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Original Research Article

Phytochemical Composition and Antihyperplasic Effect of *Fructus Hordei* G in Rats

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Abstract

Purpose: *To investigate the phytochemical profile and antihyperplasic effect of ethanol extract of Fructus hordei. G (FHE) on hyperplasia of mammary gland (HMG) in rats.*
Methods: *The Fructus hordei. G extract (FHE) was obtained by a rotary vacuum evaporator. The total flavonoids, total alkaloids and total phenolic compounds in FHE were assayed by aluminum chloride colorimetric method, acid-dye colorimetry and Folin-Ciocalteu method, respectively. HMG model rats were prepared for the experiments by injecting estradiol and progesterone. After oral administration with 150, 300 and 600 mg/kg of FHE for 30 days, breast nipple height, and serum superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), 5-hydroxytryptamine (5-HT), estradiol (E2), progesterone (P), prolactin (PRL), follicle stimulating hormone (FSH), luteinizing hormone (LH) and gonadotropin-releasing hormone (GnRH) levels of the rats were measured by ELISA kits. The pathological morphology of mammary gland tissues was examined by optical microscopy.*
Results: *The ethanol extract of FHE contained 1.88 ± 0.05 mg total flavonoids/g as quercetin equivalent (QE), 21.93 ± 0.06 mg hordenine equivalent (HE) of total alkaloids/g extract and 70.64 ± 0.11 mg total phenols/g gallic acid equivalent (GAE). The extract significantly diminished breast nipple height, MDA, 5-HT, E2, PRL, FSH and GnRH levels, and increased SOD, GSH-Px, P and LH levels ($p < 0.05$). FHE inhibited the alveolus, lobules and secretion of the mammary gland.*
Conclusion: *Fructus hordei extract has significant antihyperplasia effect on mice mammary gland by regulating serum sex hormones.*

Keywords: *Fructus Hordei, Flavonoids, Alkaloids, Phenolics, Hyperplasia Mammary gland*

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INTRODUCTION

Hyperplasia of mammary gland (HMG) is a kind of pathological hyperplasia of the lobules of the mammary gland induced by balance disorder of estrogen and progesterone [1]. HMG is related to occupation, abuse of sex hormone drugs, diet and mental pressure [2,3]. The number of patients with HMG has increased in recent years [4] and it has severe cancerous tendencies such as early breast cancer [5].

Chemical drugs are used in treating HMG, but they always cause several side effects such as menstrual disorder, and the relapse rate is very high. Hormonal drugs such as progesterone or testosterone propionate can reduce estrogen required to regulate the balance of hormone for curing HMG [6]. Tan [7] found that tamoxifen can significantly relieve the symptoms of HMG, but it still brought about some side effects such as gastrointestinal reaction, vomit or diarrhea, menstrual disorder, neuropsychiatric symptoms, headache or vertigo. Hence, patients often seek

herbal therapies outside of orthodox medical approaches.

Fructus hordei G. is a type of herb widely used in China. In the ancient book, "Zhonghuazi" of Song Dynasty, it was claimed to have been used to treat hyperplasia of mammary gland effectively [8]. It has the effect of regulating endocrine disorder and is usually used for the treatment of HMG [9,10]. In this study, we explored the effects of *Fructus hordei* G. extract (FHE) on HMG in rats. It has been reported that flavonoids, alkaloids and phenolics are the main components of *Fructus hordei* G. [11], but the contents are not known. In this study, phytochemical compositions and antihyperplasic effect of *Fructus hordei* in rats were evaluated.

EXPERIMENTAL

Materials

Fructus hordei G was collected from Bozhou City, Anhui Province in China in July 2013. Taxonomic identification of the plant was performed by Prof Jin-qiu Wang of Shandong University of Traditional Chinese Medicine in China. A voucher specimen (no. FHG 201309552) was deposited in the College of Pharmacy, Shandong University of Traditional Chinese Medicine, China for future reference. The powdered sample was placed in a thimble in a Soxhlet apparatus and extracted exhaustively with 50 % ethanol (1:40, w:v) at 60 °C for 2 h. The extract was filtered through Whatman filter paper no. 1 and the filtrate concentrated under reduced pressure at 55 °C using a rotary vacuum evaporator. The concentrated extract was then evaporated on a boiling water bath until a constant weight was obtained. The dried extract was weighed, the yield noted and kept in an air-tight container protected from light until used.

Other drugs and reagents used were Tamoxifen® tablets (Ningbo Tianheng Pharmaceutical Co. Ltd, China). E2, P, PRL, FSH, LH, GnRH, MDA, SOD, GSH-Px and 5-HT ELISA kits, and were obtained from Nanjing Jiancheng Institute of Bioengineering, Nanjing, China.

Determination of total flavonoids

Total flavonoids were analyzed using aluminum chloride colorimetric method [12]. FHE sample (600 µL ,600 µg/mL) was mixed with 600 µL of 2 % aluminum chloride solution. The mixture was allowed to stand at room temperature (27 ± 2 °C) for 10 min with intermittent shaking. The absorbance of the mixture was measured at 415 nm against a blank sample (methanol) without aluminum chloride using a UV-Vis spectrophotometer (SHIMADZU, Japan). The total flavonoids content was determined using a standard curve of quercetin (0.4–15.8 µg/mL). The content was expressed as mg quercetin equivalent/g of dry extract.

Determination of total alkaloids

Total alkaloids were determined using acid dye colorimetry method [13]. The sample (1.0 mL, 600 µg/mL) or standard hordenine ethanol solution (10 - 80 µg/mL) was mixed with phosphate buffer (2.0 mL, pH = 7), bromothymol blue solution (1.0 mL, 1 % w/v) and chloroform (3.0 mL). The mixture was allowed to stand at room temperature (27 ± 2 °C) for 1 min with shaking, then static for 60 min. The chloroform layer was obtained. The absorbance of the mixture was measured at 284 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). The content of total alkaloid compounds was expressed as mg hordenine equivalent/g of dry extract.

Content determination of total phenolics

Total phenolics were determined using Folin-Ciocalteu procedure. The sample (300 µg / mL) or standard gallic acid solution (10-120 µg / mL), 0.4 mL was mixed with the Folin-Ciocalteu reagent (1.0 mL ,diluted 1:10 with deionized water) and sodium bicarbonate solution (1.6 mL, 8.0 % w/v). The mixture was allowed to stand at room temperature (27 ± 2 °C) for 30 min with intermittent shaking. The absorbance of the mixture was measured at 765 nm using a UV-Vis spectrophotometer (SHIMADZU, Japan). The content of total phenolic compounds was expressed as mg gallic acid equivalent/g of dry extract.

Animals and model preparation

Female Wistar rats, weighing 200 - 240 g, were obtained from Shandong Center for Disease Control and Prevention, Jinan, Shandong (certificate number SYXF 2002-0008). The animals had free access to rat feed and water, and were allowed to acclimatize for at least one week before use. The rat experiments were approved by the Animal Care and Use Committee of Shandong Tumor Hospital (Approval reference no. 20130827) and was carried out in compliance with the Directive 2010/63/EU on the handling of animals used for scientific purposes [14].

Estradiol (0.5 mg/kg) was injected in the thigh muscle of the rats once every day for 25 days, then 5 mg/kg progesterone was injected once every day consecutively for 5 days. In this way, the HMG model rat was prepared [15,16]. All rats were randomly assigned to six groups of ten rats: sham group, model group, tamoxifen group (1.65 mg/kg), and FHE (150, 300 and 600 mg/kg) groups. The rats were orally administrated with the corresponding drugs once a day. Distilled water was administered in the same manner for the sham-operated and model control groups. Treatment started from the next day after the model preparation and continued for 4 weeks.

Measurement of nipple height and biochemical parameters

After 4 weeks of administration, the nipple height of the rats were measured using a vernier caliper. Serum MDA, GSH-Px and 5-HT of rats were measured by detection kits according to the procedures recommended by the manufacturer (Nanjing Jiancheng Co. Ltd), and serum SOD was measured by spectrophotometric method.

At the same time, serum E2, P, PRL, FSH, LH and GnRH of rats were measured by ELISA kits according to the procedures recommended by the manufacturer.

Histological examination

Tissues of mammary gland from each group were fixed in 10 % buffered formalin, embedded in paraffin, sectioned into 4μm thickness, stained with hematoxylineosin (H-E) and Masson-Trichrome (M-T) and examined using optical microscope. The severity of mammary gland hyperplasia was based on four parameters (hyperplasia of gland alveolus, lobule, shape and thickness of vessel and secretion).

Statistical analysis

All values are expressed as mean ± SEM. Multiple group comparisons were performed using one-way analysis of variance (ANOVA) with the aid of SPSS 18.0 software, followed by Dunnett's test to detect inter-group differences. *P* < 0.05 was considered significant in all cases.

RESULTS

Total flavonoids, alkaloids and phenolic contents of FHE

Extract yield, total flavonoids, total alkaloids and total phenolic contents of the 50 % ethanol extract of *Fructus hordei* G are shown in Table 1.

Table 1: Yield, total flavonoids, total alkaloids and total phenolics contents of 50 % ethanol extract of *Fructus hordei G*

Variable	Yield
Crude extract (% dry weight)	37.24 ± 0.05
Total flavonoid content (mg QE/g)	1.88 ± 0.05
Total alkaloid content (mg HE/g)	21.93 ± 0.06
Total phenolic content (mg GAE/g)	70.64 ± 0.11

Effect of FHE on nipple height

As shown in Table 2, the nipple height increased significantly in the model group compared with those in the control group ($p < 0.05$). Compared with model group, the middle-dose and high-dose FHE as well as tamoxifen treatment significantly decreased breast nipple height ($p < 0.05$).

Table 2: Effect of FHE on breast nipple height in HMG rats after 4 weeks of administration (mean ± SEM, n = 10)

Group	Dose	Nipple height (mm)
Control	-	0.98± 0.11
Model	-	1.56 ± 0.12*
Tamoxifen	1.65mg/kg	1.13 ± 0.12**
FHE -L	150mg/kg	1.39± 0.16
FHE -M	300mg/kg	1.12 ± 0.10**
FHE -H	600mg/kg	1.05 ± 0.13**

**P < 0.05 vs control group; **p < 0.05 vs. model group; model = HMG model rats*

Effect of FHE on serum MDA, SOD, GSH-Px and 5-HT

As shown in Table 3, MDA concentration was significantly higher in the model than in the control rats ($p < 0.01$), but they were attenuated by middle-dose, high-dose treatment of FHE and tamoxifen groups ($p < 0.05$). SOD and GSH-Px concentrations were significantly lower in the model than in the sham-operated rats ($p < 0.01$), but they were increased in middle-dose and high-dose treatment of FHE groups ($p < 0.01$).

5-HT concentration was significantly higher in the model than in the sham-operated rats ($p < 0.01$), but was attenuated by middle-dose and high-dose treatment of FHE ($p < 0.01$).

Effect of FHE on serum E2, P, PRL, FSH, LH and GnRH

As shown in Table 4, serum E2, PRL and FSH, GnRH concentrations were significantly higher in the model than in the control rats compared with model group, they were attenuated by middle-

Table 3: Effect of FHE on serum MDA, SOD, GSH-Px and 5-HT levels in HMG rats (mean ± SEM, n = 10)

Group	Dose	MDA (cB/(μmol/L)	SOD (λB/U/mg)	GSH-Px (λB/104U/L)	5-HT (pg/mL)
Control	—	1.82 ± 0.25	208.49 ± 4.58	8.25 ± 0.24	17.292 ± 0.747
Model	—	3.41 ± 0.43†	165.35 ± 5.12†	5.45 ± 0.36†	27.515 ±4.375†
Tamoxifen	1.65 (mg/kg)	2.05 ± 0.46*	176.58 ± 6.91	6.94 ± 0.25*	25.714 ± 5.097
FHE-L	150 (mg/kg)	2.61 ± 0.51	168.14 ± 5.55	5.82 ± 0.37	25.630 ± 3.844
FHE-M	300 (mg/kg)	2.38 ± 0.36*	198.65 ± 2.85**	7.20 ± 0.32**	20.670 ± 2.636**
FHE-H	600 (mg/kg)	2.19 ± 0.25*	206.21 ± 3.46**	8.67 ± 0.33**	18.179 ± 2.053**

$^{\dagger}p < 0.01$ *vs control group.* $^{*}p < 0.05$, $^{**}p < 0.01$ *vs Model group; model = HMG model rats*

Table 4: Effect of FHE on E2, P, PRL, FSH, LH and GnRH in HMG rats (mean ± SEM, n = 10)

Group	Dose	E2 (pmol/L)	P (ng/mL)	PRL (pg/mL)	FSH (IU/L)	LH (mIU/mL)	GnRH (pg/mL)
Sham	—	2.80 ± 0.34	1.41±0.16	286.37±7.08	0.08± 0.02	1.85± 0.10	1.26± 0.34
Model	—	4.85± 0.19†	0.63±0.06‡	411.26±8.91‡	0.52±0.01‡	1.08± 0.05‡	2.12 ± 0.10‡
Tamoxifen	1.65mg/kg	2.81 ± 0.28*	1.12±0.20*	414.29±15.24	0.48± 0.27	1.48± 0.10*	1.85 ± 0.12
FHE-L	150mg/kg	3.58± 0.38	0.85±0.17	368.17±17.26	0.32± 0.01	1.15± 0.15	1.62 ± 0.39
FHE-M	300mg/kg	2.97± 0.25*	1.03±0.18*	312.85±12.49*	0.18±0.02*	1.33± 0.10*	1.334± 0.32*
FHE-H	600mg/kg	2.67± 0.16**	1.13±0.17*	278.93±8.42**	0.11±0.01**	1.77± 0.09*	1.27 ± 0.35**

$^{\ddagger}P < 0.05$, $^{\dagger}p < 0.01$ *vs control group;* $^{*}p < 0.05$, $^{**}p < 0.01$ *vs model group; model = HMG model rats*

dose and high-dose treatment of FHE group. Serum P and LH concentrations were significantly lower in the model than in the control rats compared with model group, they were increased in middle-dose and high-dose treatment of FHE and tamoxifen group ($p < 0.05$).

Effect of FHE on morphology of mammary tissue

The morphology of mammary tissue in control rats was shown in Figure 1A. Intense hyperplasia of gland alveolus and lobule, secretion, vessel arborization, lymphoplasia and plasma cells were observed in mammary gland tissues of HMG model rats (Figure 1B). In tamoxifen-treated rats at week 4, the hyperplasia of lobules and gland alveolus of mammary gland lessened, secretion and lymphocyte decreased in intracavitary (Figure 1C). The mammary gland of model rats treated with high-dose and middle-dose of FHE recovered well. There was few hyperplasia in gland alveolus and lobules of mammary gland, and only few secretion and lymphocyte was seen (Figure 1E and F). The low-dose of FHE had no significant effect, the gland alveolus, lobules hyperplasia and much secretion was still seen in mammary gland tissues (Figure 1D).

DISCUSSION

Hypothalamus-pituitary-gonadal axis is one of the parts of NIM network and plays an important role in the maintenance of homeostasis of the internal environment and the normal physiological function of the organic body [17]. When extraneous stimulations act on human body, some sex hormones secrete abnormally leading to many kinds of disease such as HMG, hyperprolactinemia, hysteromyoma and infertility. The sex hormone disorder occurs in HMG rat models [18].

Our study result revealed that FHE regulated PRL, E2, P, FSH, LH and GnRH, and eliminated the hyperplasia of lobules and gland alveolus. FHE has therapeutic effects on HMG rats induced by estrogen and progestogen. It showed that E2, PRL, FSH and GnRH were remarkably decreased while P and LH concentrations significantly increased by FHE treatment compared with HMG model group.

Histopathological examination revealed that FHE remarkably alleviated the degree of HMG, number of lobules and acinars. Therefore, the mechanism of FHE in treating HMG is by way of regulating endocrinal and immune functions to inhibit the pathological proliferation of mammary gland.

MDA, SOD and GSH-Px are important indices reflecting antioxidant capacity *in vivo*. Estrogen plays an important role in HMG and breast carcinoma [19]. The results presented serum MDA concentration increased and SOD, GSH-Px concentrations decreased in model rats compared with control group. FHE decreased MDA level and increased SOD and GSH-Px levels significantly, revealing that FHE treated HMG by inhibiting lipid peroxidation to regulate free radical metabolism. Serum 5-HT concentra-

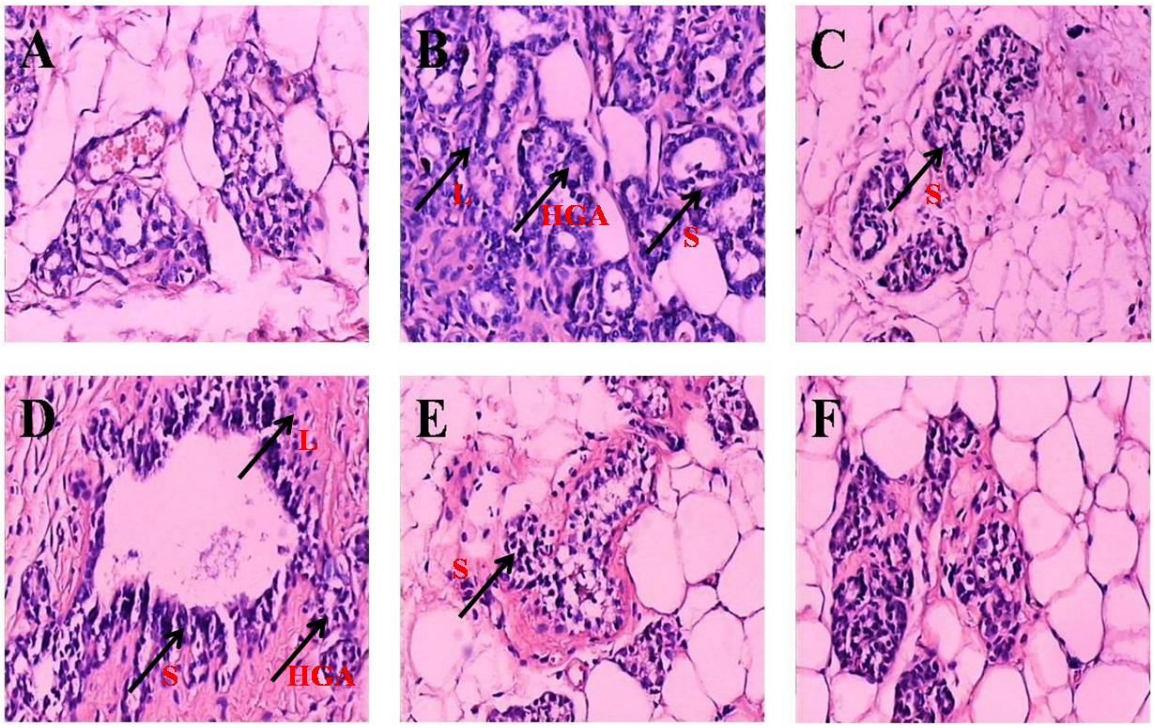

Fig. 1: Effect of FHE on pathological morphology of mammary tissue (x200). A = control group; B = model group; C = Tamoxifen group; D = Low-dose of FHE group; E = Middle-dose of FHE group; F = High-dose of FHE group; HGA = hyperplasia of gland alveolus; S = secretion; L = lymphocyte; Model: HMG model rats

tion increased significantly in HMG rats revealing that emotion and psychic factors play important roles in the occurrence of HMG. FHE decreased 5-HT concentration effectively, suggesting the regulating action of traditional Chinese herb *Fructus hordei* G [20].

The total flavonoids, alkaloids and phenols are the main compositions of *Fructus hordei* G. extract, which contributed together to the antihyperplasia effect in rats. The mechanism needs further study in future.

CONCLUSION

The study showed that *Fructus hordei* G extract has significant antihyperplasia effect on mice mammary gland, and therefore has the potential to be developed as an anti-HMG product.

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