Letter to the Editor

Hyeon-Ji Lim, Mi-Hee Kim, Kang-Yeol Yu, Kyung-Mook Lim, Kwang-Youll Lee* and Jiyoung Kim*

The extract of male bee and beehive from *Bombus terrestris* has biological efficacies for promoting skin health

https://doi.org/10.1515/tjb-2020-0019
Received January 10, 2020; accepted February 16, 2020; published online April 7, 2020

**Keywords:** beehive; *Bombus terrestris*; extract; male bee; skin health.

To the Editor,

Seventy percent of the animals living on Earth are insects, and the number of insect species reported so far is more than 1.2 million. Insects have been differentiated into so many species as they are today, probably because their adaptability to the environment is superior to that of any other animals. Recently, approaches to edible insects have been attempted as a functional food or to develop new protein sources in the future. On the other hand, insects have been used for medicinal purposes as a traditional medicine for the treatment of various diseases. In recent years, with the development of biotechnology, research on the efficacy of traditional medicines and the exploration of new medical substances have been actively conducted, and the industrial value of insects through this is very high.

*Bombus terrestris* (bumblebee) lives in a beehive as basic units consisting of queen bees, worker bees and male bees. The beehive can be eaten by humans, and its main ingredient is wood, which is made by worker bees chewing and softening. Recently, the excellent nutritional values of the pupa and the larvae from *B. terrestris* (male bees) have begun to be known. Therefore, using the extract of male bees, research on the development of functional foods, pharmaceutical substances and cosmetic raw materials is recognized as a very promising field for the purpose of developing insect industry. To date, little is known about the use of by-products derived from a beekeeping business. The purpose of this study was to find ways to utilize the by-products, such as male bee and beehive, from a beekeeping business. As a part of the purpose, it was investigated whether the extract of male bee and beehive (EMB) from *B. terrestris* has the effects in promoting skin health.

First, EMB was examined whether it has a cytotoxic effect on HaCaT cells. Cytotoxic effect by EMB on HaCaT cells was not observed (Figure 1A). The keratinocytes are important cells that play important roles in maintaining the structure and homeostasis of the epidermis [1]. Since differentiation of keratinocytes is a pivotal process to provide a skin barrier that is well tolerated by the adverse environment, disorders of keratinocyte differentiation are directly linked to skin disease. For example, defects in the skin barrier can result in overly dry skin, which can make skin conditions worse. The end product of keratinocyte differentiation, such as filaggrin, provides a natural moisturizing factor and helps maintain healthy skin. Skin can be moisturized by moisturizing factors such as hyaluronic acid and filaggrin. Hyaluronic acid not only protects skin elasticity by preventing moisture évaporation in the epidermis, but also plays an important role in cell migration, storage and diffusion of nutrients. To determine the moisturizing effect of EMB, we analyzed the levels of *HAS* (hyaluronic acid synthase)-3 and *filaggrin* mRNA induced by EMB in HaCaT cells. As a result, it was confirmed that EMB increases the expression of *HAS*-3 and *filaggrin* as compared with the control (Figure 1B, C). In order to investigate whether EMB promotes hyaluronic acid biosynthesis, hyaluronic acid contents in HaCaT cells were analyzed. As shown in Figure 1D, EMB increased the biosynthesis of hyaluronic acid in HaCaT cells compared with the control. Therefore, EMB is
estimated to be effective in enhancing skin moisturizing efficacy.

Collagen is the main protein in the extracellular space and in the various connective tissues of the body [2]. As the main component of connective tissue, collagen is the most abundant protein in mammals. Collagenase is an endopeptidase that breaks the peptide bonds in collagen [3]. Elastin is a highly elastic protein in the connective tissue of the skin, allowing the skin to retain its shape when numerous body tissues expand or contract [4]. Elastase breaks down elastin and should be suppressed to maintain skin elasticity [5]. EMB was investigated for its elastase and collagenase inhibitory activity. The results revealed that EMB inhibits elastase and collagenase activity (Figure 1E, F). Therefore, EMB is estimated to have biological efficacy in improving skin wrinkles.

In summary, we explored the utilization of EMB, a by-product derived from a beekeeping business. EMB showed various efficacies for prompting skin health. Therefore, EMB can be used a raw material for cosmetics to improve skin health. The experimental methods used in this study are described in the supplement section.

Acknowledgments: This study was supported by the “Leading Company R&D Project” from the Jeonbuk Technopark, South Korea.

Conflict of interest: The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

References


**Supplementary Material**

**Experimental**

**MTT assay in HaCaT cells**

For MTT assay, HaCaT cells were seeded in a 96-well (2 × 10^4 cells/well) treated with different doses of EMB for 24 h. After incubation, the MTT working solution was added to each well. Absorbance at 540 nm was measured using a microplate reader (Thermo).

**qRT-PCR**

qRT-PCR of HAS-3 and filaggrin was performed using HaCaT cells treated with EMB. Quantitative assessment of cDNA was performed by a SYBR Green Master Mix Kit (BioFact). The mRNA levels of HAS-3 and filaggrin were relatively assessed using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a housekeeping gene. The primer pairs (sense/antisense) for HAS-3 were 5′-CCC AGC CAG ATT TGT TGA-3′ and 5′-AGT GGT CAC GGG TTT CTT CC-3′; for filaggrin were 5′-AAG CTT CAT GGT GAT GCG AC-3′ and 5′-TCA AGC AGA AGA GGA AGG CA-3′; for GAPDH were 5′-ATT GTT GCC ATC AAT GAC CC-3′ and 5′-AGT AGA GGC AGG GAT GAT GT-3′. qRT-PCR was performed using the real-time PCR system (BioRad). Results were expressed as a fold change relative to the control.

**Measurement of hyaluronic acid production**

HaCaT cells were seeded in a 6-well plate (1 × 10^6 cells/well) and treated with EMB for 24 h. After incubation, the cell supernatant was collected and performed by the Hyaluronic acid (HA) Test kit (R&D System) according to the manufacturer’s instruction using a microplate reader (Thermo).

**Elastase and collagenase inhibition activity assay**

Elastase and collagenase inhibition activity assay was performed using EnzChek Elastase and Collagenase Assay Kit (Molecular Probes) following the manufacturer’s instruction.