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The Effect of Maize Plumule Extracts on Rat Mammary Gland Hyperplasia

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Abstract: The goal of this study was to examine the effect of maize plumule extracts on mammary gland tissue and serum hormone levels in SD rats suffering from mammary gland hyperplasia and to investigate the mechanism by which maize plumule extracts inhibit mammary gland hyperplasia. About 56 rats were subcutaneously injected with progesterone combined with estradiol benzoate for the induction of mammary gland hyperplasia and were then randomly divided into 7 groups. Maize plumule extracts were subsequently given to the rats at doses of 0, 1.25, 2.5, 5, 10, 20 and 40 mg kg^{-1} daily for 20 days. Following treatment, hormone levels in the blood were measured and the morphology of the rat mammary glands was observed by microscopy. Results showed that high doses of maize plumule extracts significantly improved the morphology of lesional tissue and significantly reduced the levels of Follicle-Stimulating Hormone (FSH), serum Estradiol (E_2) and Testosterone (T) in the serum and increased the levels of Prolactin (PRL) and serum Luteinizing Hormone (LH). However, the treatment did not have a substantial effect on serum Progesterone (P) levels. It can be concluded that maize plumule extracts containing rich adenine derivatives administered at doses of 2.5-40 mg/kg/day could effectively prevent and treat rat mammary gland hyperplasia.

Key words: Maize plumule extracts, rat, mammary gland hyperplasia, oxidative damage, hormone level

INTRODUCTION

DNA damage and mutation are direct causes of breast cancer initiation (Mobley and Brueggemeier, 2004) and oxidative damage is a major form of DNA damage (Dizdaroglu *et al*., 2002; Jun *et al*., 2007; Bitiren *et al*., 2010; Mohamed *et al*., 2011; Momtaz and Abdullahi, 2012). A large amount of statistical data has revealed that mammary gland hyperplasia is closely related to breast cancer (Yu *et al*., 2010). Recent studies have demonstrated that exogenous purine substances protect against and repair DNA damage and may have an antioxidant effect on animals and plants (Che, 2004; Wyszko *et al*., 2003). Maize plumule extracts contain rich adenine derivatives and have been shown to cause silkworms to be resistant to oxidative skin damage (Zhang *et al*., 2005) and to have antioxidation effects on *in vitro*-cultured mouse dermal fibroblasts (Li *et al*., 2005). However, research using maize plumule extracts for the prevention of rat mammary gland hyperplasia is limited. Therefore, this study investigated the role of maize plumule extracts in rat mammary gland hyperplasia. This study provides a theoretical basis for the screening and application of drugs aimed at inhibiting mammary gland hyperplasia.

MATERIALS AND METHODS

Experimental materials: Maize plumule extracts were provided by the teaching lab of the Department of Veterinary Medicine at the Veterinary School of the Northwest Agricultural and Forestry University. The serial number of the extracts used in this study was 0501 and these extracts contained 93.33% adenine derivatives. A concentrated solution of maize plumule extract was prepared by dissolving maize plumule extracts in a special reagent (prepared by the teaching lab) at a concentration of 1000 mg L^{-1}. The solution was then diluted to various concentrations prior to use.

Experimental animals: In total, 100 SD rats were purchased from the Experimental Animal Center at the Fourth Military Medical University. The average body weight of the rats was 150±5 g.

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Reagents and instruments: The concentration of the estradiol benzoate injection solution was 2 mg mL^{-1} and the serial number of this solution was 20020101. The concentration of the progesterone injection solution was 20 mg mL^{-1} and the serial number was 20011102. Both reagents were manufactured by Shanghai General Pharmaceutical Co., Ltd. The analytical grade formalin, hematoxylin, eosin and anhydrous ethanol used were domestically manufactured and the Prolactin (PRL), Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Estradiol (E_2), Progesterone (P) and Testosterone (T) Radioimmunoassay (RIA) kits were purchased from Tianjin Biological Products Co., Ltd.

The FJ-6010 model automatic γ counter was manufactured by the state-owned Xi'an 262 Plant in China. The AO microtome was made in the USA and the OLYMPUS CX41 microscope was made in Japan.

Experimental methods

Rat Mammary Gland Hyperplasia Model: SD rats were randomly divided into either the A or B Group. Group A represented the mammary gland hyperplasia group and contained 80 rats in total. The rats were subcutaneously injected with 0.5 mg kg^{-1} estradiol benzoate daily for 20 days. Subsequently, the rats were then subcutaneously injected with 5 mg kg^{-1} progesterone daily for 5 days. Group B represented the control group and contained 20 rats. Saline was injected into the control rats in the same manner as the experimental group. The rats were allowed to recover for 35 days after treatment (Wyszko *et al*., 2003). Mammary gland tissue specimens were prepared from eight rats in Groups A and B. Tissues were fixed in formalin and embedded in paraffin and histological sections were observed under a microscope. The rats in Group A displayed ductal hyperplasia, acinar hyperplasia and cystic hyperplasia whereas the rats in Group B were all negative for hyperplasia indicating that the rat mammary gland hyperplasia model had been successfully established (Yu *et al*., 2010).

Experimental animal groups: The treated rats were divided into eight groups. Eight rats from Group B represented the normal controls (Group I) and were fed by gavage with 2 mL saline daily. From Group A, 56 rats were divided into 7 groups numbered II-VIII which each contained 8 rats. Group II was used as the Hyperplasia Model control; the rats in this group were fed by gavage daily with 2 mL of the saline that was used to dilute the concentrated maize plumule extract solution. Groups III-VIII were used as maize plumule extract treatment groups; the rats in these groups were fed by gavage daily with 2 mL of maize plumule extracts at concentrations of 1.25, 2.5, 5, 10, 20 and 40 mg kg^{-1}, respectively. All rats were fed for 20 days.

Morphological assessment and specimen preparation: The diameter and height of the second and third pair of nipples located near the SD rat groin were measured with a vernier caliper. About 3 days after the end of treatment, an eyeball was removed and blood was withdrawn from the rats at 4:30 pm. The blood samples were incubated at 37°C for 1 h and were then centrifuged at 3000 rpm min^{-1} for 15 min at a low temperature to separate the serum. Intact mammary gland tissue surrounding the rat groin was isolated and fixed in formalin.

Morphological observation and serum hormone level measurement: Mammary gland tissue was embedded in paraffin, sectioned and stained with Hematoxylin and Eosin (H and E). Sectioned slides were observed under the microscope. The levels of Prolactin (PRL), Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), Estradiol (E_2), Progesterone (P) and Testosterone (T) were measured from the serum by radioimmunoassay. This assay was performed strictly according to the manufacturer's instructions.

Data analysis: Significant differences between the groups were analyzed using the statistical analysis software SPSS Version 13.

RESULTS AND DISCUSSION

Effect of maize plumule extracts on the morphology of the SD rat nipple: As shown in Table 1, the nipple diameter and height of the rats in the hyperplasia model control Group II and the treatment Groups III-IV were significantly larger than those in normal control Group I ($p<0.01$). Group V also exhibited a mild significant difference compared with normal control Group I ($p<0.05$). However, Groups VI-VIII were not significantly different from normal control Group I. Compared with Hyperplasia Model

Table 1: Comparison of nipple diameter and height of SD rats in the eight experimental groups (n = 8, mean ±SE)

	Nipple diameter (mm)		Nipple height (mm)	
Groups	Second pair	Third pair	Second pair	Third pair
I	1.06±0.22^{B}	1.02±0.29^{B}	1.73±0.03^{B}	1.73±0.09^{B}
II	1.51±0.22^{A}	1.57±0.36^{A}	2.25±0.05^{A}	2.19±0.19^{A}
III	1.49±0.15^{A}	1.45±0.05^{A}	2.19±0.02^{A}	2.14±0.21^{A}
IV	1.41±0.16^{A}	1.40±0.11^{A}	2.06±0.08^{Ab}	2.03±0.07^{Ab}
V	1.28±0.04^{ab}	1.27±0.26^{ab}	1.82±0.04^{aB}	1.80±0.13^{aB}
VI	1.19±0.15^{b}	1.15±0.12^{b}	1.79±0.07^{B}	1.79±0.17^{B}
VII	1.07±0.25^{B}	1.09±0.32^{B}	1.77±0.01^{B}	1.77±0.09^{B}
VIII	1.07±0.12^{B}	1.06±0.17^{B}	1.76±0.03^{B}	1.76±0.12^{B}

Compared to the normal control Group I: $^{a}p<0.05$, $^{A}p<0.01$. Compared to Hyperplasia Model Group II: $^{b}p<0.05$, $^{B}p<0.01$

Group II, the nipple diameters of rats in treatment Groups V-VI were significantly decreased ($p<0.05$) and Groups VII and VIII exhibited an even more pronounced significant decrease ($p<0.01$). The nipple height of rats in treatment Group IV was significantly less than that of rats in Hyperplasia Model control Group II ($p<0.05$). The nipple height of rats in Groups V-VIII was significantly reduced compared to rats in Hyperplasia Model control Group II ($p<0.01$).

Effect of maize plumule extracts on the morphological histology of the rat mammary gland: From the microscopic observation of the rat mammary gland tissue sections (Fig. 1) and the data shown in Table 2, researchers found that the ductal epithelial cells of rats in normal control Group I were organized in a normal manner. The lumen was not expanded and there was no epithelial shedding inside the lumen. In addition, the nucleoli were unclear and there were 2-6 lobular acini (Fig. 1a). In hyperplasia model control Group II, ductal epithelial cells displayed substantial proliferation, a pseudostratified or papillary structure and prominent nucleoli. Furthermore, the ducts were dilated, shed epithelial cells and secretions were found inside the lumen and there were 19-28 lobular acini. There was also hyperplasia in the acinar epithelial cells, acini and the fibrous tissue surrounding the lobular area (Fig. 1b). Compared with Hyperplasia Model control Group II as the dose of maize plumule extracts increased, the incidence of ductal epithelial cell differentiation exhibiting mild to moderate anomalies was reduced in the treated groups. Additionally, the diameter of the nucleus for the treated groups was similar to that of the normal control Group I. Furthermore, nuclear atypia was reduced, chromatin and nucleoli did not show abnormal changes and regular mitotic patterns were occasionally observed. Treatment Groups VI-VIII also had a significantly reduced number of acini ($p<0.01$). The size of certain acini was decreased and ductal dilation was not substantial. Moreover, all of these features were similar to those of the normal control Group I (Fig. 1c-h).

Effect of maize plumule extracts on the hormone levels in serum: As shown in Table 3, the serum levels of E_2 in

Table 2: Comparison of the number of mammary ducts and lobular acini in rats

Groups	Number of mammary ducts	Number of lobular acini
I	2.99 ± 0.83^{B}	3.92 ± 1.11^{B}
II	8.51 ± 2.26^{A}	23.57 ± 4.38^{A}
III	7.60 ± 2.18^{A}	24.56 ± 3.07^{A}
IV	7.52 ± 1.79^{A}	21.51 ± 4.13^{A}
V	6.39 ± 1.97^{Ab}	14.38 ± 3.29^{AB}
VI	3.19 ± 1.13^{B}	5.15 ± 1.13^{B}
VII	4.09 ± 0.98^{B}	4.39 ± 1.34^{B}
VIII	4.07 ± 1.19^{B}	4.86 ± 1.16^{B}

Table 3: Changes in the serum hormone levels of rats with mammary gland hyperplasia

Groups	E_2 ($pg\cdot mL^{-1}$)	P ($ng\cdot mL^{-1}$)	T ($ng\cdot mL^{-1}$)	PRL ($ng\cdot mL^{-1}$)	LH ($IU\cdot L^{-1}$)	FSH ($IU\cdot L^{-1}$)
I	15.71 ± 2.230^{B}	7.08 ± 0.49^{B}	6.27 ± 1.24	1.98 ± 0.86	1.25 ± 0.05^{B}	12.96 ± 1.37^{B}
II	76.67 ± 14.62^{A}	2.19 ± 0.19^{A}	12.45 ± 2.42^{a}	1.92 ± 0.95	2.33 ± 0.13^{A}	28.51 ± 6.22^{A}
III	43.58 ± 24.39^{A}	2.14 ± 0.21^{A}	8.51 ± 0.71	2.57 ± 0.88	2.19 ± 0.02^{A}	10.49 ± 0.15^{B}
IV	41.39 ± 33.29^{Ab}	2.13 ± 0.07^{A}	6.48 ± 0.87^{B}	2.27 ± 0.47	2.06 ± 0.08^{Ab}	10.64 ± 0.29^{B}
V	28.64 ± 11.32^{B}	1.90 ± 0.13^{a}	6.53 ± 0.99^{B}	2.63 ± 0.72^{AB}	1.82 ± 0.04^{aB}	10.64 ± 0.29^{B}
VI	27.80 ± 7.300^{B}	2.29 ± 0.17	7.13 ± 1.12^{b}	2.84 ± 0.75^{AB}	1.79 ± 0.07^{B}	10.02 ± 0.26^{B}
VII	32.75 ± 4.180^{ab}	2.37 ± 0.09	6.56 ± 0.53^{B}	2.59 ± 0.48^{b}	1.77 ± 0.01^{B}	10.01 ± 0.18^{B}
VIII	24.95 ± 6.550^{B}	2.46 ± 0.12	5.98 ± 1.23^{B}	2.74 ± 0.36^{AB}	1.76 ± 0.03^{B}	9.65 ± 0.36^{B}

Compared to normal control Group I: $^{a}p<0.05$, $^{A}p<0.01$; Compared to hyperplasia model Group II: $^{b}p<0.05$, $^{B}p<0.01$

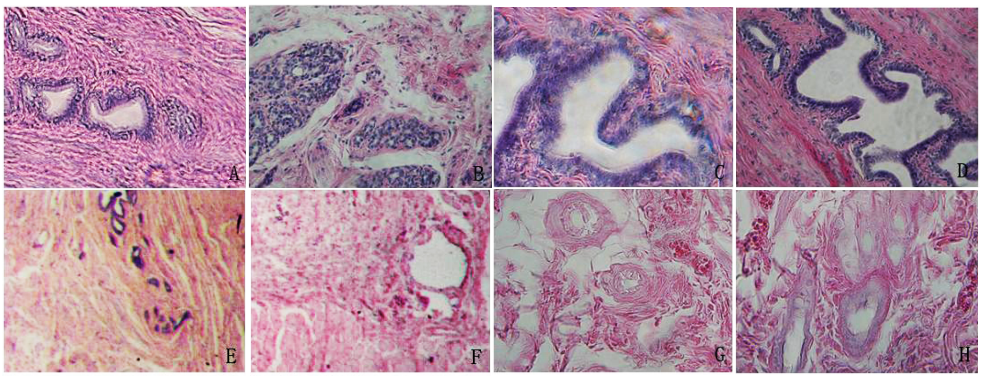

Fig. 1: H and E staining of SD rat mammary gland histological sections; A) Normal mammary gland Group I (H and E, 20x); B) Mammary gland hyperplasia model Group II (H and E, 20x); C-H) Mammary gland hyperplasia treated with different doses of maize plumule extracts (Groups III-VIII) (H and E, 20x)

Groups II-IV were significantly increased to a greater degree than were those of the normal control Group I. With the exception of Group VII as the dose of the maize plumule extracts increased, serum E_2 levels in the treated groups displayed significant differences. Compared with Hyperplasia Model Group II, the serum E_2 levels in treatment Groups IV-VIII were significantly decreased. The degree of the difference between Groups V-VIII was highly significant. The serum FSH levels in the treated groups were not significantly different from those of the normal control Group I. However, serum FSH levels were significantly reduced compared to those in Hyperplasia Model Group II. As the treatment dose increased, the degree of the reduced serum P levels in the treated groups changed from highly significant (III and IV) to significant (V) to not significant (VI-VIII) compared with normal control Group I however, no significant differences were observed with hyperplasia model control Group II. There were no significant differences between the serum T levels of Group III and those of hyperplasia model control Group II. Although, Group VI demonstrated a mildly significant reduction, all other groups displayed highly significant reductions in serum T levels compared to hyperplasia model control Group II. Serum PRL levels in the treated Groups (V-VIII) were significantly increased compared to hyperplasia model control Group II whereas serum LH levels in the treated Groups (V-VIII) were significantly decreased compared to hyperplasia model control Group II.

Previous studies have shown that maize plumule extracts which contain large amounts of purine derivatives and their metabolites have unique biological activities (Zhang *et al*., 2005). In addition, research has shown that maize plumule extracts can reduce peroxidase activity inhibit the production of oxygen free radicals, promote the synthesis of free amino acids into protein and protect DNA against oxidative damage (Wyszko *et al*., 2003). Maize plumule extracts also have dose-dependent anti-aging and cytokine activities and play a critical role in regulating human myeloid leukemia cell differentiation and apoptosis. These effects are mediated by reductions in intracellular ATP content during the process of cell differentiation and by the ability of the extracts to interfere with mitochondrial membrane potential and oxygen free radical accumulation (Ishii *et al*., 2002). Under normal physiological conditions in animals, oxidation and reduction reactions are maintained in a dynamic balance. Active oxygen species and antioxidant systems are the main factors that contribute towards maintaining this balance. The production of large amounts of oxygen free radicals or the weakening of antioxidant system function can lead to oxidative damage (Che, 2004). A study by Mobley and Brueggemeier (2004) demonstrated that both endogenous and exogenous estrogen can mediate DNA oxidative damage which can then directly initiate mammary gland hyperplasia and breast cancer. As researchers know, cancer remains one of the leading causes of death in developed countries for many reasons including non-selectiveness of commonly used anti-cancer drugs that often influence non-specific rather than tumour-specific targets. The reason is that cancer cells are characterized by the ability to divide and multiply in an uncontrolled manner whereby a set of specific proteins modulate cell division processes, proteomics seems to be a suitable tool for seeking out molecular mediators of anti-cancer drugs action and resistance thus the challenge today is to shift from the identification of drug response and disease biomarkers to more time-consuming process of revealing the biochemical mechanism that connects a specific protein with a disease or cellular response to a drug (Li *et al*., 2009; Wang *et al*., 2011; Yu *et al*., 2010; Zhang *et al*., 2006) The study has shown that maize plumule extracts can function as an exogenous hormone (Shi and You, 2005).

CONCLUSION

Researchers found that the administration of maize plumule extracts within a certain dose range inhibited mammary gland hyperplasia which was manifested as reduced nipple diameter; lower nipple height; the decreased incidence of lobular hyperplasia; lower ductal epithelial proliferation; significantly decreased levels of FSH, E_2 and T and increased levels of PRL and LH. However, the mechanism by which maize plumule extracts regulate and block estradiol-induced mammary gland hyperplasia, reduce the biological effects of estrogen on target cells and inhibit mammary gland hyperplasia requires further study.

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