BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902019000418785

Article

Effect of rape flower on benign prostatic hyperplasia in rats

Song-Chan Han*, Young-Hak Yun

Department of Pathophysiology, Pyongyang Medical College, Kim Il Sung University, Taesong District, Pyongyang, Democratic People's Republic of Korea

We were carried out to investigate the efficacy of Rape (Rapeseed, *Brassica napus* L.) flower on BPH (benign prostatic hyperplasia) in rats. We found that the extract from Rape flower prevented hyperplasia in testosterone-induced BPH model, the relevant animal model of human BPH. Extract reduced the weight of prostate and induced significantly cell apoptosis in prostate in BPH model. In addition, the extract controlled expression of TGF-β1 in prostate gland and promoted urinary output in dose-dependence in BPH model. Our data provide that Rape flower may be useful for treatment of BPH.

Keywords: Rape flower. Rapeseed. BPH. LUTS. TGF-β1.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is one of the most common disease in men. All around the world BPH and associated lower urinary tract symptoms (LUTS) are common clinical problems in urology. This pathology affects 50% of men over the age 50 and 90% of men over the age 80. BPH is associated with increased incidence of prostate cancer (Orsted, Bojesen, 2013). Current treatments for BPH are based on three main strategies: inhibition of 5-α-reductase (5αR), attenuation of gonadotropin-releasing hormone, and blocking of α-adrenoreceptors. Recently, Inhibitors of 5-α-reductase such as Finasteride suppress conversion of testosterone into a more potent metabolite, 5α-dihydrotestosterone (DHT) and become the considerable and standard therapy of BPH (Lepor, 2006). These improve lower urinary tract symptoms by less than 35-50% and cause some side effects. Unlike these chemical medicines, herbal medicines are very useful for treatment of a lot of diseases and have no obvious side effects.

Rapeseed (*Brassica napus* L.), also known as rape, oilseed rape, rapa and rappi, is a bright yellow flowering member of the family Brassicaceae (mustard or cabbage family). *Brassica napus* is cultivated mainly for its oil-rich seed, the third largest source of vegetable oil in the world (OED Online, 2012).

Rapeseed is grown for the production of animal feed, vegetable oil for human consumption, and biodiesel; leading producers include the European Union, Canada, the United States, Australia, China and India.

Korea has long history of Rapeseed cultivation. Rapeseed (*Brassica napus* L.) is also cultivated for the economic benefits such as oil-rich seed and animal feed in our country.

Few studies have reported biological components of Rape pollen for the treatment of some diseases (Wu, Lou, 2007; Han *et al.*, 2007) but there has been no research to use its flower as herbal medicine for the treatment of BPH.

***Correspondence:** S.-C. Han. Department of Pathophysiology. Pyongyang Medical College. Kim II Sung University. Taesong District, Pyongyang. Democratic People's Republic of Korea. Email: pmed5@ryongnamsan.edu.kp (Song-Chan Han)

MATERIAL AND METHODS

Animals

Male adult Wistar rats (11-12 week old, 250~300g) were provided by Laboratory Animal Centre of Pyongyang Medical College, Kim Il Sung University. All rats were housed with regular diet for two weeks before the experiment.

Preparation of the ethanol extract from Rape flowers

Rape (Rapeseed, *Brassica napus* L.) flowers collected from the suburban area of Pyongyang, DPR of Korea were authenticated by National botanical institute of DPR of Korea and extracted by Traditional Medicine Centre of Pyongyang Medical College, Kim Il Sung University.

The extract used in the present study was prepared using the traditional ethanolic method. Briefly, 3 kg of dry flowers including pollen were immersed in 3 L of 90% ethanol with intermittent shaking for 24 h, and then refluxed for 3 h by heating. The filtrate was evaporated below 45 °C under reduced pressure. The residue (yield: 9.1%) was designated as an alcoholic extract. The extract was quantified by a HPLC assay to contain main components: total flavonoid at 9.4%.

Model of BPH

Rat model of BPH was based on prostate overstimulation by subcutaneous injection of testosterone propionate (10 mg/kg, daily, for 30 days) after castration. (Geller *et al*., 1969; Van *et al*., 2001).

Experimental design

Animals were divided into the following groups as Control, BPH model, Extract and Finasteride. Each group consisted of 9 rats. On the 7th day of testosterone injection, animals were given either extract of Rape flowers (***Extract group***) or Finasteride (***Finasteride group***) or left untreated (***model***). Rats of Extract group were orally administrated with extract at daily doses of 30 mg/kg and 60 mg/kg, respectively. Rats of Finasteride group were orally administrated with finasteride (10 mg/kg) daily (Cayatte *et al*., 2006). All rats were sacrificed by decapitation on the 31st day of the experiment.

Assessments

Relative weight of the ventral lobe: The ventral lobes of prostate were collected by dissection and lobes weight (mg) divided by total body weight (g).

The positive rate of cell apoptosis in prostate was evaluated by flow-cytometry(FCM) analysis as percentage (%).

Total RNA was isolated from samples of prostate tissus by using acid guanidium thiocyanate-phenol-chloroform (AGPC) protocol (Kingston, 1995; Chomczynski, Sacchi, 1987) and oligo (dT) 16 primer (Stahlberg *et al*., 2004) was used in the reverse transcription reaction. mRNA expression levels of SRD5A2 (5-α-steroid reductase type 2), IGF-1 and TGF-β1 genes in prostate cell was examined by the reverse transcription-polymerase chain reaction (RT-PCR) assay (GAPDH gene, internal control). Expression ratio (target gene/GAPDH) was used to evaluate gene expression.

24 h urine amount was measured by using urine collection box for rats.

Statistical analysis

All results are expressed as mean±SE. Differences between other groups were tested by using analysis of variance (ANOVA). Where significant effects were found, T-test was performed. $p < 0.05$ and $p < 0.01$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Benign prostatic hyperplasia (BPH) also commonly called benign prostatic hypertrophy can be described clinically or pathologically. Clinical BPH is commonly viewed as benign enlargement of the prostate, which contributes to an array of urinary voiding difficulties that can range from bothersome to significantly impacting quality of life among older men (Roehrborn, 2011). Pathologic BPH is the histological determination of non-neoplastic new prostatic growth in adult men. Autopsy studies have revealed that the prevalence of pathologic BPH increases markedly after the 4th decade and is found in up to 90% of men over age 80 (Berry *et al*., 1984). The high prevalence of BPH in older men has led some to consider prostatic hyperplasia to be a ubiquitous result of aging (Ho, Habik, 2011). The precise molecular mechanisms underlying the induction, maintenance, and

development of clinical sequelae resulting from BPH are incompletely understood.

The flowers of Rape cultivated in our country become potential or important source of brassinosteroids including brassinolide and flavonoids, especially Naringenin, Kaempferol and Quercetin.

Here we tested the effects of exract from Rape flowers in rat model of BPH.

Figure 1 shows relative weight of the ventral lobe according to the dose (30, 60 mg/kg) of extract from Rape flowers.

Animal models of BPH are based on prostate overstimulation by sex hormones (Geller *et al.*, 1969; Van *et al.*, 2001). In testosterone-induced model, administration of testosterone causes hyperplasia in ventral lobes of the rat prostate, analogous to morphological changes in human BPH (Scolnik, Servadio, Abramovici, 1994; Altavilla *et al.*, 2012; Rick *et al.*, 2013).

As positive control, Finasteride prevented ventral lobe enlargement in prostate but not Extract (Figure 1).

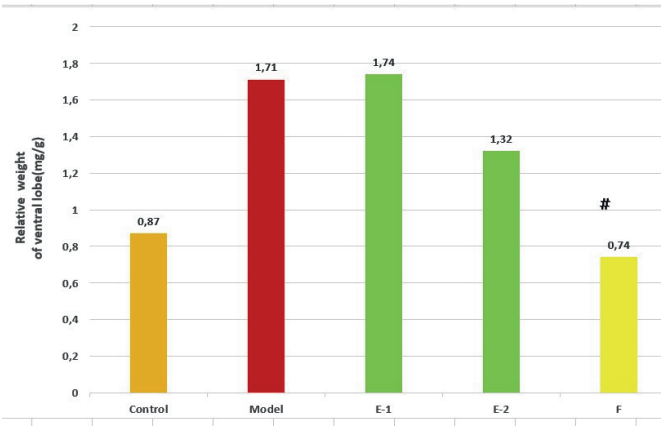

FIGURE 1 - Relative weight of the ventral lobe. Model: BPH, E-1: Extract-30 mg/kg, E-2: Extract-60 mg/kg, F: Finasteride-10mg/kg. The data were stated as mean ± SE. Superscript sign shows significant difference: #–from Model, #p < 0.05.

The mechanisms of BPH are multifactorial and are not yet recognized in the all details. Cell growth in the normal prostate is regulated by a delicate balance between cell death and cell proliferation. Disruption of the molecular mechanisms that regulate these processes may underline the abnormal growth of the prostate. In BPH, the imbalance of cell proliferation and programmed cell death (apoptosis) leads to continuous stromal growth. Common medication interrupts stromal cell proliferation but has only little effect on inducing stromal cell apoptosis.

In present study, we investigated the extract from Rape flowers for its ability to induce cell apoptosis of prostate in BPH model. After administration with different doses of Extract (30, 60 mg/kg), the positive rate of cell apoptosis in the prostate was measured using FCM analysis. Figure 2 shows the effects on the positive rate of cell apoptosis in the prostate.

In Figure 2, the positive rate of cell apoptosis in Extract group with 60 mg/kg and Finasteride group increased significantly compared with Model group. But extract at dose of 30 mg/kg had no effect on cell apoptosis induction in prostate enlargement ($P > 0.5$). Finasteride is now being used to treat benign prostatic hyperplasia (BPH). Inhibitors of 5α-reductase such as finasteride have been shown to reduce the size of BPH tissues by inducing apoptosis through caspase dependent pathway (Bozec *et al.*, 2005). In Figure 2, apoptosis induction was obviously increased after finasteride treatment. ($p<0.01$) From this result, we suggest that extract from Rape flowers in dose-dependence could reduce prostate enlargement in BPH by triggering apoptosis. Therefore, Rape flowers might be a promising candidate for the treatment of BPH as herbal medicine.

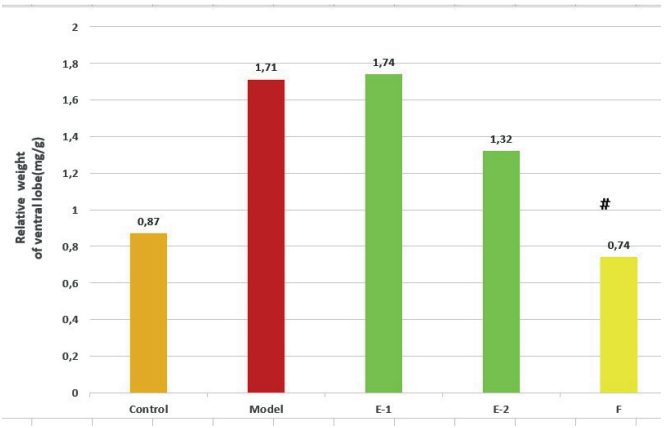

FIGURE 2 - Positive rate of cell apoptosis in the prostate. Model: BPH, E-1: Extract-30 mg/kg, E-2: Extract-60 mg/kg, F: Finasteride-10 mg/kg. The data were stated as mean ± SE. Superscript sign shows significant difference: #–from Model, #p < 0.05, ##p < 0.01.

It is well known that SRD5A2 gene encodes the prostatic (or type II) steroid 5α-reductase, which catalyses the irreversible conversion of testosterone to

dihydrotestosterone (DHT), the most active androgen in the prostate.

In addition, high serum insulin-like growth factor-1(IGF-1) level has been implicated as a possible risk factor for the development of BPH or prostate cancer. It was reported that IGF-1 level in patients with prostate volume > or = 50 mL were significantly higher than those in prostate volume < or = 30 mL ($P < 0.05$). A positive correlation between the serum levels of IGF-1 and PV displayed ($r = 0.58$) (Yu *et al.*, 2003). These observations implicate IGF-1 as important factor during the progression of BPH.

Transforming growth factor β (TGF- β) is a multi-functional cytokine that plays a fundamental role during embryonic development and tissue homeostasis in metazoans. Changes in TGF- β signalling are implicated in prostate cancer and benign prostatic hyperplasia (BPH), two of the most common diseases affecting ageing males. Because TGF- β 1 inhibits the growth of stromal cells and induces the differentiation of stromal cells to smooth muscle cell (SMC), its expresson plays an important role in the mechanism of BPH (Gu *et al.*, 2006). The levels of mRNA expression SRD5A2, IGF-1 and TGF-β1 in prostate tissus were examed through RT-PCR to find out the effect of extract from Rape flowers. Figure 3 shows mRNA expression levels of SRD5A2, IGF-1 and TGF-β1 in prostate.

mRNA expression levels of SRD5A2 and IGF-1 were significantly lower and mRNA expression level of TGF-β1 was significantly higher in Finasteride group than in Model group. ($p<0.05$) In Extract group with only 60 mg/kg, mRNA expression levels of SRD5A2 and IGF-1 showed a decrease from model values respectively, but this difference was not statistically significant and mRNA expression level of TGF-β1 increased significantly compared to Model group. There was no significant difference in mRNA expression level of TGF-β1 between Finasteride group and Extract group with 60 mg/kg. (Figure 3)

The usual treatments of benign prostate hyperplasia (BPH) including the α-blockers, the inhibitors of the 5α-reductase and the phytotherapy drugs allow significant improvements of the lower urinary tracts symptoms (LUTS). However, some patients are not responders or have side effects due to the treatments. Other therapeutic approaches described in the literature are possible in order to alleviate the LUTS. We evaluated the diuresis of extract by measuring 24h urine amount.

Figure 4 shows 24 h urine amount in all groups. 24h urine amount in both Extract groups increased significantly compared to the model ($p<0.05$) and had a tendency to increase in Finasteride group. ($p>0.05$) As shown in Figure 4, increased urine amount showed a clear dose-dependence of extract on diuresis in BPH model. Although inhibitors of 5α-redutase such as finasteride have been used to the treatment of BPH, these medicines cause some side effects in patients. Therefor experts have attempted to solve thise problems by using herbal medicines. In present study, the effects of extract on BPH model could be related to available componemts in Rape flowers. Futhermore, no obvious side effects of extract were detected during the expemental period time. We sugest that Rape flowers may be useful for the treatment of BPH as herbal medicine.

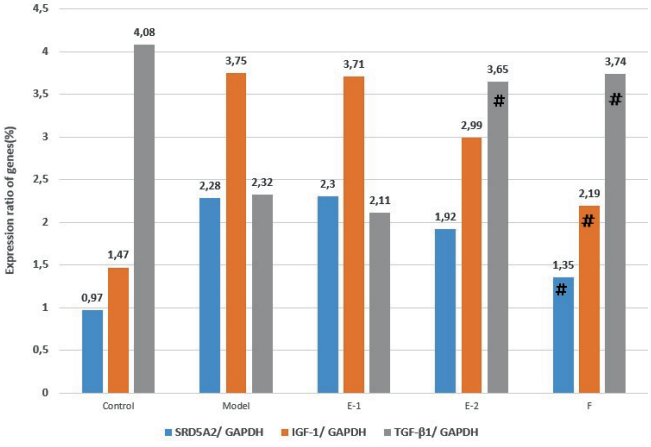

FIGURE 3 - Expression ratio of SRD5A2, IGF-1 and TGF-β1. Model: BPH, E-1: Extract-30 mg/kg, E-2: Extract-60 mg/kg, F: Finasteride-10 mg/kg. The data were stated as mean ± SE. Superscript sign shows significant difference: #–from Model, #$p < 0.05$.

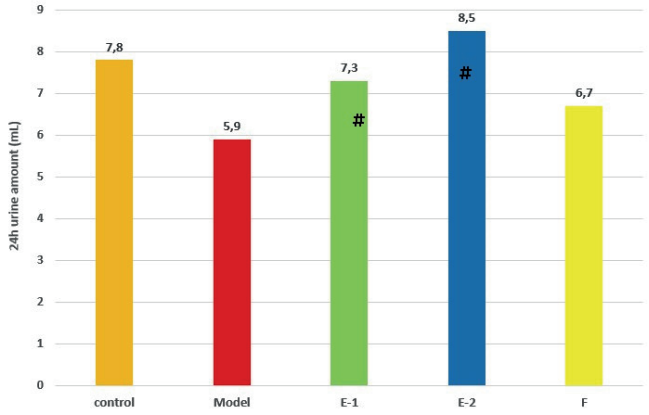

FIGURE 4 - Effect on 24 h urine amount. Model: BPH, E-1: Extract-30 mg/kg, E-2: Extract-60 mg/kg, F: Finasteride-10mg/kg. The data were stated as mean ± SE. Superscript sign shows significant difference: #–from Model, #$p < 0.05$.

CONCLUSION

Our present results demonstrate that extract from Rape flowers at 60 mg/kg induces cell apoptosis in prostate, increases mRNA expression of TGF-β1 in prostate gland and promotes urinary output in rat model of BPH. Furthermore, our results could open new avenues for the treatment of BPH by Rape flowers.

ACKNOWLEDGMENTS

We would like to thank Dr. Guangchol Kim. for the preparation of Rape (*Brassica napus* L.) flowers extract and appreciate Mr. Songchol Mun. for skilled technical assistance in present study.

REFERENCES

Altavilla D, Minutoli L, Polito F, Irrera N, Arena S, Magno C, et al. Effects of flavocoxid, a dual inhibitor of COX and 5-lipoxygenase enzymes, on benign prostatic hyperplasia. Br J Pharmacol. 2012;167(1):95-108.

Berry SJ, Coffey DS, Walsh PC, Ewing LL. The development of human benign prostatic hyperplasia with age. J Urol. 1984;132(3):474-479.

Bozec A, Ruffion A, Decaussin M, Andre J, Devonec M, Benahmed M, Mauduit C. Activation of caspases-3, -6, and -9 during finasteride treatment of benign prostatic hyperplasia. J Clin Endocrinol Metab. 2005;90(1):17-25.

Cayatte C, Pons C, Guigonis J-M, Pizzol J, Elies L, Kennel P, Rouquie D, Bars R, Rossi B, Samson M. Protein profiling of rat ventral prostate following chronic finasteride administration: identification and localization of a novel putative androgen-regulated protein. Mol Cell Proteomics. 2006;5(11):2031-43.

Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987;162(1):156-159.

Geller J, Angrist A, Nakao K, Newman H. Therapy with progestational agents in advanced benign prostatic hypertrophy. JAMA. 1969;210(8):1421-1427.

Gu H, Dong ZX, Wang CB, Yuan YF, Hou JH. Role of bFGF and TGF- β 1 in primary cultured prostatic stromal cells. Zhonghua Nan Ke Xue. 2006;12(10):917-22.

Han HY, Shan S, Zhang X, Wang NL, Lu XP, Yao XS. Down-regulation of prostate specific antigen in LNCaP cells by flavonoids from the pollen of Brassica napus L. Phytomedicine. 2007;14(5):338-43.

Ho CK, Habib FK. Estrogen and androgen signaling in the pathogenesis of BPH. Nat Rev Urol. 2011;8(1):29-41.

Kingston RE. Preparation and analysis of RNA, In: Ausubel FM, Brent R, Kinston RE, et al. (editors). Short protocols in molecular biology. John Wiley & Sons; 1995. p. 4-27.

Lepor H. The role of gonadotropin-releasing hormone antagonists for the treatment of benign prostatic hyperplasia. Rev Urol. 2006;8(4):183-189.

OED Online. Dictionary.oed.com. Retrieved 2012-04-22. Available from: http://eol.org/pages/583918/details.

Orsted DD, Bojesen SE. The link between benign prostatic hyperplasia and prostate cancer. Nat Rev Urol. 2013;10(1): 49-54.

Rick FG, Abi-Chaker A, Szalontay L, Perez R, Jaszberenyi M, Jayakumar AR, et al. Shrinkage of experimental benign prostatic hyperplasia and reduction of prostatic cell volume by a gastrin-releasing peptide antagonist. Proc Natl Acad Sci U S A. 2013;110(7):2617-2622.

Roehrborn CG. Male lower urinary tract symptoms (LUTS) and benign prostatic hyperplasia (BPH). Med Clin North Am. 2011;95(1):87-100.

Scolnik MD, Servadio C, Abramovici A. Comparative study of experimentally induced benign and atypical hyperplasia in the ventral prostate of different rat strains. J Androl. 1994;15(4):287-297.

Ståhlberg A, Håkansson J, Xian X, Semb H, Kubista M. Properties of the reverse transcription reaction in mRNA quantification. Clin Chem. 2004;50(3):509-515.

Van Coppenolle F, Slomianny C, Carpentier F, Le Bourhis X, Ahidouch A, Croix D, et al. Effects of hyperprolactinemia on rat prostate growth: evidence of androgeno-dependence. Am J Physiol Endocrinol Metab. 2001;280(1):E120-129.

Wu YD, Lou YJ. Brassinolide, a plant sterol from pollen of Brassica napus L., induces apoptosis in human prostate cancer PC-3 cells. Pharmazie. 2007;62(5):392-5.

Yu JP, Wu XM, Chen JG, Liu WM, Yang QX. Analysis of serum insulin-like growth factor-1 and insulin-like growth factor-binding protein-3 in benign prostatic hyperplasia. Zhonghua Nan Ke Xue. 2003;9(5):341-3.

Received for publication on 25th September 2018
Accepted for publication on 18th December 2018