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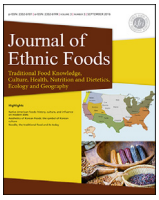

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# Function of Korean black ginseng: Improvement of andropause symptoms by a complex extract of black ginseng and fenugreek in TM3 Leydig cells and aged rats

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ABSTRACT

*Background:* Korean ginseng (*Panax ginseng* C.A. Meyer), the native ginseng of Korea, has traditionally been widely used for thousands of years in Korea. Various biological functions of Korean ginseng have been reported. However, there have not been many studies on its effect on andropause. As women reach menopause, they show a sudden decrease in female hormone levels; men also undergo similar endocrinologic changes. Andropause is also referred to as late-onset hypogonadism, male hormone deficiency syndrome, and male climacteric syndrome. It is linked to increasing age among men and serum testosterone deficiency and specific clinical and biochemical symptoms that accompany it. To improve such andropause symptoms, male hormone supplementation therapy has been attempted. However, owing to various adverse effects, the development of improved therapeutic agents that are safe, both psychologically and physically, is in demand. The present study examined the andropause symptom-alleviating effects of black ginseng and fenugreek complex extract to develop a natural therapeutic agent with minimal adverse effects.
*Methods:* In the present study, we measured the anti-oxidant effect of black ginseng and fenugreek complex extracts using Cell viability of TM3 mouse leydig cells. The mechanism of apoptosis was assessed Erk and Akt kinase activity. We also investigated whether these complex extracts could affect on male hormone and muscle endurance. After administrating black ginseng and fenugreek complex extracts for 4 weeks. The aged rats were sacrificed and checked the testosterone hormone and forced swimming test.
*Results:* The extract increased cell viability, which had been reduced after oxidative stress, confirming the antioxidant effects of the complex, and this effect involved regulation of Erk kinase activation. Moreover, in a hormone-deficient animal model, after oral administration of the complex extract, the group that received 100 mg/kg showed significantly increased total and bioavailable testosterone levels. Besides the levels of sex hormones, those of luteinizing hormone and follicle-stimulating hormone that promote spermatogenesis were also elevated in a forced swimming test for verification of muscle endurance and motor functions, the group that received 100 mg/kg of the complex extract showed improved motor function and increased muscle endurance.
*Conclusion:* Thus, it is believed that the complex extract of black ginseng and fenugreek may be used in the future as a preventive and therapeutic agent for alleviating the symptoms of andropause.
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## 1. Introduction

Korea ginseng (*Panax ginseng* C.A. Meyer), the native ginseng of Korea, has traditionally been widely used for thousands of years in Korea, as well as China and Japan, as an *elixir* mostly for promoting good health. Currently, the outstanding efficacies of ginseng and its components are also being reported. However, studies on overall aspects of ginseng, including its history of origin, are still lacking. The present study aims to take a brief look at ginseng and black ginseng, one of its processed products. We believe that an additional comprehensive review of ginseng,

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its processed products, and fenugreek will be needed in the future.

### 1.1. History of Korean ginseng

For over 2,000 years, Korean ginseng (*P. ginseng* C.A. Meyer), a native plant of Korea, has been used in Northeast Asia as an important medicinal herbs for protecting vitality. In Shen-Nung Pharmacopeia (神農本草經), the oldest herbal pharmacopeia in Asia, it is written that ginseng protects the five viscera and supplements vitality [1]. Ginseng has the scientific name of *Panax ginseng* C.A. Meyer (1843), and, looking at its word origin, *pan* means all and *axos* means medicine, which combined gives the meaning *panacea*. The first record of ginseng being discovered by an alchemist as a tonic or elixir is in a Chinese book from the pre-Han era (BCE 33–48), about 2,000 years ago. In Hanwon (翰苑), there is a record of ginseng being found in Dah-Jang Mountain (長多山) in Koryo (probably Gae Jang Mountain) and record of "Koryo ginseng packaging and transport method…" is found in Gookjungbaekrok (國定百錄). Moreover, Myungibyugok (名義別錄) (456–536) contains a record of Baekje (白帝) ginseng being gifted as a tribute when Song (梁) was the emperor (武帝).

More than that, considering that there were frequent travels between Goguryeo (高句麗) and Cao Wei (魏; CE 220–265), and that there were 92 records of Goguryeo's bringing tribute (儅使朝貢), it can be surmised that the ginseng from Goguryeo was also gifted as tribute [2]. The first record of such ginseng being cultivated can be found in *Dohkyungchobon* (圖經草本, CE 1061), which was written during the time of Song (梁) of China. Furthermore, there are records of it in Sanrimgyeongje (山林經濟) by Hong of Joseon dynasty as well, while the first record of *gah-sam*, which was the term given to ginseng cultivated in Korea, was in the Annals of the Joseon Dynasty, the Veritable Record of King Chongjo [3,4]. In these records, cultivation of Korean ginseng began as early as CE 1000 or so during the Koryo Dynasty, and sharing of ginseng cultivation technology began as writing and distribution of books happened throughout the King Sejong (CE 1419–1450) and King Sukjong (CE 1675–1720) periods during the Joseon Dynasty, when the expression *san-sam* (wild ginseng) was used [5]. Such ginseng is cultivated not only in Korea, but also in China, Northeast Asia, and far eastern regions of Russia. In addition to Korean ginseng (*P. ginseng* C.A. Meyer), there is also American ginseng (western ginseng, *hwa-gi-sam*), *Panax quinquefolius* L., which are being cultivated in the USA and Canada, as well as China in recent times. *Panax notoginseng* (Burk.) F.H. Chen cultivated in China and *Panax japonicus* D.A. Meyer cultivated mainly in Japan are being used commercially.

### 1.2. Types of Korean ginseng

Types of ginseng can be categorized largely into fresh ginseng in its natural form and its processed forms: white, red, and black ginseng. White ginseng can be subcategorized as straight, curved, and semicurved ginseng, depending on its dried form, and the names refer to the ginseng processing methods that involve drying by heat or sunlight. Red ginseng can be subcategorized as heaven-, earth-, and good-grade ginseng, which refer to ginseng that has been dried after being steam cooked or by other methods using raw ginseng, or fresh ginseng with its skin retained. These ginseng processing methods increase ginsenoside content in ginseng and thus such processing methods are being studied. Particularly in Korea, red ginseng has been developed into a variety of products, such as candy, jelly, powder, and extract, which are being enjoyed by not only Koreans but also people worldwide, and, as such, studies related to its processing are being actively pursued. In addition to red ginseng processing methods, ginseng processed by steaming it nine times, then drying it nine times to take on an exterior color close to black is referred to as black ginseng, which has been reported to have increased new ginsenosides. Unlike active studying of red ginseng, the effects of black ginseng are being studied gradually, while the known effects of black ginseng include anticancer, antithrombin, and immune boosting effects. However, there have not been many studies on its effect on andropause.

### 1.3. Fenugreek (Trigonella foenum-graecum L.)

Fenugreek, also used in the present study, is an annual plant belonging to the Fabaceae family, which reaches a height of 0.7–1 m and has pod-shaped fruit. Its leaves have a nutty scent, and it is rich in carotene and consumed as a vegetable. Throughout history, it has been used as a medicinal plant, especially its seeds, in China, India, and Egypt, and has been used as a medicinal ingredient and spice in curries, while it is known to have a bitter taste, its warm temperament is believed to help protect vitality [6]. Pharmacologically, fenugreek is known to possess a variety of effects, such as antidiabetic and anticholesterolemic. However, even though a recent study reported it being effective against male infertility, there have been no studies on its alleviating effects on andropause symptoms [7,8].

Accordingly, the present study aimed to investigate what effects a complex extract comprised of black ginseng and fenugreek (BF) has on andropause.

### 1.4. Andropause symptoms

It is known that each year, men experience decreases in total and free testosterone by 0.4% and 1.2%, respectively, and such decrease in male hormone levels has an impact on sexual function, bone metabolism, muscle and body fat distribution, mood, and cognitive function. As such, decrease in male hormones associated with aging is referred to as partial androgen deficiency in the aging male. Moreover, various symptoms that fall under the category of andropause symptoms caused by decreased sex hormone level are collectively referred to as late onset hypogonadism in men. Typical symptoms include clinical and biochemical manifestations such as nervousness, emotional instability, depression, vertigo, sleep disorder, hypodynamia, memory impairment, diminished work performance, hyposexuality, and reduced muscle strength [9,10].

Andropause symptoms can cause significant damage to quality of life and have negative impacts on various bodily functions [11].

Various therapeutic modalities are being studied to alleviate and treat such andropause symptoms, and the most commonly used therapy is a male hormone replacement method using oral medications, injections, and transdermal agents. However, caution should be exercised when using hormone replacement therapy, and such therapy should only be administered based on accurate diagnosis since it may result in various adverse effects, such as enlarged prostate, growth in prostate cancer tumor size, exacerbation of sleep apnea, gynecomastia, polycythemia, and increased risk of cardiovascular diseases [12]. Therefore, it is believed that if treatments to increase testosterone hormone levels can be developed from foods and natural products, then the adverse effects can be prevented and alleviated in the treatment of andropause. Accordingly, there is an urgent need to develop foods and natural products as therapeutic agents with fewer adverse effects that can prevent and improve andropause symptoms.

In the present study, BF were used to produce the complex extract of BF with an optimal ratio of the two for investigating their efficacy in alleviating andropause symptoms. As a result, the

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