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Article

Comparative assessment of effect of malt with different bud length on prolactin in hyperprolactinemia rat

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Malt is the mature fruit of *Hordeum vulgare L.* after germination and drying and has been applied for treatment female abnormal galactorrhea. Previous studies have showed total alkaloids in malt have anti-HPRL effect. However, total alkaloids of malt change with the growth cycle, and the specified levels of total alkaloids in different bud length of malt have not been decided. To determine the definitive level of total alkaloids in different buds of malt and the most suitable bud length for clinical application by comparing effects on hyperprolactinemia rat. During the budding of malt, the content of total alkaloids first increased and then decreased, and it peaked at a bud length of 0.75 cm. Treated the HPRL model rats with different buds of malt, the PRL level was decreased, the number of PRL-positive cells and the mRNA expression level in the pituitary were significantly declined, and the number of dopamine D1 and D2 receptors in the hypothalamus was increased. The above changes were most significant in 0.75 cm bud. These results suggest that in terms of the content of effective substance and the effects on HPRL model rats, a malt bud length of 0.75 cm is optimal for clinical application.

Keyword: Malt. Alkaloid. Endocrine system. Hormone. Dopamine receptors.

Abbreviations: E2, estradiol; P, progesterone; PRL, prolactin; HPRL, hyperprolactinemia; DRD2, D2 receptor; MCP, metoclopramide; BMT, bromocriptine.

INTRODUCTION

Hyperprolactinemia (HPRL) is defined as galactorrhea beyond the lactation period caused by an abnormal increase of prolactin (PRL) level in human body which are attributed to drugs or pathological factors (e.g., pituitary adenoma). An important group of patients in whom dopamine agonists are contraindicated are patients with antipsychotic-induced HPRL, caused mainly by the use of the typical antipsychotics (phenothiazines, haloperidol, and thioxanthenes) and by some of the atypical agents (amisulpride, risperidone, and paliperidone) (Ranjbar *et al.*, 2015; Krysiak *et al.*, 2016; Lee *et al.*, 2012). Although a reduction in dose or switching to a PRL sparing antipsychotic may be effective for decreasing PRL levels, these treatment strategies may carry the risk of an exacerbation or relapse of psychotic symptoms (Kelly *et al.*, 2013).

Malt is the mature fruit of *Hordeum vulgare L.* after germination and drying, which is of wide geographical distribution and grows in all areas of China. Malt is the sole Chinese medicine with a function of delectation recorded in the Chinese ancient medical books and has been applied for the treatment of female abnormal galactorrhea in Chinese clinical or folk practice (Xiong *et al.*, 2014). The malt is traditionally processed according to the following procedures: the fresh mature barley seeds are selected, and then induced for budlet germination under a certain temperature or humidity (Mark *et al.*, 2013; Farzaneh *et al.*, 2017); through the budding, the starch, protein and fat are decomposed to produce various

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secondary metabolites (e.g., digestive enzymes and alkaloids) (Zhuang *et al*., 2015; Zeng *et al*., 2012); and then the biological activities (e.g., promotion of digestion or regulation of milk secretion) are formed (Song *et al*., 2014).

As shown by the current studies, the abnormally increased PRL level in HPRL model rats was significantly decreased by the decoction or extract of malt (Wei *et al*., 2009; Zhu *et al*., 2015), possibly via activating dopamine receptors in rat hypothalamus. As the main effective substance for PRL reduction, total alkaloids (e.g., hordenine and gramine) in malt can not only decrease the PRL level in HPRL model rats (Kim *et al*., 2013; Larsson *et al*., 2011), but also increase the estrogen and progesterone (P) levels and restore the balanced between hormones (Hu *et al*., 2012). It is considered that pituitary PRL secretion is regulated mainly through the hypothalamic neuro-endocrine dopaminergic system (Brooks, 2012; Brown *et al*., 2016). Dopamine, that is released from tuberoinfundibular dopaminergic (TIDA) and delivered into anterior lobe, is considered the major physiological regulator of PRL secretion. D2 receptor (DRD2) shows an important effect on short-loop feedback mechanism of PRL regulation, and robust PRL expression is observed in DRD2-null mice (Wei *et al*., 2017; Wang *et al*., 2012; Tsuboi *et al*., 2013; Nakano *et al*., 2010).

According to our preliminary study, the content of total alkaloids in malt varied with the place of origin, and hordenine was not found in unbudded barley seeds (An, Chen, 2014; Li *et al*., 2016). The content of alkaloids varied with different buds of malt, and it did not reach a peak at the bud length of 0.5 cm (He *et al*., 2017). Although being stipulated as 0.5 cm in Chinese Pharmacopoeia (2015) (National Pharmacopoeia Commission, 2015), the bud length of malt is found as very different in many buds of malt available on market, and there are no uniform standards for the preparation process of malt (He *et al*., 2017), thus it is difficult to ensure the quality of malt. Therefore, we presumed that varying efficacy for HPRL may be produced by different buds of malt. In order to verify this hypothesis, we detected the effects of decoction prepared with different buds of malt on the following indices in HPRL model rats: serum levels of PRL, P and estradiol (E2); expression of dopamine D1 and D2 receptors in the hypothalamus, and mRNA expression in PRL cells in the pituitary.

MATERIAL AND METHODS

Herbal preparation and identification of the quality

Preparation of fresh malt (He *et al*., 2017): The fresh barley seeds (batch No.: 20161215, marked as "S") were bought from Bozhou City, Anhui Province, which were identified as mature seeds of Hordeum vulgare L. (Poaceae) by Chinese pharmacist Chen Yonggang, deputy director of Pharmacy Department, the Third Hospital of Wuhan. The following procedures were implemented: removing the impurities from fresh barley seeds, washing up, adding a sufficient amount of water, soaking for 5 h, budding in a constant temperature & humidity incubator (25 ºC, 70%), sprinkling water daily by a ratio of water: barley seed weight = 1:1, stopping the budding after reaching the required bud length, and drying up at 70 ºC under a normal pressure.

Measurement method of bud length: Totally 100 budded barley seeds were selected, the shells were removed, and the length of light-yellow stripy plantule was measured and averaged. Different buds of malt (i.e., 0.25 cm, 0.50 cm, 0.75 cm, 1.00 cm, 1.25 cm, 1.50 cm, 1.75 cm and 2.00 cm) were collected and sequentially marked as A~H (Figure 1).

The samples of different buds of malt (Mark S and A~H) were grinded and sieved. Then, 10.00 g of the most coarse powder was weighed, put into a conical flask with a plug, and added with a 5-folds amount of methanol solution (80%). Thereafter, the mixture was ultrasonically extracted 3 times, 45 min each, and filtered. The drug residue and container was washed with methanol solution (80%) in sequence, and the filtrates were mixed and evaporated up in a water bath (60 ºC). Finally, the residue was dissolved with HCl solution (0.03%) and added with water to the required volume in a 10 mL volumetric flask, ultrasonically mixed uniformly, and filtered with a 0.22 μM millipore filter membrane. The product was the sample solution for quality analysis.

Determination of the content of total alkaloids (An, Chen, 2016): 1.0 mL of the sample solution prepared in Section 2.1 was precisely pipetted into a separating funnel, added with 4.0 mL H_3PO_4 buffer solution (pH 6.0) first and then 2.0 mL Bromothymol Blue acidic dye solvent, mixed and preserved for 10 min, and then added with 50 mL methenyl trichloride, fully shaken for 5 min and preserved for 1 h. Thereafter, the methenyl trichloride solution was separated. 1.0 mL HCl solution

(0.03%) was used and subject to the same procedures as above, and the product served as the blank solution. The absorbance of solutions was measured at 415 nm. The absorbance of 5 hordenine standard product concentrations was measured repeatedly for 3 times, and then the standard curve was plotted to give a formula of Y = 320.3X - 13.06, wherein R=0.9987; X: absorbance, Y: content of total alkaloids. And the content of total alkaloids was thereby calculated.

HPLC analyses were performed with a Dionex U-3000 series (Shanghai, China) equipped with a SR-3000Solvent Rack, a LPG-3400SDN Quaternary Pump, a WPS-3000SL Auto sampler, an injector with a 100 µL loop, a TCC-3000RS Column compartment, a DAD-3000RS detector and Chromeleon 7 chromatography workstation. A Phenomenex Luna-C8 column (4.6 mm × 250 mm, 5 µM) (Guangzhou, China) was used. The mobile phase consisted of 0.05 mol/L potassium dihydrogen phosphate (adjusting pH to 7.10 with Triethylamine) (A) and methanol (B). The isocratic elution was 95:5. The flow rate was kept at 1.0 mL/min and the column temperature was maintained at 25 ºC. The injection volume was 20 µL and the detective wavelength was selected at 226 nm (Li *et al.*, 2016).

The major peaks in barley malt extract were identified as hordenine by comparison of the retetion times. The content of hordenine was quantified by comparison of the area under curve of the sample with an injection of a standard solution of itself.

HPRL rat model establishment and drug administration

Animals and treatments

The experimental protocol was approved by the Animal Care and Use Committee and was carried in compliance with the Animal Welfare Act and the NIH guidelines (NIH publication number 80-23, revised 1996). Female Sprague-Dawley rats aged 8 weeks old and weighing 200-220 g were obtained from Hubei Center for Diseases Control and Prevention, Wuhan, Hubei, China. The animals were housed in a temperature controlled facility (21 ± 1 ºC, 55 ± 5% relative humidity) and were kept on a 12 h light/dark cycle, with water and standard chow available *ad libitum* at least one week before use.

To prepare the extract the herb were ground into crude powder and extracted with purified water three times (8 volumes of water for 45 min boiling). The combined filtrate was evaporated under reduced pressure below 60 ºC. The yield of the extract was 25%.

Animal experimentation was conducted as described previously (Hu *et al.*, 2012). To generate the experimental model of HPRL, 100 animals were given intraoeritoneal (i.p.) metoclopramide (MCP, 75 mg/kg, twice daily), a dopamine inhibitor for 10 days. This model has been widely used for investigation of HPRL (Krysiak *et al.*, 2016). Additional 10 rats were injected with saline as the control group. At day 10, blood was drawn from the tail vein of both untreated and MCP-treated animals for measurement of PRL, E2, P. Since all MCP-treated animals displayed an at least 80% elevation of serum PRL concentrations compared to an averaged level of untreated controls, all of them were used for experimental treatment and received gastric water extract of barley malt at different length of budlet (0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 cm, labeled as group A to H) with a dose of 9.5 g/kg, respectively, or i.p. injection of 0.4 mg/kg bromocriptine (BMT) for 30 days in a random manner (n=10 per group). The selection of these doses was based on the dosages used in humans, as previously reported (Hu *et al.*, 2012). Blood samples were collected at the completion of experimental treatment and sera were separated for the measurement of PRL, E2, and P.

Hormone assay

The concentrations of PRL, E2, and P in rat sera was determined using enzyme-linked immunosorbent assay (ELISA) (Calbiotech, USA). Assay sensitives were less than 0.5 ng/mL for PRL and P, and less than 2 pg/mL for E2, and the intra-assay coefficients of variation were less than 10%. All the samples were measured in the same assay in duplicate.

Immunohistochemistry

The immunohistochemical staining and the immunocytochemical PRL-positive cells were performed by Wuhan Google Biotechnology Co., Ltd (Wuhan, China). Briefly, sample was embedded in paraffin after being undertaken using 4% PBS-buffered paraformaldehyde to fix overnight. Sample was deparaffinized and rehydrated after being cut at 5 µm. The sides were washed in distilled water and incubated with 5% goat serum containing 1 BSA at 25 ºC for 1

h after being used to heat in microwave. Subsequently, hypothalamus tissue sides were incubated with mouse D2 dopamine receptor (anti-D2R, 1: 600, Santa Cruz, CA, USA) and mouse D1 dopamine receptor (anti-D1R, 1: 500, Santa Cruz, CA, USA), and the pituitary tissue sample was incubated with primary mouse antibody prolactin (anti-PRL, 1:1000, Santa Cruz, CA, USA) at 4 ºC overnight, and then a second antibody solution containing rabbit anti-mouse antibody (1:400, Alexa Fluor 647, Jackson Immuno Research), followed by incubation with streptavidin conjugated with horseradish peroxidase. Sides were washed by buffer PBS for four times each five minutes. A DAB peroxidase substrate was added to sides and incubated for 5 min. Definitively sides were washed by distilled water followed by dehydration, transparency and sealing. The quantification of PRL-positive cells of ten thousand cells per study group was evaluated by using Leica LAS Power Mosaic. The visible nuclear and cellular profiles of cells in the plane of section were screened out. The PRL-positive cells in digital micrographs were determined by taking from each section and processing by Adobe Photoshop CS21. Samples were viewed under a light microscope (400 ×) and positive cell number was measured using a digital image processing software (ImageJ).

Real time PCR

Total RNA was obtained from pituitary tissues of rat using Trizol reagent (Sangon co., Shanghai, China). The cDNA was synthesized by a First Strand cDNA Synthesis Kit (Thermo, NY, USA) according to the manufacturer's direction. The PCR primers and their cycling conditions were set as directed by Shanghai Sangong Biotechnology. The reverse transcription reaction was performed with a SYBR-Green Kit (Shanghai, China) following these conditions: 95 ºC for 10 min, followed by 40 cycles of 95 ºC for 15 s, and then a 30 s annealing step at 60 ºC. A melting curve was accomplished from 75 ºC to 95 ºC, increasing the 1 ºC by 20 s. The results were performed by the $2^{-\Delta\Delta Ct}$ method. The relative expression of mRNA was normalized to β-actin.

The following primers were used for PCR amplification: β-actin: forward primer: 5'-TGCTATGTTGCCCTAGACTTCG-3' and reverse primer 5'-GTTGGCATAGAGGTCTTTACGG-3'; PRL: forward primer: 5'-AACAAGCCCAGAAAGTCCCTC-3'; and reverse primer 5'- GGAGTTGTGACCAAACCAAGTAGA-3'.

Statistical analysis

The cells with brown-yellow cytoplasm indicated by immunohistochemistry were considered as positive cells. The integral optical density (IOD) of positive cells was obtained by analysis with Image-Pro Plus 6.0 software, the unit area IOD in each group was calculated, and the amplification multiple of PRL mRNA was finally calculated from PCR results. All data were statistically analyzed by SPSS 16.0 software, and expressed as mean ± SEM. The inter-group comparison was performed with *t* test. $P < 0.05$ or $P < 0.01$ indicated that the difference was statistically significant.

RESULTS AND DISSCUSION

Results

Identification and quantification of Alkaloid substance in malt

During the budding process of malt, the content of total alkaloids first decreased, then increased and thereafter slightly decreased, and peaked at a bud length of 0.75 cm (Figure 2). A difference of 33.3 μg/g was found between 0.75 cm bud of malt group and 0.50 cm bud of malt group. The content of hordenine first increased and then decreased, with a large change; its content in 1.25 cm bud was about 5 folds of that in 0.25 cm bud; and hordenine was not found in fresh barley seeds (Kim *et al.*, 2013). These results were shown in Table I, Figure 1 and Figure 2.

Influence on the serum levels of PRL, P and E2

As shown in Figure 3, the serum levels of PRL and P were significantly increased in the model group ($P < 0.01$), but that of E2 was markedly decreased ($P < 0.01$). Compared with the model group, there was an evident decrease in the serum levels of PRL and P ($P < 0.05$; $P < 0.01$), but a significant increase in that of E2 ($P < 0.01$) within BMT-positive drug group and the groups of different buds of malt. These findings proved that the decoction of different buds of malt could decrease the serum levels of PRL and P but increase that of E2. In the BMT-positive drug group and 0.75 cm bud of malt group, the serum PRL level was most close to that in the normal group, indicating that the PRL level in HPRL rats was best regulated by 0.75 cm bud.

TABLE I – The content of Total alkaloid and hordenine in different buds of malt

No.	Bud length (cm, mean)	Total alkaloids (μg/g)	Mean	Hordenine (μg/g)	Mean
S	0.00	70.21 72.14	71.18	0 0	0
A	0.25	57.34 54.77	56.06	10.59 11.24	10.92
B	0.49	74.71 73.43	74.07	29.82 28.89	29.36
C	0.74	90.16 88.55	89.35	38.07 36.60	37.34
D	0.98	87.58 90.16	88.87	39.45 40.06	39.76
E	1.24	82.43 86.94	84.69	48.67 54.02	51.35
F	1.51	80.83 83.08	81.95	30.85 31.95	31.40
G	1.76	76.32 77.29	76.81	33.24 34.34	33.79
H	2.04	71.18 72.78	71.98	34.51 36.29	35.40

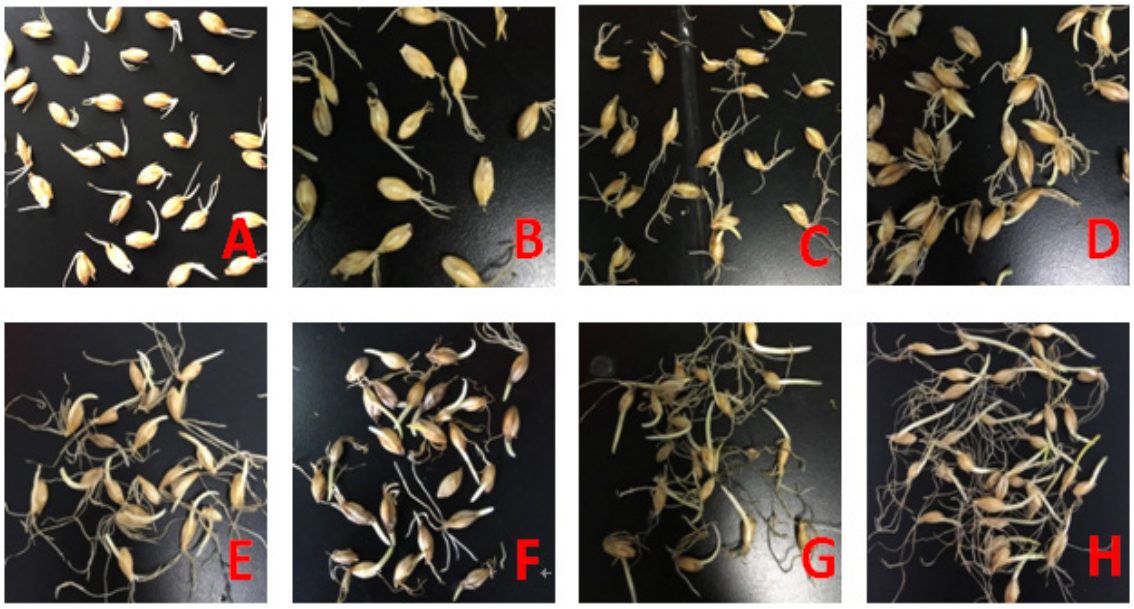

FIGURE 1 – Different bud of *F. H. Germinatus.* (A) 0.25 cm; (B) 0.50 cm; (C) 0.75 cm; (D) 1.00 cm; (E) 1.25 cm; (F) 1.50 cm; (G) 1.75 cm; (H) 2.00 cm.

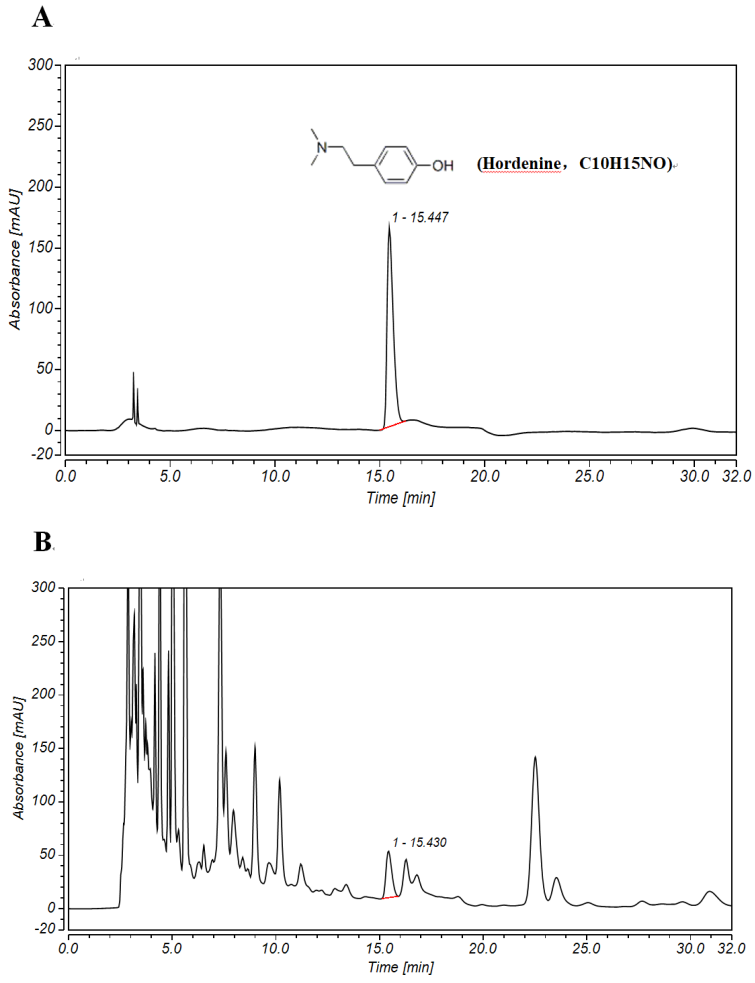

FIGURE 2 – The HPLC fingerprints of reference substance and malt samples: (A) hordenine; (B) 0.75 cm bud of barley malt.

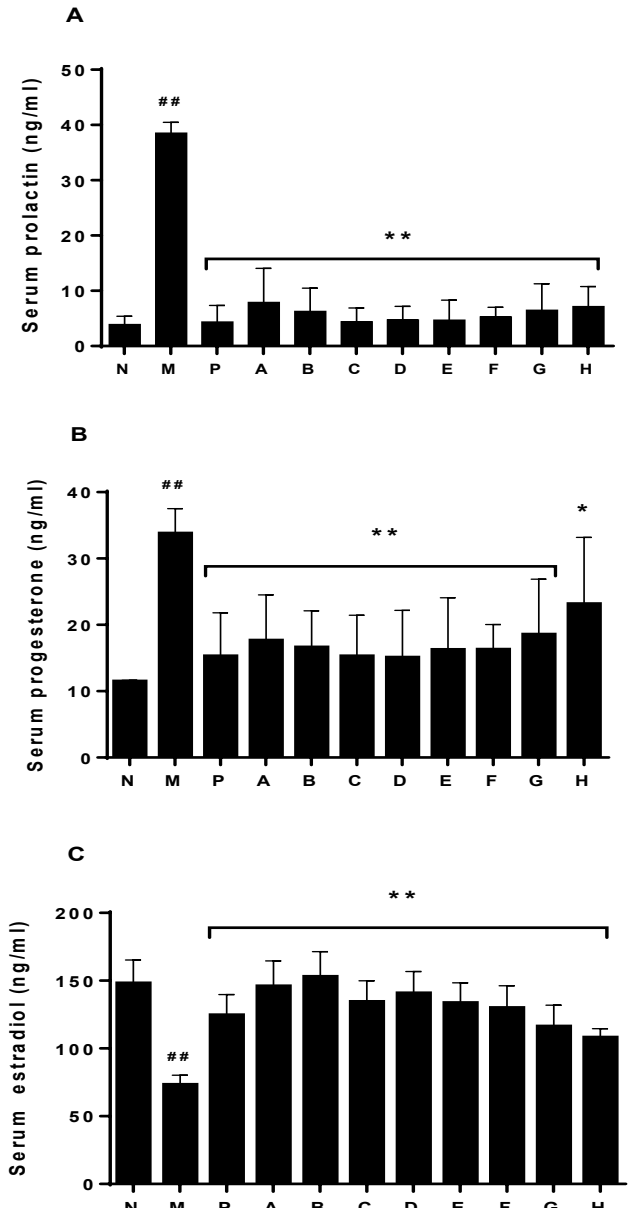

FIGURE 3 – The effects of decoction of different buds of malt on the contents of PRL, P and E2 in serum of HPRL rat model. Rat model of HPRL was produced by injection of 75 mg/kg MCP for 10 days, followed by treatment with or without 9.46 g/kg decoction of different buds of malt (A to H) or 0.4 mg/kg BMT (P) for 30 days. 0.9% salt solution-treated rats were served as model group (M). Normal group were non-modeling and treated (N). After the completion of experimental treatment, serum of rats in each group was collected serum PRL (A), P (B) and E2 (C) levels were determined by ELISA. Data are expressed as mean ± SEM (n = 9). $^{\#\#}P < 0.01$ vs. normal group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. model group.

Positive response of PRL cells in rat pituitary

A positive response of PRL cells was indicated by the brow-yellow cytoplasm (Figure 4). Compared with the normal group, the IOD of rat PRL cells was significantly increased in the model group and various bud of malt groups ($P < 0.01$). Compared with the model group, the IOD of PRL cells in rat pituitary was significantly decreased in BMT-positive drug group and various bud of malt groups ($P < 0.01$) (Figure 5). These data suggested that the number of PRL-positive cells in HPRL rats could be decreased by the decoction of different buds of malt, and such an effect was most significant in 0.75 cm bud of malt group.

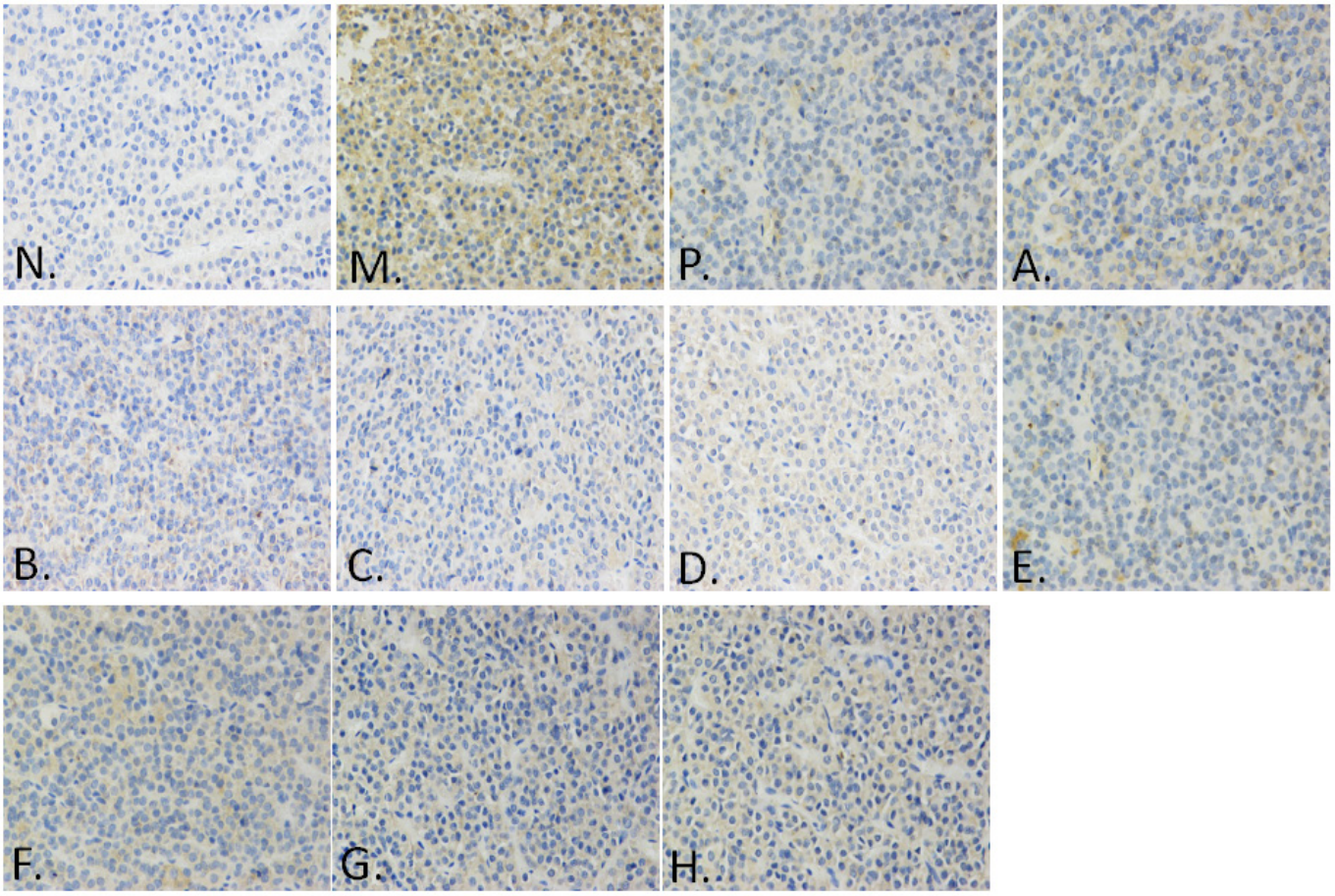

FIGURE 4 – PRL cell positive expression in the pituitary was observed by microscope (original magnification, 400 ×). The positive expression of PRL cell in the pituitary was brown in cytoplasm, and the darker the color was, the greater its positive expression was. (N) normal group; (M) model group; (P) bromocriptine group; (A) 0.25 cm bud of malt group; (B) 0.50 cm bud of malt group; (C) 0.75 cm bud of malt group; (D) 1.00 cm bud of malt group; (E) 1.25 cm bud of malt group; (F) 1.50 cm bud of malt group; (G) 1.75 cm bud of malt group; (H) 2.00 cm bud of malt group.

Prolactin mRNA expression in rat pituitary

In each group, the PRL mRNA expression of PRL cells was tested in the rat pituitary tissues. As compared with the normal group, the mRNA expression level of P was significantly increased in the model group ($P < 0.01$) and some bud of malt groups (i.e., 0.50 cm, 0.75 cm and 1.0 cm) ($P < 0.05$, $P < 0.01$), indicating that the mRNA expression of PRL cells in rat pituitary was not influenced by 0.25 cm, 1.25 cm, 1.50 cm, 1.75 cm and 2.00 cm buds. There was no statistically significant difference between some bud of malt groups (i.e., 0.5~1.25 cm) and normal group ($P > 0.05$), which revealed that the mRNA expression of PRL cells in rat pituitary was down-regulated in these four bud of malt groups at the same level as the normal group. Compared with the model group, the mRNA expression level of PRL was significantly different in BMT-positive drug group and some bud of malt groups (i.e., 0.50 cm, 0.75 cm, 1.00 cm and 1.25 cm); and that in 0.75 cm bud of malt group was most approximate to the normal group, with the best effect of down-regulation (Figure 6).

Expression of dopamine D1 and D2 receptors in rat hypothalamus

A positive response of dopamine D1 and D2 receptors in rat hypothalamus was indicated by the brow-yellow cytoplasm; the higher IOD was, the greater positive response would be (Figure 8 and 9). The expression level of dopamine D1 receptor in rat hypothalamus was significantly decreased in the model

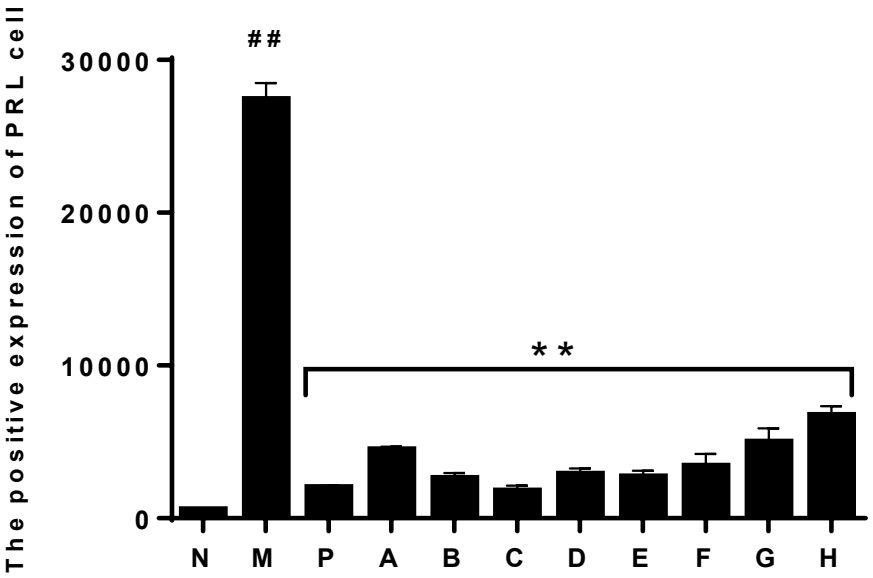

FIGURE 5 – The effects of decoction of different buds of malt on the positive expression of PRL cells in the pituitary gland of HPRL rat model. Rat model of HPRL was produced by repeated injection with 75 mg/kg MCP for 10 days, followed by treatment with or without 9.46 g/kg decoction of different buds of malt (A to H) or 0.4 mg/kg BMT (P) for 30 days. 0.9% salt solution treated rats were served as model group (M). Normal group were non-modeling and treated (N). At the completion of experimental treatment, tissues of pituitary gland of rats in each group were collected respectively. The positive expression of PRL cells were determined by immunohistochemistry. Data are expressed as mean ± SEM (n = 4). $^{\#\#}P < 0.01$ vs. normal group, $^{**}P < 0.01$ vs. model group.

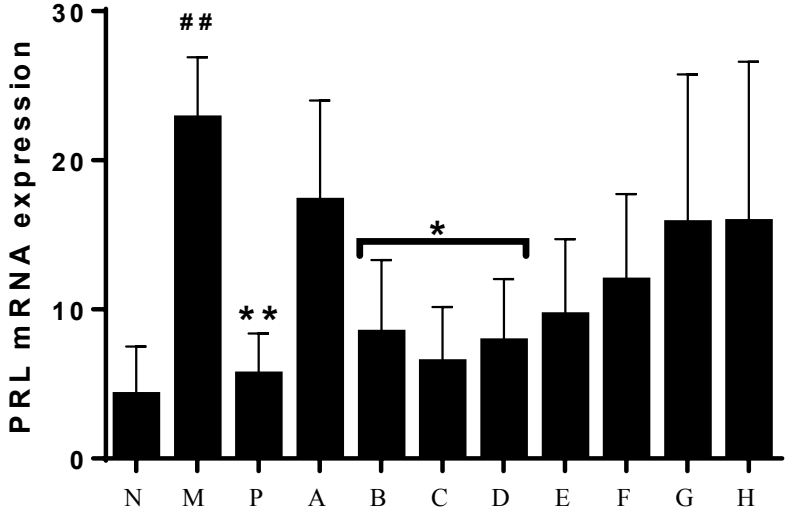

FIGURE 6 – The effects of decoction of different buds of malt on the expression of PRL mRNA in the pituitary gland of HPRL rat model. Rat model of HPRL was produced by back subcutaneous repeated injection with 75 mg/kg MCP, for 10 days, followed by treatment with or without 9.46 g/kg decoction of different buds of malt (A to H) or 0.4 mg/kg BMT (P) for 30 days. 0.9% salt solution treated rats were served as model group (M). Normal group were non-modeling and treated (N). At the completion of experimental treatment, tissues of pituitary gland of rats in each group were collected respectively. The expression of PRL mRNA was determined by PCR. Data are expressed as mean ± SEM (n = 6). $^{\#\#}P < 0.01$ vs. normal group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. model group.

group, BMT-positive drug group and various bud of malt groups ($P < 0.01$); compared with the model group, it was markedly increased in BMT-positive drug group and various bud of malt groups ($P < 0.05$, $P < 0.01$). This indicated that the number of dopamine D1 receptor in the hypothalamus of HPRL model rats was slightly increased by the decoction of different buds of malt, but it was still significantly lower than that in the normal group (Figure 7). Therefore, the further studies were required for the role of changes in the number of dopamine D1 receptor in the process that the malt regulated PRL secretion.

Compared with the normal group, the expression level of dopamine D2 receptor in rat hypothalamus was significantly decreased in the model group ($P < 0.01$); compared with the model group, it was evidently increased in BMT-positive drug group and some bud of malt groups (i.e., 0.25 cm, 0.50 cm, 1.50 cm, 1.75 cm and 2.00 cm) ($P < 0.05$, $P < 0.01$); in 0.75 cm bud of malt group, it was most close to the normal group (Figure 7). Therefore, the expression level of dopamine D2 receptor in the hypothalamus of HPRL model rats was best regulated by the decoction of 0.75 cm bud; and dopamine D2 receptor should be the main target of delectation for malt.

DISCUSSION

HPRL is a common disease of dysfunction in the hypothalamus-pituitary-ovary axis, with the following characteristic symptoms: PRL elevation (PRL > 25 ng/mL), amenorrhea, galactorrhea, anovulation and infertility (Farzaneh *et al.*, 2017). Since galactorrhea is closely relevant to PRL level, the HPRL rat model was used in our study to investigate the delectation effect of malt. This animal model is well-established, easy to operate, and easy to judge the experimental results. Some studies suggested that the change in the levels of P and E2 could be caused by the change of PRL level (Pacchiarotti *et al.*, 2015). In our study, the levels of PRL and P were effectively decreased by different buds of malt, producing an effect of negative feedback to increase E2 level to the normal one; the best regulation of PRL was found in 0.75 cm bud of malt group.

The PRL secretion in the pituitary is inhibited by dopamine in the hypothalamus. In cyclic adenosine monophosphate-protein kinase A system (cAMP-PKA) signal pathway of dopamine receptors, D1 and D2 receptors both belong to G protein-coupled receptor family, but counteracts on the expression of cAMP-PKA pathway (Pennacchio *et al.*, 2017). By coupling with Gi protein, D2 receptor decreases the cAMP content in the hypothalamus and inhibit the production of PKA, thus reducing the phosphorylation level of proteins in cells and the PRL secretion (Gerlo *et al.*, 2005; Nakano *et al.*, 2010). In the present study, the number of dopamine D1 receptor in the hypothalamus was increased but not to a normal level in various bud of malt groups, indicating that the regulation of D1 receptor on PRL is not significant. The number of dopamine D2 receptor in the hypothalamus was significantly increased; in the bud of malt groups of 0.75~1.25 cm, it approached that in the normal group; the best effect of increase was observed in 0.75 cm bud of malt group. These findings suggest that the delectation effect of malt is mainly mediated by dopamine D2 receptor in the hypothalamus, thus inhibiting the production and secretion of PRL. However, further study is still required to determine the exact signaling pathways. It was confirmed in the preliminary study that alkaloids are the basic substances for the delactation effect of malt. In HPRL model rats, the serum PRL was regulated less after no administration of alkaloid extract (9.45 g/kg) than after the intragastric administration of alkaloid extract at a high dose (18.26 g/kg) and a low dose (4.56 g/kg) (Hu *et al.*, 2012).

In conclusion, we have proven that 0.75 cm bud of malt contains the highest level of total alkaloids, and demonstrates the strongest effect on the secretion and regulation of PRL in HPRL model rats. In terms of dose-effect relation, the content of alkaloids in malt is suggested to be added as an index into the quality control standard for malt. Based on our results, it is recommended that in the future industrialized processing operation, the bud length of malt should be about 0.75 cm, so as to ensure the effects of malt in the clinical application of traditional Chinese medicine.

CONFLICT OF INTEREST

Authors have declared that there is no conflict of interest.

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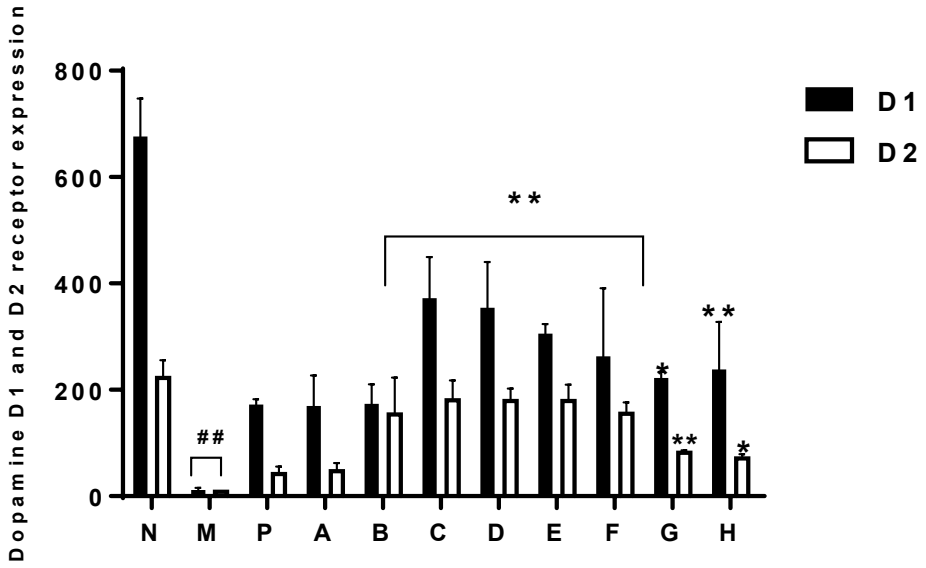

FIGURE 7 – The effects of decoction of different buds of malt on the expression of dopamine D1, D2 receptor in the hypothalamus gland of HPRL rat model. Rat model of HPRL was produced by back subcutaneous repeated injection with 75 mg/kg MCP, for 10 days, followed by treatment with or without 9.46 g/kg decoction of different buds of malt (A to H) or 0.4 mg/kg BMT (P) for 30 days. 0.9% salt solution treated rats were served as model group (M). Normal group were non-modeling and treated (N). At the completion of experimental treatment, tissues of hypothalamus gland of rats in each group were collected respectively. The expression of dopamine D1, D2 receptor was determined by immunohistochemistry. Data are expressed as mean ± SEM (n = 6). $^{\#\#}P < 0.01$ vs. normal group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. model group.

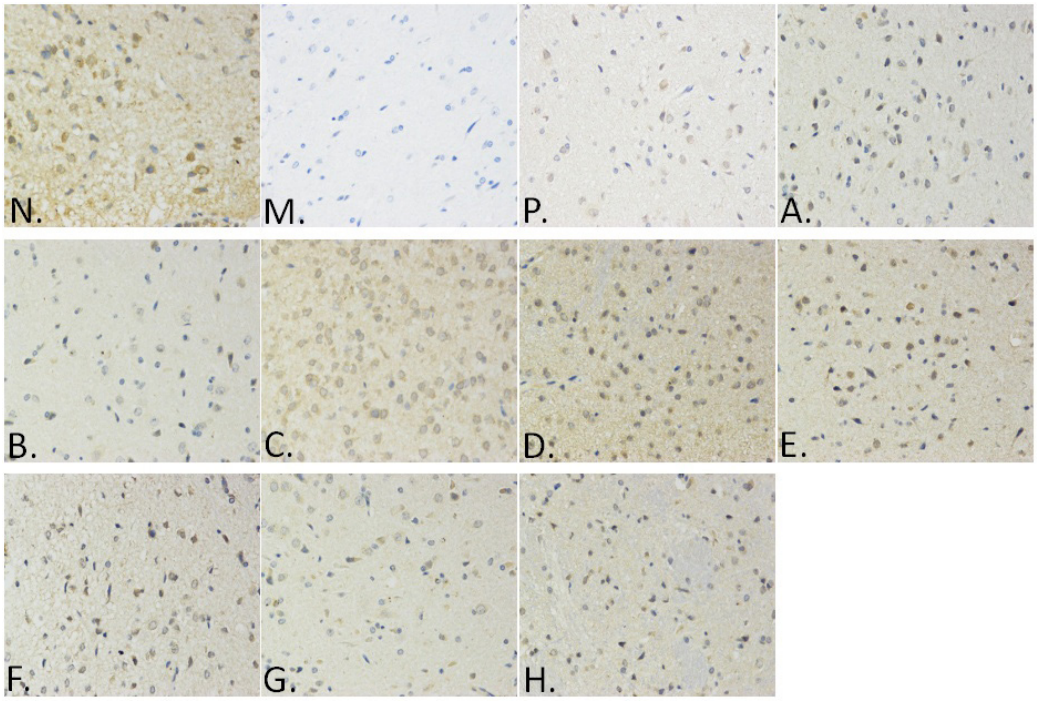

FIGURE 8 – Dopamine D1 receptor expression in the hypothalamus was observed by microscope (original magnification, 400×). The positive reaction of dopamine D1 receptor in the hypothalamus was brown in cytoplasm, and the darker the color was, the greater its positive expression was (N) normal controls; (M) model group; (P) bromocriptine group; (A) 0.25 cm bud of malt group; (B) 0.50 cm bud of malt group; (C) 0.75 cm bud of malt group; (D) 1.00 cm bud of malt group; (E) 1.25 cm bud of malt group; (F) 1.50 cm bud of malt group; (G) 1.75 cm bud of malt group; (H) 2.00 cm bud of malt group.

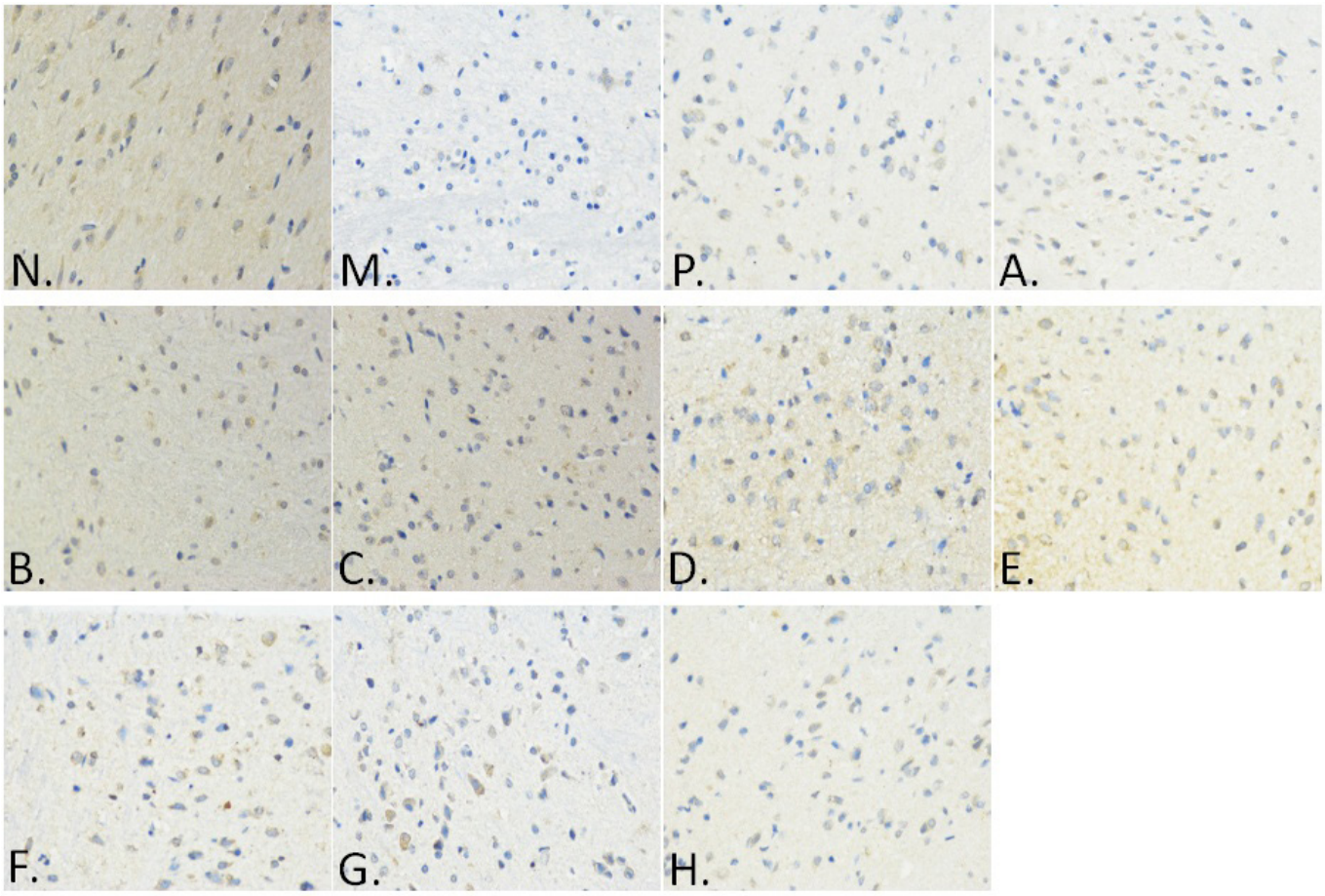

FIGURE 9 – Dopamine D2 receptor expression in the hypothalamus was observed by microscope (original magnification, 400×). The positive reaction of dopamine D2 receptor in the hypothalamus was brown in cytoplasm, and the darker the color was, the greater its positive expression was (N) normal controls; (M) model group; (P) bromocriptine group; (A) 0.25 cm bud of malt group; (B) 0.50 cm bud of malt group; (C) 0.75 cm bud of malt group; (D) 1.00 cm bud of malt group; (E) 1.25 cm bud of malt group; (F) 1.50 cm bud of malt group; (G) 1.75 cm bud of malt group; (H) 2.00 cm bud of malt group.

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