. Real-Time PCR

To analyze the mRNA expression of sodium/iodide symporter (NIS), thyroglobulin (TG), and thyroid peroxidase (TPO), total RNA was extracted using RiboEX (GeneAll Biotechnology Co., Ltd., Seoul, Republic of Korea) according to the manufacturer's instructions. A total of 2 μg of RNA from synthesized cDNA was obtained using a cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). Real-time polymerase chain reaction (RT-PCR) was conducted using the Power SYBR Green PCR Master Mix (Thermo Fisher Scientific) according to the manufacturer's instructions on a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA). The sequences of primers used in these experiments are described in Table 1 [14].

Table 1. Primer sequences used in RT-PCR analysis. The relative mRNA expression levels were normalized using GAPDH mRNA expression.

Name	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
NIS	GCTGTGGCATTGTCATGTTC	TGAGGTCTTCCACAGTCACA
TG	GAATTGCTGGCAGATGTTCAG	GGGCACTGAGCTCCTTGTAG
TPO	TCTGGCATCACTGAACTTGC	CGGTGTTGTCACAGATGACC
GAPDH	ACAGCAACAGGGTGGTGGAC	TTTGAGGGTGCAGCGAACTT

2.6. Western Blotting

Cells were lysed with RIPA buffer (150 mM sodium chloride, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 50 mM Tris-HCl [pH 7.5], and 2 mM ethylenediaminetetraacetatic acid [EDTA]) (Biosesang, Inc., Gyeonggi-do, Republic of Korea) containing protease inhibitors (Quartett, Berlin, Germany) and phosphatase inhibitors (Roche Applied Science, Penzberg, Germany). The protein concentration was measured using a bi-cinchoninic acid (BCA) protein assay (Thermo Fisher Scientific). Protein extracts (50 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on a 4–20% Mini-PROTEAN TGX Precast Protein Gel (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Inc.). Membranes were then blocked with 3% bovine serum albumin (BSA) in Tris-buffered saline containing 0.5% Tween 20 (TBS-T; iNtRON Biotechnology, Gyeonggi-do, Republic of Korea) for 1 h at 15–25 °C and then incubated overnight with specific antibodies (1:1000); phospho-CREB (Ser133) (87G3) rabbit monoclonal antibody (#9198; Cell Signaling Technology, Danvers, MA, USA), CREB (48H2) rabbit monoclonal antibody (#9197; Cell Signaling Technology), phospho-Akt (Ser473) rabbit polyclonal antibody (#9271; Cell Signaling Technology), Akt rabbit polyclonal antibody (#9272; Cell Signaling

Technology), phospho-p44/42 MAPK (Erk1/2) (Thr202/Thr204) rabbit polyclonal antibody (#9101; Cell Signaling Technology), p44/42 MAPK (Erk1/2) rabbit polyclonal antibody (#9102; Cell Signaling Technology), TPO rabbit polyclonal antibody (ab203057; Abcam, Cambridge, MA, USA), proliferating cell nuclear antigen (PCNA) (F-2) mouse monoclonal antibody (sc-25280; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), and β-actin (C4) mouse monoclonal antibody (sc-47778; Santa Cruz Biotechnology, Inc.) in TBS-T containing 1% BSA with gentle shaking at 4 °C. The secondary antibodies used (1:10,000) were HRP-conjugated goat anti-rabbit (sc-2004) or mouse IgG (sc-2005; Santa Cruz Biotechnology, Inc.), and signals were detected using the Clarity Western ECL Substrate (Bio-Rad Laboratories, Inc.). The band density was measured using ImageJ software (version 1.52, USA) and the relative intensities of bands were normalized to β-actin.

2.7. Statistical Analysis

Statistical analysis was performed using Prism software (version 7.0; GraphPad Software Inc., San Diego, CA, USA). Values are presented as mean ± standard deviation (SD). The statistical significance of group differences was determined using two-tailed *t*-tests, and $p < 0.05$ was considered statistically significant. Each experiment was performed in triplicate.

3. Results

3.1. Aloe vera Affects Thyroxine Levels Released form FRTL-5 Cells

Thyroxine levels in cell culture supernatants were released from FRTL-5 cells after treatment with AV (Figure 1). To determine whether the presence or absence of TSH is correlated with altered secretion of thyroxine, thyroxine levels were measured using an ELISA kit with (6H) and without TSH (5H). Thyroxine release was higher in 5H media than in the control (Figure 1A), whereas it was lower in 6H media than in the control (Figure 1B).

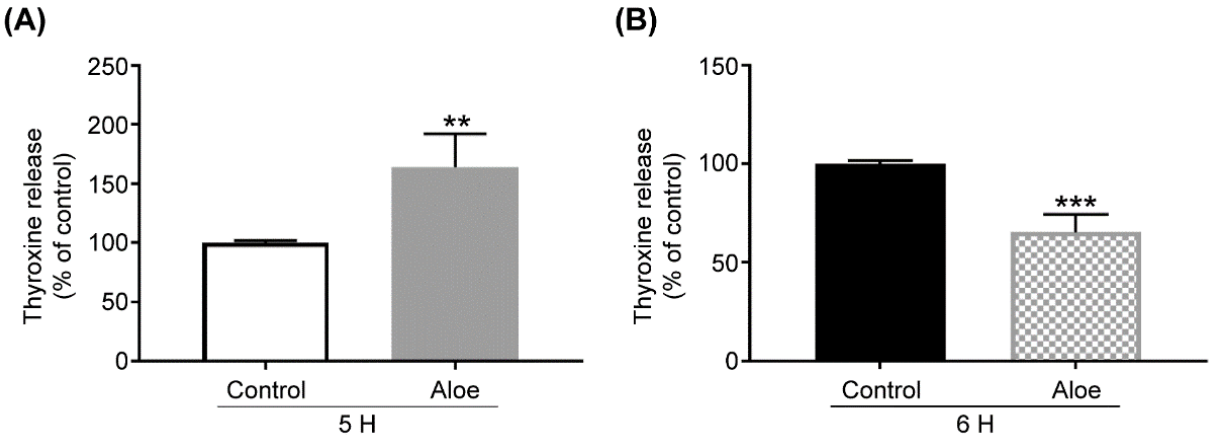


Figure 1. Effects of Aloe (AV) on the regulation of thyroxine release in FRTL-5 cells. Cells were treated with 10 μg/mL AV in 5H (**A**) and 6H (**B**) media conditions, and thyroxine levels released into culture media were measured. Data were represented as means ± SD ($n = 3$); ** $p < 0.01$, *** $p < 0.001$ versus control.

3.2. Aloe vera Does Not Affect Cell Proliferation

We investigated whether cell numbers were related to AV-regulated thyroxine release. The results of cell proliferation and PCNA protein expression, which are events associated with the control of eukaryotic DNA replication, showed that AV did not affect cell proliferation in both 5H and 6H media conditions (Figure 2).

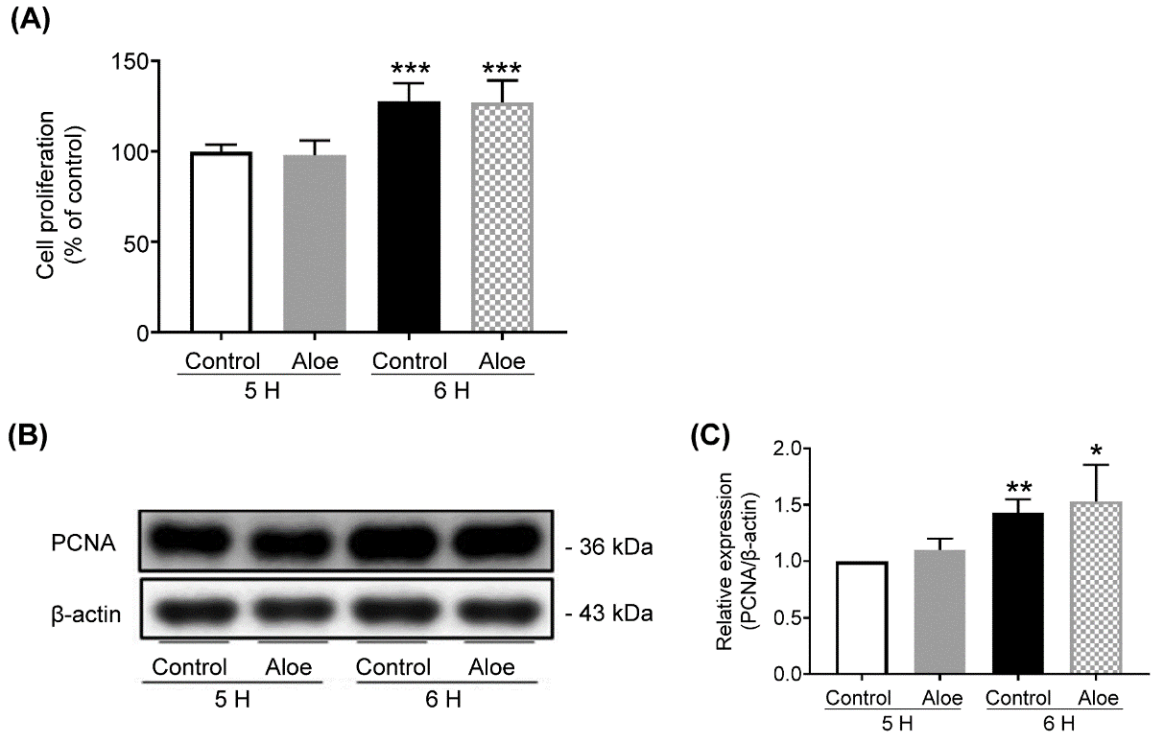


Figure 2. Effects of Aloe (AV) on the cell proliferation and PCNA protein expression in FRTL-5 cells. Cells were treated with 10 μg/mL AV in both 5H and 6H media conditions. Cell proliferation (**A**) and PCNA protein expression were measured, and densitometric analysis of PCNA was performed (**B**,**C**). Data were represented as means ± SD (n = 3); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus 5H control.

3.3. Aloe vera Regulates TPO Expression at the Transcription and Protein Levels

Because thyroid-specific genes such as NIS, TG, and TPO are related to thyroxine synthesis and release, we next investigated the effects of AV on the mRNA expression of these genes. AV did not affect the mRNA expression of NIS and TG in both 5H and 6H media (Figure 3A,B). However, the mRNA expression of TPO was slightly increased in 5H media and significantly decreased in 6H media (Figure 3C). Similarly, TPO protein expression was increased in 5H media and decreased in 6H media (Figure 3D,E).

3.4. Aloe vera Affects the Protein Expression of Phosphorylated ERK and Phosphorylated CREB

We also investigated the molecular mechanism by which AV mediates the expression of signal molecules. The PKA, PKC, and Akt signaling pathways are pivotal in regulating thyroid function [15]. We hypothesized that AV -induced regulation of thyroxine release initiates signaling events that lead to the activation or inactivation of molecules related to PKA, PKC, or Akt signaling pathways. Figure 4A,B shows that AV promoted ERK activation in 5H media and inactivation in 6H media. *Aloe vera* did not activate CREB phosphorylation significantly in 5H, but decreased phosphorylation in 6H media (Figure 4A,D). In contrast, AV did not affect Akt activation in either media (Figure 4A,C).

3.5. Aloe vera Affects ERK Phosphorylation via PKA Not PKC

To investigate the upstream factor involved in AV-induced ERK phosphorylation in 5H media, we inhibited PKA and PKC activation using H89 and GF 109203X inhibitors, respectively. We also measured the protein expression of phosphorylated CREB and phosphorylated ERK. We found that the inhibition of PKA attenuated the phosphorylation of CREB at both basal levels and in the AV treatment group (Figure 5A,B). In addition, H89 inhibited AV -induced phosphorylation of ERK (Figure 5A,C). Inhibition of PKC attenuated phosphorylation of CREB at both basal levels and in the AV treatment group, but not that of ERK (Figure 5D–F).

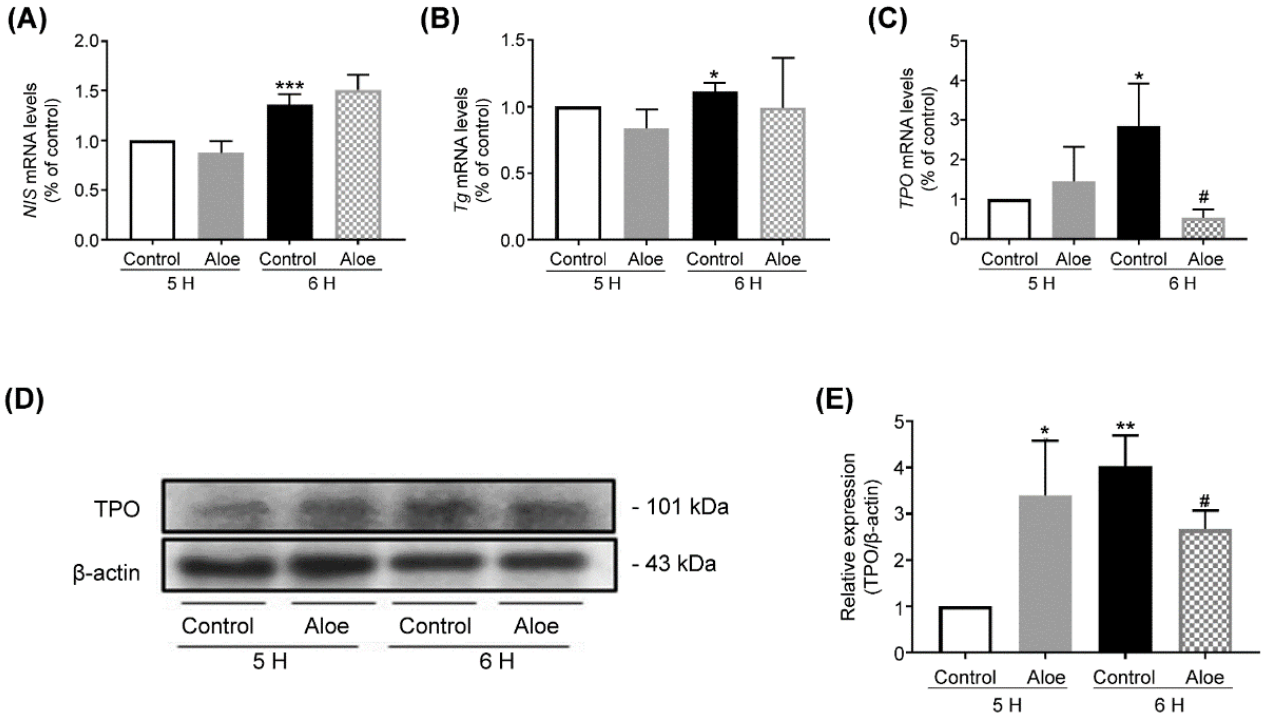


Figure 3. Effects of Aloe (AV) on the expression of factors involved in thyroid hormone synthesis and release by mRNA and protein level in FRTL-5 cells. (**A**–**C**) Cells were treated with 10 μg/mL AV in both 5H and 6H media conditions, and mRNA expression of NIS, TG, and TPO were measured. * $p < 0.05$, *** $p < 0.001$ versus 5H control; # $p < 0.05$ versus 6H control. (**D**) Cells were treated with 10 μg/mL AV in both 5H and 6H media conditions, and protein expression of TPO were measured. (**E**) Densitometric analysis of TPO. Data were represented as means ± SD ($n = 3$); * $p < 0.05$, ** $p < 0.01$ versus 5H control; # $p < 0.05$ versus 6H control.

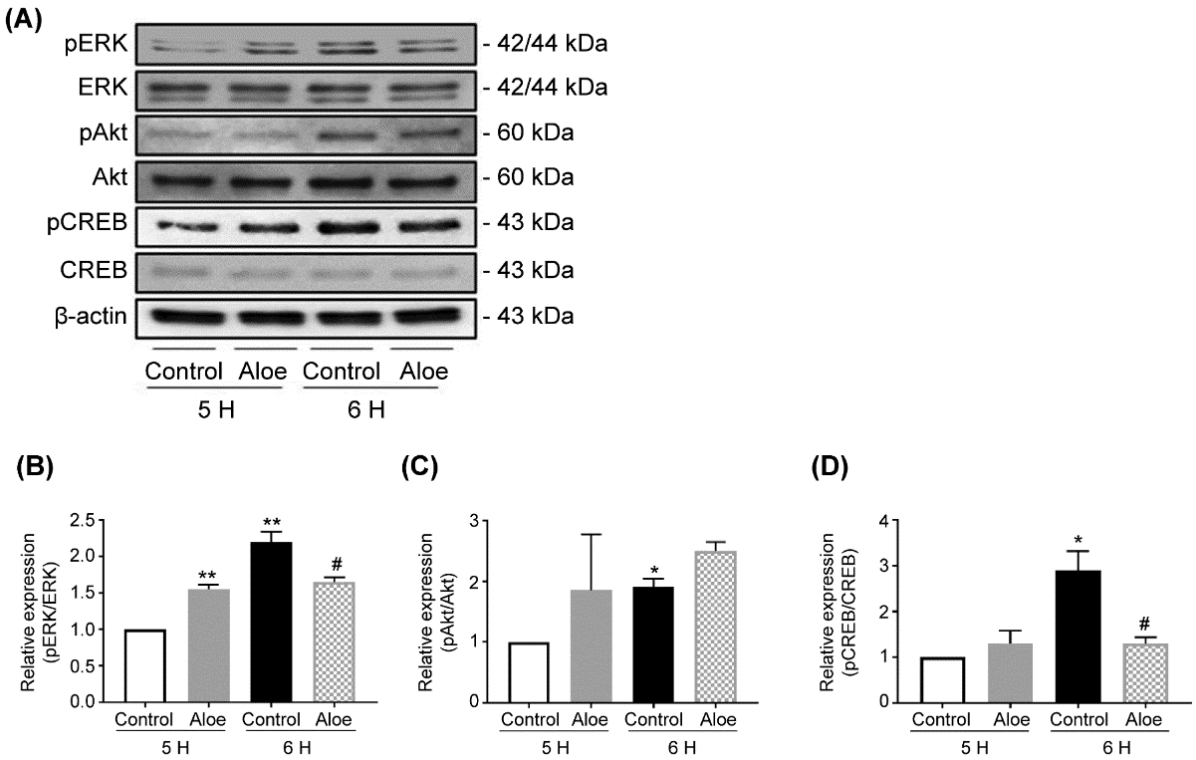


Figure 4. Effects of Aloe (AV) on the phosphorylation of ERK, CREB, and Akt. (**A**) Cells were treated with 10 μg/mL AV in both 5H and 6H media conditions, and protein expression of phosphorylated ERK,

CREB, and Akt were measured. (**B**–**D**) Densitometric analysis of pERK, pAkt, and pCREB. Data were represented as means ± SD (n = 3); * $p < 0.05$, ** $p < 0.01$ versus 5H control; # $p < 0.05$ versus 6H control.

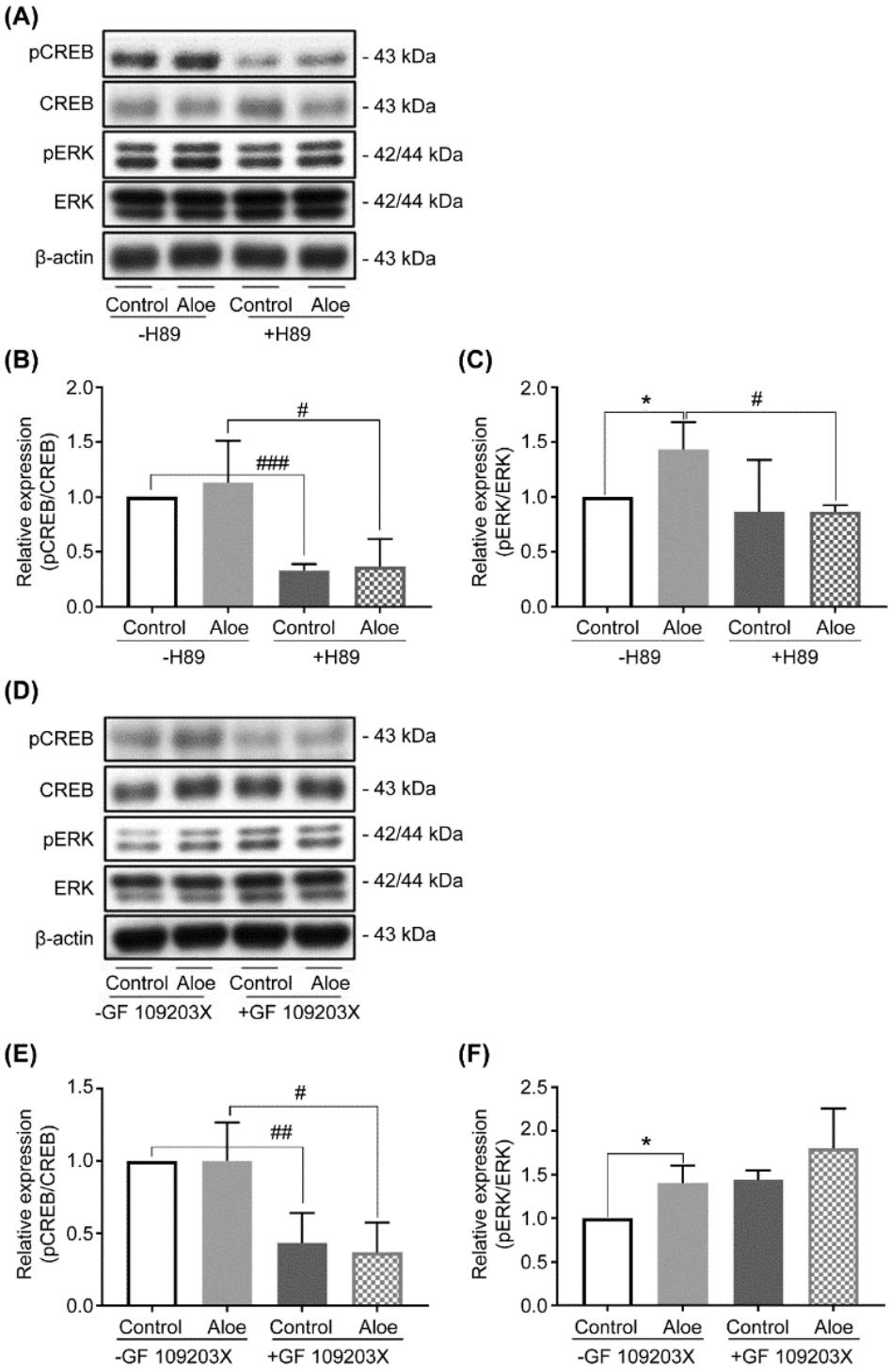


Figure 5. Suppression of Aloe (AV) protein level by PKA inhibitor H89. (**A**) Cells were treated with PKA inhibitor (H89) and 10 μg/mL AV in 5H media conditions and measured protein expression of phosphorylated ERK and CREB. (**B**,**C**) Densitometric analysis of pCREB and pERK. (**D**) Cells were treated with PKC inhibitor (GF 109203X) and 10 μg/mL AV in 5H media conditions and measured protein expression of phosphorylated ERK and CREB. (**E**,**F**) Densitometric analysis of pCREB and pERK. Data were represented as means ± SD (n = 3); * $p < 0.05$, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$.

4. Discussion

We demonstrated that AV regulates thyroid hormone production through the PKA pathway and TPO in FRTL-5 thyroid cells. Maintaining a constant level of hormones is a key factor in maintaining homeostasis in the body. Thyroid hormones are required for normal growth and development, and increase the basal metabolic rate of nearly every cell in the body. Therefore, an imbalance of thyroid hormones, such as through excessive or insufficient production in the body, may cause several symptoms reflecting metabolism.

The production and secretion of thyroid hormones are highly regulated by complicated mechanisms in thyroid follicles located in the thyroid gland. This process involves several processes, including iodine transport into the cells by NIS, TG synthesis, iodination of the tyrosine on TG, conjugation of iodinated tyrosine residues by TPO, endocytosis, proteolysis of colloid containing iodinated TG, and secretion of thyroid hormones [16,17]. Each of these processes is enhanced by the binding of TSH to the TSH receptor. Stimulation of the TSH receptor by TSH activates G protein-coupled receptor effectors and signaling pathways such as the cAMP/PKA/ERK, cAMP/PKA/CREB, PKC/ERK, and PI3K-Akt pathways [15]. Of these pathways, the cAMP mainly plays a role in thyroid follicular cells and modulates the expression of thyroid-specific genes such as NIS, TG, and TPO [18].

In this study, AV increased and decreased thyroxine levels in media without (5H) and with TSH (6H), respectively (Figure 1). The effects of AV were not related to the number of cells (Figure 2); thus, AV may regulate the release of thyroxine in thyroid cells, and could be an effective candidate for affecting the role of TSH. Some studies have reported that herbal medicine can reverse the symptoms of hypothyroidism induced by propylthiouracil (PTU) or L-thyroxine in rats. *Cuscuta Semen* and Insamyangyoung-tang aqueous extracts were shown to cure PTU-induced hypothyroidism in rats [19–22]. Similarly, Yangkyuksanhwa-tang, Palmulgunja-tang, Cheongpyesagan-tang, and Yukwooltang have been shown to effectively reverse hyperthyroidism induced by sodium levothyroxine in rats [21,22]. However, these herbal medicines have effects on either hypo- or hyperthyroidism, but not both, and differ from AV in this respect. In this study, we tested the ability of AV to regulate thyroxine release in two media conditions representing hypo- (5H) and hyperthyroidism (6H media) in FRTL-5 cells which are widely used to study thyroid function [23]. AV grows and is functional in media containing six hormones, including TSH. Therefore, an increase in thyroxine release in 5H media could compensate for the deficiency in thyroxin production, whereas a decrease in thyroxine release in 6H media could mean an excessive reduction in thyroxine production. AV may regulate thyroid function to maintain a constant level of thyroid hormones in the body and is, thus, a potential candidate for thyroid disease therapies. In clinical practice, *Aloe barbadensis* Linne juice has been reported to improve Hashimoto's thyroiditis-related subclinical hypothyroidism [24]. In male mice, AV decreased both triiodothyronine and thyroxine levels and could be effective for the treatment of mild hyperthyroidism [25]. However, there is a need for further research on AV-regulated thyroxine release in both hypothyroidism and hyperthyroidism animal models.

We found that AV affected the mRNA and protein expression of TPO (Figure 3), which is a rate-limiting enzyme in thyroid hormone production and works on the iodination and conjugation of tyrosine on TG. In hyperthyroidism, anti-thyroid drugs such as PTU and methimazole are known to interact with TPO to inhibit its function and decrease thyroid hormone synthesis [26]. It has also been reported that the anti-thyroid mechanisms of many compounds and flavonoids inhibit TPO activity in pigs [27]. AV may have similar characteristics to these chemical substances in terms of targeting TPO function in hyperthyroidism. Conversely, anti-TPO autoantibodies attack the enzyme that synthesizes thyroid hormones, leading to too few thyroid hormones in hypothyroidism [28]. Symptoms of hypothyroidism are treated with hormone replacement therapy using levothyroxine, which is a synthetic thyroxine. It has been reported that *Aloe barbadensis* Linne juice inhibits TPO auto antibodies, and improves thyroid function in subclinical hypothyroidism patients [24].

We suggest that AV can be used to treat hypothyroidism due to its ability to increase TPO protein expression. The ability of AV to increase TPO could be induced by the inhibition of TPO autoantibodies. Thyroid peroxidase could be a key factor in AV-regulated thyroxine release and the presence of tyrosine, the main constituent of the thyroid hormone, in *Aloe barbadensis* leaves could be one of the reasons for its curative effects on hypothyroidism [24].

In thyroid cells, cAMP-mediated signaling pathways that activate PKA and ERK mediate thyroid cell proliferation and function induced by TSH [29]. CREB, a transcription factor, plays important role in controlling the growth and function of FRTL-5 cells [30]. Our observations showed that AV affected ERK phosphorylation in both 5H and 6H media and CREB phosphorylation in 6H media, but not Akt phosphorylation in 5H and 6H media (Figure 4). Akt is also known to play an important role in FRTL-5 cells growth and cell cycle progression [30]. Thus, ERK and CREB, but not Akt, may be related to AV-regulated thyroid hormone. Akt might be related to the proliferation of FRTL-5 cells induced by TSH. We hypothesized that AV did not affect cell proliferation (Figure 2), because there was no change in Akt phosphorylation levels. Results from a few previous studies also suggest that quercetin inhibits cell proliferation by down regulating Akt phosphorylation in FRTL-5 cells [23].

To determine whether AV-induced ERK activation in 5H media is dependent on PKA or PKC, we inhibited PKA and PKC by H89 and GF109203X inhibitors, respectively. Phosphorylation of ERK was inhibited by the H89 inhibitor, but not by the GF109203X inhibitor. These results indicate that AV-induced ERK phosphorylation occurs mainly through cAMP-mediated signaling pathways. AV may induce thyroxine release via the PKA/ERK pathway and TPO (Figure 6A). Moreover, it may reduce thyroxine release by inhibiting the PKA/CREB, PKA/ERK pathways and TPO (Figure 6B).

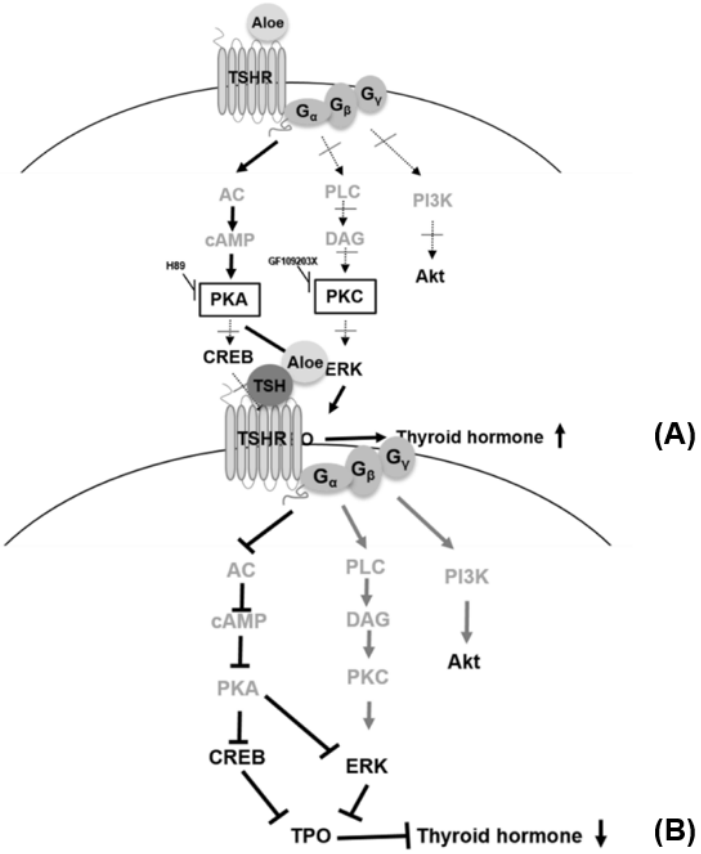


Figure 6. Proposed model showing the pathway by which Aloe (AV) regulate thyroid hormone production in FRTL-5 cells. (**A**) pathways induced by AV in 5H media. AV-induced ERK phosphorylation

occurs mainly through cAMP-mediated signaling pathways and AV may induce thyroxine release via the PKA/ERK pathway. (**B**) pathways induced by AV in 6H media. AV may reduce thyroxine release by inhibiting the PKA/CREB, PKA/ERK pathways and TPO. Abbreviations: cAMP, cyclic adenosine monophosphate; CREB, cAMP response element-binding protein; ERK, extracellular signal-related kinase; PKA, protein kinase A; PKC, protein kinase C; TPO, thyroid peroxidase; TSHR; thyroid-stimulating hormone receptor.

This study shows that AV could potentially act as an alternative therapy for both hypo- and hyperthyroidism. Nevertheless, there is a limitation in vitro that could be addressed in future research. A study focused on the stimulation of the production and release of thyroid hormone following the administration of AV extract should be performed in vivo.

Author Contributions: Conceptualization and design of experiments: B.-S.K.; Conducting of experiments: H.G. and J.-A.R.; Analysis of data: H.G. and J.-A.R.; and Manuscript writing: H.G. and J.-A.R. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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