Effect of *Phellinus linteus* water extract on benign prostatic hyperplasia

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**Abstract**

Benign prostatic hyperplasia (BPH) is one of the most common diseases among elderly men. As the old-age population is increasing recently, it is to our interest to observe the growing BPH within them. In BPH, the dihydrotestosterone (DHT) acts as promotes prostate growth. It inhibits enzyme 5α-reductase that is involved in the conversion of testosterone to the DHT activity which reduces the excessive prostate growth. Through experiments, the effects of *Phellinus linteus* water extract performed on the BPH rats were induced by testosterone treatments. For 12 weeks, Sprague-Dawley rats were treated with testosterone for the induction of BPH. Rats were divided into four experimental groups: the not treated group (N), the testosterone injection and D.W treatment group (TN), the testosterone injection and *Phellinus linteus* treatment group (TP) and testosterone injection and finasteride treatment group (TF). Prostate weight, volume and weight ratio in the TP group and the TF group were significantly lower than the TN group. Testosterone and DHT levels in the TN group were significantly higher than that of the N group. And the TP group was significantly decreased than that of the TN group. While prostates of control rats revealed severe acinar gland atrophy and stromal proliferation; the TP and TF groups showed atrophic symptoms and were lined by flattened epithelial cells, thus, the stromal proliferation is relatively low as compared to the TN group. These suggest that *Phellinus linteus* water extracts may be an useful remedy for treating the benign prostatic hyperplasia.

**Key Words:** *Phellinus linteus*, benign prostatic hyperplasia, acne symptoms, hair growth, dihydrotestosterone

**Introduction**

Benign prostatic hyperplasia (BPH) is a common urological disorder in men. Its prevalence increases with age and may affect every 3 out of 4 men in their sixties [1-3]. As the main endocrine changes, sexual hormones and aging act as elements of enlarged prostate [4]. Recently, there has been a continuous increase in the number of patients with benign prostatic hyperplasia in South Korea due to an increase in the number of old-aged people, and thus, prostate has emerged as important medical concerns [5].

The molecular mechanism of benign prostatic hyperplasia has not yet been clearly revealed. However, the hypertrophy of prostate, caused by excessive dihydrotestosterone (DHT) is estimated as the mechanism that oversupplies testosterone in blood, and leads to vast amount of DHT synthesis via the action of 5α-reductase in the prostate; the synthesized DHT combines with androgen receptor with consequent generation of benign prostatic hyperplasia [6,7]. Furthermore, secretion from androgen of prostate cells decreases as the male patients become old while the receptors of prostate cells increase to balance endocrine system as DHT is combined with other parts [8]. This process is estimated to be related to the increased histological, ocular and clinical prevalence rate cases due to an increase [9,10] in age, which leads to cell hyper-plasia and changes in tissues. In other words, the level of oxidative stress increases with an increase in age. If the antioxidant system inside the body cannot protect the body from oxidative damages [9], the senile change occurs in the prostate with consequent developments of benign prostatic hyperplasia.

*Phellinus linteus*, also called as *Phellinus baumii*, is a white-colored rotting fungi which belongs to the hymenochaetaceae family, and is grown or planted in China, Cambodia and Japan. *Phellinus linteus* contains various nutrients including saccharides, proteins, vitamins, minerals and large amounts of β-glucan. Recently, *Phellinus linteus* has been used as health food and medicine for cancer treatment [11-13]. Currently, there are ongoing researches on benign prostatic hyperplasia in Korea and other countries. The antioxidant [14,15], anticancer and immunopotentiation [16,17] effects of various functional mushrooms which contain large amounts of psychological active substances are being studied actively. However, sufficient research on benign prostatic hyperplasia is still lacking. Hence, in this regard, the present study is conducted to verify the stability and efficacy of *Phellinus linteus* on benign prostatic hyperplasia. By doing so, rats with such symptoms generated by injecting testosterone were being used to verify the efficacy of *Phellinus linteus*. 

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Materials and Methods

Preparation of water extracts from Phellinus linteus

For this experiment, *Phellinus linteus*, which was cultivated in Kumhwang Bio, located in Sangok-ri, Munsan-eup, and Jinju-si, Korea, was purchased and it was used as experimental material by extraction and concentration after drying. After confirming whether the extraneous material was mixed, material was rinsed. After weighing *Phellinus linteus*, the first extraction after 12 hours was conducted at 95°C and adding water to generate 20 times more than the material weight. The second extraction was conducted 12 hours later at 95°C by adding five times more water than the material weight. After mixing the first and the second extracts, filtration was performed, and the *Phellinus linteus* extracts were obtained through 95°C sterilization with a concentrator. The β-glucan content of *Phellinus linteus*, used in this experiment, was 6 g per 100 g in the fruiting body of *Phellinus linteus*.

Manufacturing of the animal model of BPH by the administration of testosterone to aged rats

The hypothesis that human Benign Prostatic Hyperplasia occurs due to aging or male hormone excess is dominant. Therefore, based on this hypothesis, this experiment divides groups into the aged group, the testosterone intervention group, the administration group of controlled drugs, and the administration group of olfactory materials by using age rates of over 12 weeks as test models of Benign Prostatic Hyperplasia under the judgment that this group division shall be effective in reducing prostate at the appropriate levels under the influence of drugs. Accordingly, in this experiment, the model of Benign Prostatic Hyperplasia caused by aging or the excess of testosterone was manufactured by giving subcutaneous injections with testosterone of 1.5 mg/corn oil 0.1 ml/kg for 30 days, after diluting testosterone of male Sprague-Dawley rats which reached 12 weeks and also completed the acclimatization period of the corn oil. The research was performed in accordance with the guidelines established by the International University of Korea Institutional Animal Care and Use Committee (IUK 2011-06).

The allocation of experimental groups and experimental methods

Ten rats were assigned to each group and the allocation of groups was divided into normal group, control group, the administration group of *Phellinus linteus*, and positive control group. Subcutaneous injection was given to the normal group with 0.1 ml/kg, and oral administration was made only with physiological saline of 5 ml/kg. While subcutaneous injection was given to the administration group of *Phellinus linteus* for 30 days with the mixture of testosterone, oral administration was made by using zonde one time per day with 1.725 ml/kg of the *Phellinus linteus* extract. In terms of the finasteride administration group, which is the positive control group, subcutaneous injections of testosterone were given for 30 days, oral administration was also made one time per day by using zonde with 1 ml/kg by suspending finasteride to physiological saline.

Body weight measurement

The first weight measurement was made on the commencement date of the experiment, and again in the last sampling, which was the closing date of the experiment. For weight measurements, an electronic scale was used for all rats before their morning feed. In order to minimize weight errors for weight movements, rats were placed into a plastic bowl where their weight could be recorded stably.

The measurement of prostate ratio according to volume and weight of the prostate

The day following the last administration, body weight measurements and blood samplings were made. When sacrificing a rat, fat or foreign materials around the prostate were removed to measure the maximum and minimum lengths of the prostate horizontally and vertically. The volume of the prostate was calculated according to the formula: Prostate volume (cm³): 1/2 (a × b²) (a, longer dimension; b, shorter dimension), while the size of the prostate was calculated with prostate weight ratio (mg/100 g of BW) = prostate weight (mg) × 100 (g)/Body weight (g). The weight of the prostate was measured by using the electronic scale after measuring the size of the prostate and after removing the moisture around the prostate.

Hematologic analysis

When measuring hematologic testosterone, blood tests were taken from the hearts of rats on the last day of the experiment. Configuration of blood was used at 3,000 rpm for 20 minutes and testosterone was measured by using the testosterone EIA kit (Cayman chemical company, USA) after storing at -40°C through isolating serum. DHT concentrations were measured with BMG LABTECH EIA reader, which is the product of the FLUO star OPTIMA by using the rat dihydrotestosterone EIA kit (cusabio, USA).

Histological changes of the prostate

After fixing each prostate into the 10% neutral buffered formalin solution, a paraffin block was made after dehydration by inserting ethanol of 60%, 80%, 95%, 100% respectively. Thereafter, each prostate tissue was sliced with the thickness of 4 μm by using microtome and it was attached to the gelatin-coated slide. To dye the tissue section, paraffin was removed by immersing each prostate into xylene and rehydration was