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Improvement in post-partum uterine involution in rats treated with *Apios americana**

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Abstract: Objective: *Apios americana*, a plant used as a staple ingredient of native American diets, has various properties, including anti-cancer, anti-hyperglycemic, hypotensive, and anti-inflammatory activity. In Japan, Apios is used as a post-natal medication. After parturition, women undergo a period of recovery as they return to pre-pregnancy conditions. However, few health products that aid post-partum recovery are on the market. We explored whether Apios can accelerate the post-partum recovery process, in particular the involution of the uterus. Methods: Female rats kept in individual cages were mated with two male rats, with the exception of the control group (female rats without mating, on basal diet; *n*=6). After delivery, rats were divided into five groups based on their diet: basal diet (model; *n*=6); basal diet+oral intake at 5.4 g/kg of Chanfukang granules (a Chinese patent medicine preparation for post-partum lochia) (positive; *n*=6); basal diet containing 10% Apios powder (low; *n*=6); basal diet containing 20% Apios powder (medium; *n*=6); basal diet containing 40% Apios powder (high; *n*=6). Five days later, uteri and spleens were weighed. Uterus and spleen indices for each rat were calculated by dividing visceral weight by the total weight. Hormone and cytokine concentrations were measured using enzyme-linked immunosorbent assay (ELISA). Histological analysis of uteri was completed using hematoxylin and eosin (H&E) staining. Expression of matrix metalloproteinases and inhibitors in uteri was measured by western blotting. Results: Our results showed that Apios treatment reduced the post-partum uterus index and regulated the hormone concentrations. Moreover, we found that the process of uterine involution was accelerated, based on morphological changes in the uterus. In addition, our results indicated that Apios alleviated the inflammatory response induced by the involution process. Transforming growth factor β was also found to be regulated by Apios. There were significant downregulation of matrix metalloproteinases and upregulation of their inhibitors by Apios, which suggested that Apios increased the rate of the collagen clearance process. Conclusions: These results, based on experimental observations at the molecular and protein levels, verified our hypothesis that Apios can improve uterine involution, and demonstrated the potential application of Apios in post-partum care.

Key words: *Apios americana*; Uterine involution; Cytokine; Matrix metalloproteinase
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1 Introduction

The uterus undergoes dramatic changes throughout adult life, particularly during pregnancy. After delivery, the uterus begins remodeling to return to pre-pregnancy size and condition through the process

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of "uterine involution." However, impaired tissue processes may lead to gynecological complications, such as post-partum hemorrhage, infections, and placental abnormalities (Rosen, 2008; WHO, 2018). About 830 women die from preventable diseases related to pregnancy and childbirth globally every day. In 2015, roughly 303 000 women died during pregnancy and following childbirth (Alkema et al., 2016).

There is an urgent, unmet clinical need to reduce the incidence of maternal mortality and uterine subinvolution. Post-partum care is important for accelerating the process of involution and avoiding the appearance of post-partum complications, for instance, injecting oxytocin for promoting uterine contractions to avoid the risk of bleeding (Wang et al., 2017; WHO, 2018), or giving an adequate dose of iron to avoid anemia (WHO, 2015). However, few functional foods are available to aid post-partum uterine involution, although pineapple has been suggested as having a positive impact (Harianja et al., 2017).

Apios americana, a perennial vine with edible beans and tubers (Reynolds et al., 1988), was a plant used as a staple ingredient of native American diets. Now it is widely used in many countries, especially in Japan (Hoshikawa and Juliarni, 1995). It contains a high level of protein (Morales and Foster, 2009), good-quality starch (Kikuta et al., 2012), and a wide variety of fatty acids and minerals (Wilson et al., 1986; Kinugasa and Watanbe, 1992). In addition to the main nutrients, secondary metabolites, including isoflavones and saponins, are also found at high levels in Apios (Krishnan, 1998; Nara et al., 2011; Chitisankul et al., 2015). It is reported that Apios and its extracts have antioxidant activity (Takashima et al., 2013) and anti-cancer (Zhang et al., 2011), anti-hyperglycemic (Kawamura et al., 2015; Yan et al., 2017), hypotensive (Kuramoto et al., 2013), and anti-inflammatory effects (Cui et al., 2016). Apios can also be used as a post-natal medication and for fatigue relief (Hoshikawa and Juliarni, 1995). This study aimed to determine the influence and mechanism of action of Apios on the process of uterine involution.

During uterine involution, extracellular matrix (ECM) remodeling is required, including cell proliferation and apoptosis (Takamoto et al., 1998; Shkurupiy et al., 2011). Most common mediators known to be involved in uterine involution appear to be similar to those involved in menstruation (Salamonsen, 2003), including cytokines (Galvão et al., 2011), growth factors (Yoshii et al., 2014), and matrix-degrading enzymes (Takamoto et al., 1998; Nguyen et al., 2016). We hypothesized that Apios accelerates uterine involution. Therefore, in this study, we investigated endometrial and myometrial changes via direct measurements of uterine weight and hematoxylin and eosin (H&E) staining of post-partum rat uteri. We also investigated endocrine changes by determining the hormones, growth factors, and cytokines related to inflammatory responses in serum. Finally, we explored the activity of matrix metalloproteinases (MMPs) and the tissue inhibitor of metalloproteinase 1 (TIMP-1) at the protein level in uteri by western blotting.

2 Materials and methods

2.1 Sample preparation

Tubers of Apios farmed in Hangzhou, Zhejiang Province, China were used for this study. A ground powder was obtained from boiled tubers after hot air drying at 50 °C.

2.2 Animals

The experimental protocols were approved by the Ethics Committee of Laboratory Animal Care and Welfare, School of Medicine, Zhejiang University, Hangzhou, China. All experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Female Sprague-Dawley rats weighing 200–300 g (8–10 weeks old) were obtained from the Experimental Animal Centre of Zhejiang Academy of Medicine Sciences, Hangzhou, China, certificate No. SCXK(Zhe)2017-0001. The animals were housed in a breeding room at a controlled temperature (20–24 °C) and with a 12-h light/dark cycle. Animals were allowed free access to basal food and water before beginning the experiment. The female rats, kept in individual cages, were mated with two male rats with the exception of the control group (female rats without mating, on basal diet; n=6). The formation of a vaginal plug indicated the first day of pregnancy. After delivery, rats were divided into five groups based on their diet: basal diet (model; n=6); basal diet+oral intake at 5.4 g/kg of Chanfukang granules (a Chinese patent medicine preparation for post-partum lochia) (positive; n=6); basal diet containing 10% Apios powder (low; *n*=6); basal diet containing 20% Apios powder (medium; *n*=6); basal diet containing 40% Apios powder (high; *n*=6).

2.3 Tissue collection

Rats in the six groups were given free access to the above experimental diets for five days. During the experiment, body weight and dietary intake were measured. After five days of dietary intervention, the rats were fasted for 12 h and then anaesthetized after intra-peritoneal injection of 10% chloral hydrate (0.3 mL/100 g), and then blood samples from the abdominal artery were collected into tubes and centrifuged to separate the serum. Uteri and spleens were also promptly removed and weighed. Uterus and spleen indices were calculated as the visceral weight measurements divided by the total weight of each animal.

2.4 Enzyme-linked immunosorbent assays (ELISA)

Serum concentrations of estrogen (E), progesterone (P), tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), IL-6, immunoglobulin A (IgA), IgG, transforming growth factor β1 (TGF-β1), and TGF-β3 were analyzed using commercially available assay kits (Elabscience Biotechnology, Wuhan, China).

2.5 Histological analysis

Half of the “Y” type uteri were immediately fixed in 10% buffered paraformaldehyde for 24 h at 4 °C. Subsequently, standard pieces of uteri were embedded in paraffin after sequential dehydration in a graded ethanol series, and sliced into 5-μm thick sections before being stained with H&E stain. After H&E staining, the thickness of the endometrium was measured using a light microscope with a calibrated ocular scale.

2.6 Western blot analysis

Total uterine protein extracts were prepared using a radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, China) according to the instructions of the manufacturer. The homogenate was centrifuged at 9168*g* for 10 min at 4 °C. The supernatant was collected and the protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit (Solarbio Science & Technology Co., Ltd., Beijing, China). Total protein was denatured in 5× sodium dodecyl sulfate-polyarylamide gel electrophoresis (SDS-PAGE) Sample Loading Buffer (Beyotime Biotechnology, Shanghai, China) at 100 °C. Equal amounts of proteins were separated by SDS-PAGE and then transferred to Immobilon®-P polyvinylidene difluoride (PVDF) transfer membranes (Merck Millipore Ltd., Massachusetts, USA). The membranes were blocked with 5% low fat dried milk and incubated with rabbit-derived monoclonal antibody to MMPs (MMP2, 1:1000; MMP7, 1:1000; MMP9, 1:1000; MMP13, 1:500; MMP14, 1:5000), TIMP-1 (1:250), and β-actin (1:1000), followed by goat-anti-rabbit-IgG coupled to horseradish peroxidase (1:10000). The bound antibody was detected using an enhanced chemiluminescence (ECL) detection system. β-Actin was used as a loading control. Densitometry analysis was performed using ImageJ 1.48v software (Wayne Rasband, National Institutes of Health, USA).

2.7 Statistical analysis

The results were expressed as mean±standard deviation (SD). Data from the ELISA and western blot tests were analyzed using a one-way analysis of variance (ANOVA) with Duncan's multiple range test and *P* values of <0.05 were considered to be statistically significant.

3 Results

3.1 Effects of Apios on body weight gain and food intake

Body weight and food intake were recorded during the experiment (Table 1). There were no significant changes in body weight among the different groups during the five days of the experiment. Moreover, Apios intervention did not affect the food intake of the rats.

3.2 Decreases in the uterus and spleen indices by Apios treatment

One of the most significant phenomena during pregnancy is the enlargement of the uterus. We determined the weight of uteri at post-partum Day 5 (Fig. 1a). The uterus indices of the model and positive groups were higher than that of the control group. However, low, medium, and high doses of Apios intervention significantly reduced the index, especially in the high dose Apios group. We also tested the spleen index (Fig. 1b). The spleen index of the model group was significantly higher than that of the control group. The spleen indices of the positive, low, medium, and high groups were lower than that of the model group, which indicated that Apios can reduce spleen enlargement caused by parturition.

Table 1 Body weight and food intake of Sprague-Dawley rats

Group	Initial weight (g)	Final weight (g)	Food intake (g/d per rat)
Model	393.2±42.3	412.5±24.0	40.1±5.1
Positive	410.5±31.3	420.5±13.4	42.2±5.5
Low	399.2±26.6	406.7±25.7	39.4±3.1
Medium	408.0±16.0	400.8±24.2	38.6±3.7
High	392.5±44.7	392.7±29.1	39.2±2.9
Control	401.7±18.8	407.7±28.1	39.1±1.3

Model, post-partum rats on basal diets; Positive, post-partum rats on a basal diet plus 5.4 g/kg of Chanfukang granules; Low, post-partum rats on a basal diet containing 10% Apios powder; Medium, post-partum rats on a basal diet containing 20% Apios powder; High, post-partum rats on a basal diet containing 40% Apios powder. Control, non-pregnant rats on a basal diet. Values are presented as mean±SD (*n*=6)

3.3 Regulation of serum sex hormone concentrations by Apios treatment

To determine the effects of Apios on serum sex hormone concentrations in post-partum rats, we determined the estrogen and progesterone concentrations in serum. Estrogen secretion was higher in the low, medium, and high groups than in the model group (Fig. 1c). In contrast, progesterone secretion was lower in the low and medium groups (Fig. 1d). Though the two sex hormone concentrations showed different trends, the concentrations of both tended to return to the non-pregnant situation.

3.4 Improvements and histological changes in the post-partum uterine horn by Apios treatment

We investigated the effect of Apios on tissue repair processes in post-partum uteri (Fig. 2). In the control group, uterine cavity morphology was normal, the endometrium was thick, and myocytes were arranged neatly (Figs. 2a and 2b). This was in contrast to tissue from of the model group in which the uterine cavity morphology was abnormal, the endometrium was thinner, and the arrangement of myocytes was

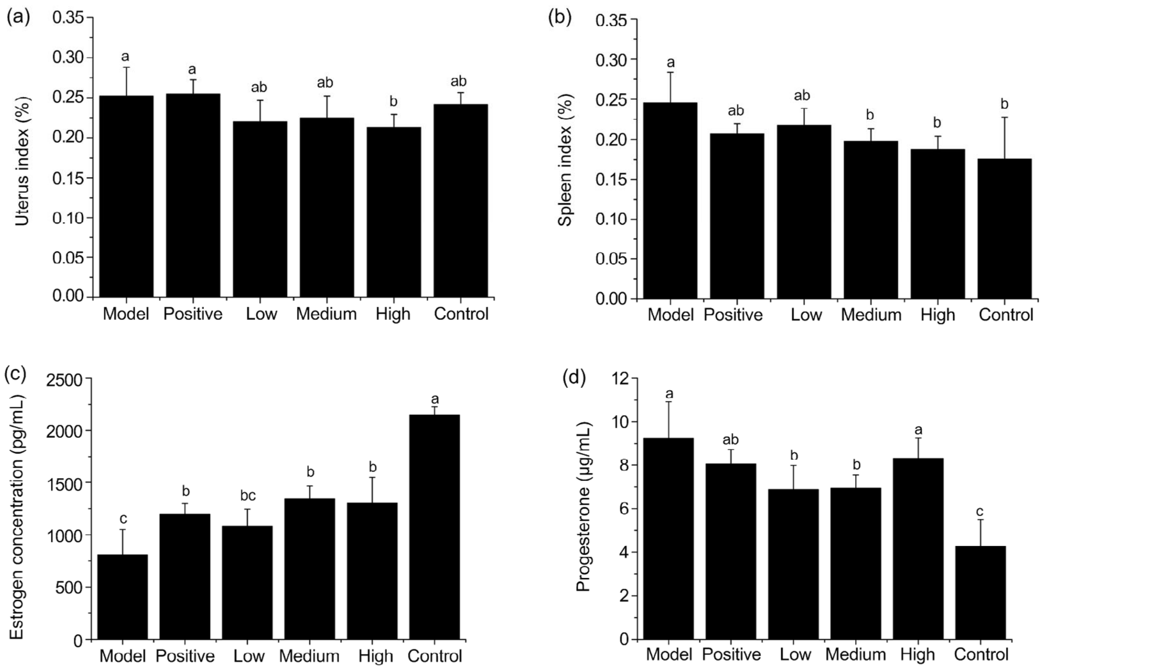

Fig. 1 Regulation of the uterus index, spleen index, and concentrations of sex hormones by Apios treatment

(a) Uterus index on post-partum Day 5. (b) Spleen index on post-partum Day 5. Both indices were calculated by dividing visceral weight measurements by the total rat weight. (c) Estrogen concentrations in fasting serum on post-partum Day 5. (d) Progesterone concentrations in fasting serum on post-partum Day 5. Model, post-partum rats on a basal diet; Positive, post-partum rats on a basal diet plus 5.4 g/kg of Chanfukang granules; Low, post-partum rats on a basal diet containing 10% Apios powder; Medium, post-partum rats on a basal diet containing 20% Apios powder; High, post-partum rats on a basal diet containing 40% Apios powder; Control, non-pregnant rats on a basal diet. Values are presented as mean±SD (*n*=5 or 6 per group). Bars with different letters are significantly different among the experimental groups ($P<0.05$)

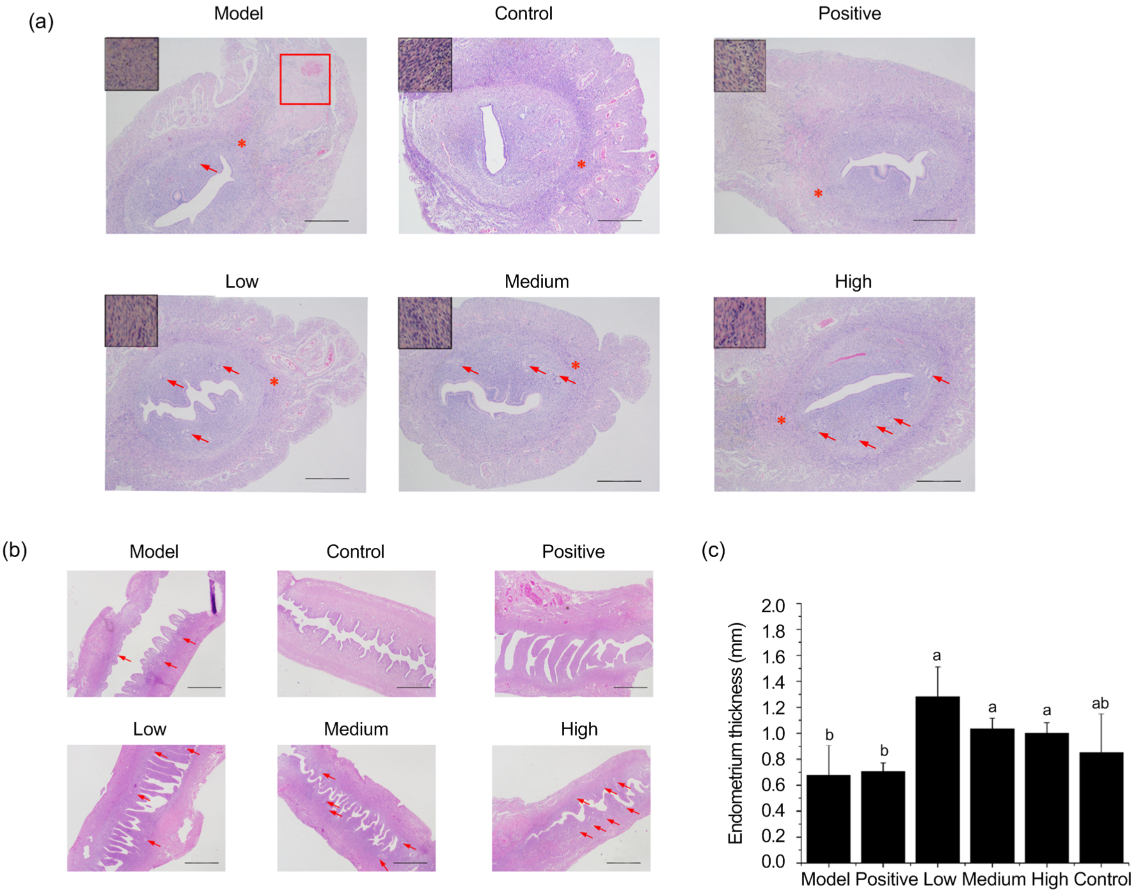

Fig. 2 Changes in uterine histology in post-partum rats following Apios intervention

(a) H&E staining on cross sections of the uterus on post-partum Day 5 (bar=500 μm). (b) H&E staining on lengthwise sections of the uterus on post-partum Day 5 (bar=1000 μm). (c) The thickness of the endometrium in lengthwise sections of the uterus on post-partum Day 5. The area of vascular hyalinization is indicated by the box. The areas of gland proliferation are indicated by arrows. The upper left corner of the thumbnail is an enlarged view of the location of the asterisk. Model, post-partum rats on a basal diet; Positive, post-partum rats on a basal diet plus 5.4 g/kg of Chanfukang granules; Low, post-partum rats on a basal diet containing 10% Apios powder; Medium, post-partum rats on a basal diet containing 20% Apios powder; High, post-partum rats on a basal diet containing 40% Apios powder; Control, non-pregnant rats on a basal diet. Values are presented as mean±SD (n=4). Bars with different letters are significantly different among the experimental groups (P<0.05)

irregular. Moreover, there was a large area of vascular hyalinization (Fig. 2a). After Apios intervention, uterine cavity morphology tended to be normal, the endometrium was thicker than that in the model group, and myocytes began to line up. In addition, the number of glands had increased and the level of vascular hyalinization had decreased. We also determined the thickness of the endometrium on lengthwise sections of uteri. After Apios intervention, the endometrium became thicker than that of the model group (Fig. 2c).

3.5 Alleviation of inflammation by Apios treatment

To understand whether Apios treatment can alleviate the inflammatory response in uterine involution, we examined proinflammatory cytokines and immunoglobulins in serum. TNF-α, IL-1, IL-6, IgA, and IgG concentrations in the model group were significantly higher than those in the control group (Fig. 3). TNF-α secretion in the positive group was similar to that in the model group (Fig. 3a), but was down-regulated by Apios intervention. The same trends were observed for IL-1 (Fig. 3b) and IL-6 (Fig. 3c), which indicated that Apios can reduce the concentrations of proinflammatory cytokines. Serum concentrations of IgA (Fig. 3d) and IgG (Fig. 3e) in the Apios-treated group were lower than those in the model group, indicating that Apios can reduce the intensity of the immune response.

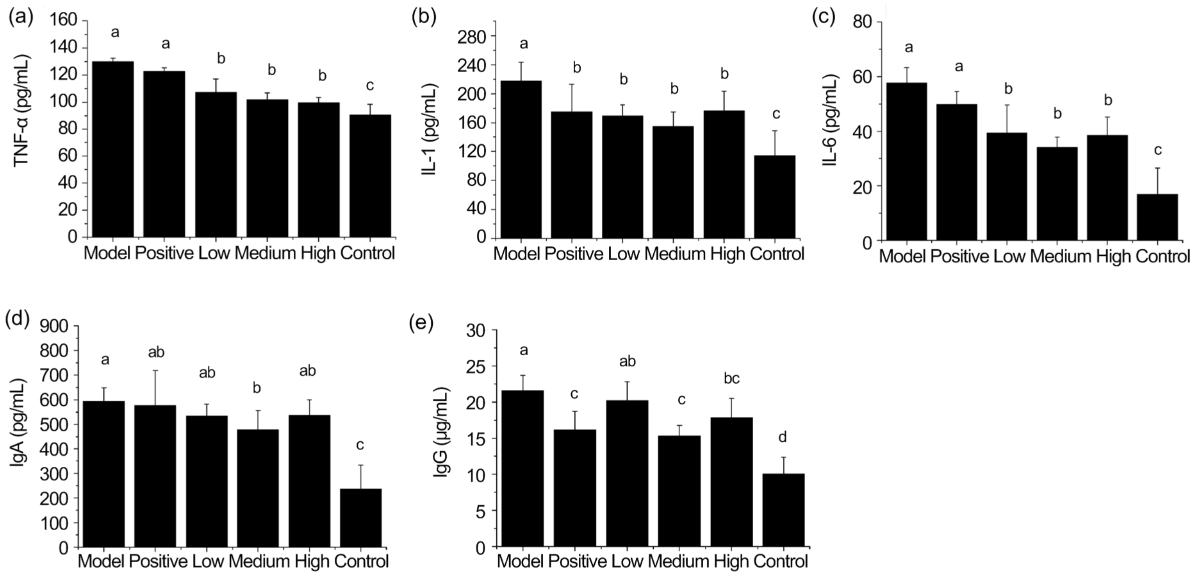

Fig. 3 Regulation of the concentrations of proinflammatory cytokines and immunoglobulins by Apios treatment

(a) Tumor necrosis factor α (TNF-α) concentrations in fasting serum on post-partum Day 5. (b) Interleukin 1 (IL-1) concentrations in fasting serum on post-partum Day 5. (c) IL-6 concentrations in fasting serum on post-partum Day 5. (d) Immunoglobulin A (IgA) concentrations in fasting serum on post-partum Day 5. (e) IgG concentrations in fasting serum on post-partum Day 5. Model, post-partum rats on a basal diet; Positive, post-partum rats on a basal diet plus 5.4 g/kg of Chanfukang granules; Low, post-partum rats on a basal diet containing 10% Apios powder; Medium, post-partum rats on a basal diet containing 20% Apios powder; High, post-partum rats on a basal diet containing 40% Apios powder; Control, non-pregnant rats on a basal diet. Values are presented as mean±SD (n=5 or 6). Bars with different letters are significantly different among the experimental groups (P<0.05)

3.6 Regulation of transforming growth factor secretion by Apios treatment

To further explain the effect of Apios on the process of uterine involution, we tested the concentrations of transforming growth factors related to tissue repair. The serum concentrations of TGF-β1 in the Apios-treated groups were higher than that in the control group (Fig. 4a), but lower than that in the model group. However, the trends for TGF-β3 were opposite (Fig. 4b). After Apios intervention, a trend towards an increase in TGF-β3 secretion was observed. The ratio of TGF-β3 to TGF-β1 was also significantly increased by Apios treatment (Fig. 4c).

3.7 Improvement in the levels of matrix metalloproteinases and inhibitors in post-partum uteri by Apios treatment

To elucidate the role of the degradation of collagen in post-partum uteri influenced by Apios, the expression of various MMPs and TIMP was analyzed semi-quantitatively by western blotting. The content of MMP9, MMP2, and proMMP2 in the Apios treatment groups was lower than that in the model group (Figs. 5b and 5c). The levels of MMP7, MMP13, and MMP14 in the Apios treatment groups were also lower than those in the model group (Figs. 5d–5f). However, the level of TIMP-1 increased after Apios intervention (Fig. 5g).

4 Discussion

In women, the uterus is known to undergo physiological involution during a period of 6–8 weeks after delivery. Accelerating the involution process can be a way to avoid post-partum complications. In our study, we established an experimental animal model to prove our hypothesis that treatment with Apios can improve uterine involution. We found that Apios decreased uterus and spleen indices, and regulated sex hormones in post-partum rats. Moreover, Apios improved the morphological changes in post-partum uteri. The molecular mechanisms involved may include the alleviation of inflammation, the

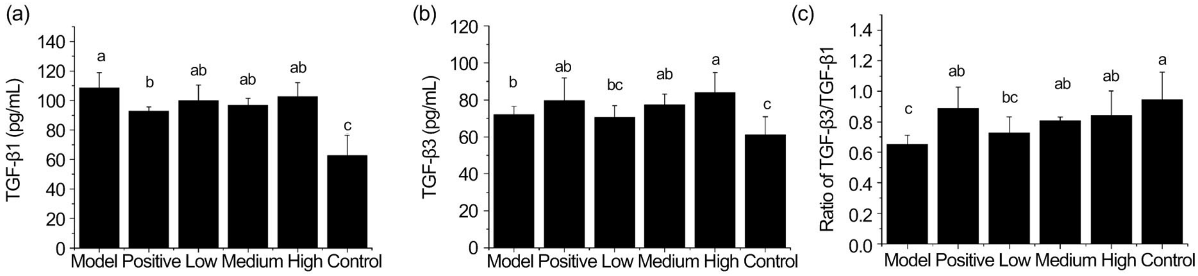

Fig. 4 Regulation of concentrations of transforming growth factor by Apios treatment

(a) Transforming growth factor β1 (TGF-β1) concentrations in fasting serum on post-partum Day 5. (b) Transforming growth factor β3 (TGF-β3) concentrations in fasting serum on post-partum Day 5. (c) The ratio of TGF-β3 to TGF-β1 in serum levels on post-partum Day 5. Model, post-partum rats on a basal diet; Positive, post-partum rats on a basal diet plus 5.4 g/kg of Chanfukang granules; Low, post-partum rats on a basal diet containing 10% Apios powder; Medium, post-partum rats on a basal diet containing 20% Apios powder; High, post-partum rats on a basal diet containing 40% Apios powder; Control, non-pregnant rats on a basal diet. Values are presented as mean±SD (*n*=5 or 6). Bars with different letters are significantly different among the experimental groups (*P*<0.05)

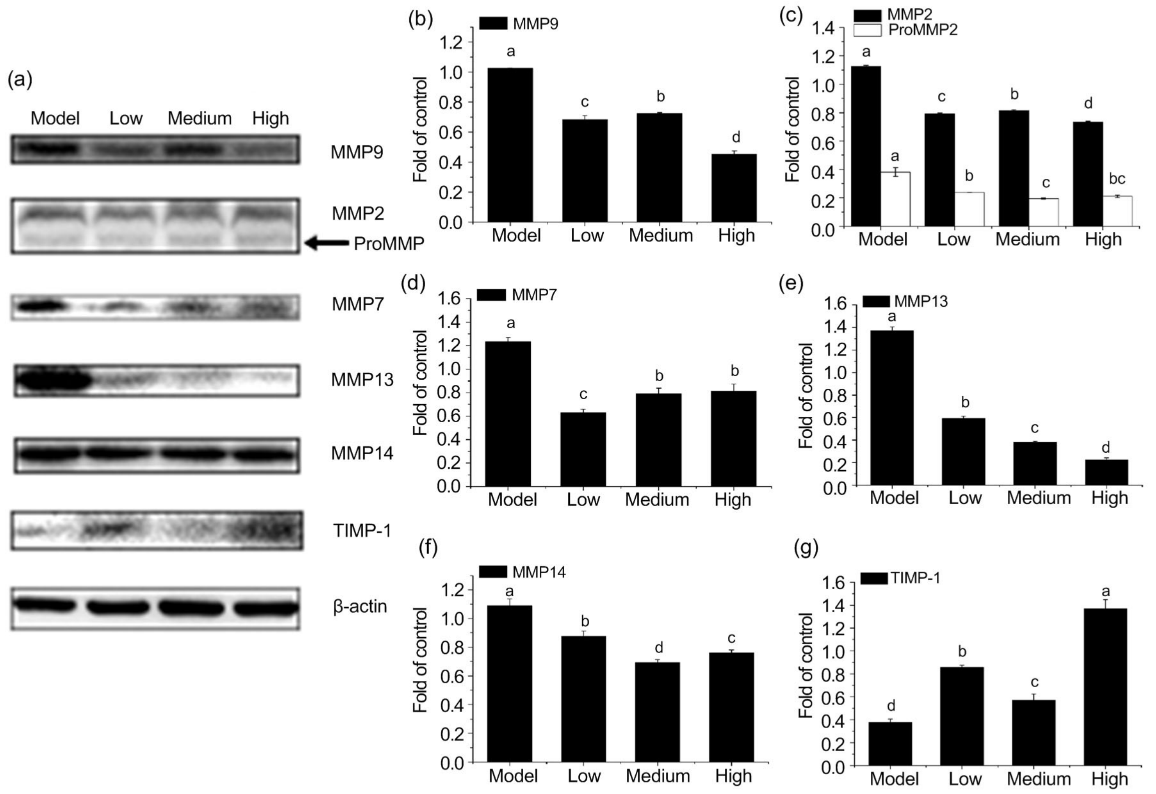

Fig. 5 Regulation of the protein expression of matrix metalloproteinases (MMPs) and its inhibitor (TIMP-1) in post-partum uteri by Apios treatment

(a) Protein levels of MMP9, MMP2/proMMP2, MMP7, MMP13, MMP14, and TIMP-1 on post-partum Day 5. The intensity of bands corresponding to MMP9 (b), MMP2/proMMP2 (c), MMP7 (d), MMP13 (e), MMP14 (f), and TIMP-1 (g) in the uterine horn on post-partum Day 5 was corrected for β-actin (control). Model, post-partum rats on a basal diet; Low, post-partum rats on a basal diet containing 10% Apios powder; Medium, post-partum rats on a basal diet containing 20% Apios powder; High, post-partum rats on a basal diet containing 40% Apios powder. Values are presented as mean±SD (*n*=5 or 6). Bars with different letters are significantly different among the experimental groups (*P*<0.05) regulation of transforming growth factors, and a reduction in levels of MMPs. Several lines of evidence suggest that Apios may be a potential food for the improvement of uterine involution.

Firstly, after Apios treatment, the degree of uterine remodeling was accompanied by an accelerated reduction in uterine weight and improvements in hormone levels. Uterine involution involves tissue destruction and subsequent repair (Alan and Liman, 2012). Changes in the size of the uterus are the most intuitive indicator of the process. Apios treatment can accelerate a return to normal uterine weight, especially at high doses. Sex hormone levels, especially estrogen concentrations, are coincident with endometrial repair (Ferenczy et al., 1979). In the rat, serum concentrations of estradiol and progesterone decrease rapidly after delivery, but begin to rise on Day 2 post-partum (Takamoto et al., 1998). Our results showed that the serum concentrations of estrogen in the Apios treatment group were higher than that in the model group. Estrogens appear to be a major requirement for the growth of mammary ducts (Grota and Eik-Nes, 1967). Therefore, Apios may help lactation. Grota and Eik-Nes (1967) found a slight decrease in serum progesterone concentrations from Day 3 post-partum. Our results indicated that Apios intervention may help speed up this process (Fig. 1d).

Secondly, Apios helped post-partum uterine reconstruction as shown by histology. After parturition, the endometrium and myometrium begin remodeling (Al-Bassam et al., 1981; Gray et al., 2001). The glands in the endometrium increase and smooth muscle cells begin to regain ordinary form and orientation (Henell et al., 1983). Our results showed that Apios treatment increased the numbers of glands, thickened the endometrium, and promoted the orderly arrangement of muscle cells. In addition, in the sub-involuted uterus, vessels are often filled with red blood cells and thrombi, the walls of which show deposition of hyaline (Petrovitch et al., 2009). Apios intervention could prevent this phenomenon (Fig. 2).

Thirdly, Apios alleviated the secretion of proinflammatory cytokines. The uterus is sterile during the whole of pregnancy, but after parturition, microorganisms may easily contaminate the uterine lumen, as observed frequently in cattle (Griffin et al., 1974; Sheldon et al., 2002). From Day 0 (the parturition day) to Day 14, the number of uterine bacteria increased, and then the bacteria declined to normal levels within 60 d (Elliott et al., 1968). During this period, contamination stimulated the immune response with phagocytes recruited to the uterine lumen, releasing proinflammatory cytokines, such as TNF-α, IL-1, and IL-6. These cytokines can cause an acute phase protein response to further increase phagocyte mobilization (Sheldon, 2004). These changes indicated that these proinflammatory cytokines will decrease to normal levels when the uterine involution process is completed.

Galvão et al. (2011) found that from Week 1 to Week 7 post-partum, gene expression of proinflammatory cytokines (TNF-α, IL-1β) in the endometrium of normal cows decreased with time. IL-6 gene expression changed slightly. Kasimanickam et al. (2013) found that serum concentrations of IL-1β decreased after one week and whereas IL-6 concentrations did not change significantly. However, TNF-α concentrations decreased during the first five weeks and increased at Week 7 in normal post-partum cows, which was contrary to these observed inflammatory conditions. This may be because cows in those groups had undergone body condition loss, causing an adipocyte-induced increase in TNF-α after five weeks.

In our study, serum concentrations of TNF-α, IL-1, and IL-6 were lower in the Apios-treated groups than in the model group, which indicated that Apios intervention can inhibit pathogenic proliferation. Immunoglobulin concentrations in uterine secretions reflect the microbial challenge and the clinical recovery conditions in the endometrium (Azawi, 2008). LeBlanc et al. (1988) found that IgA and IgG concentrations in post-partum uterine fluids significantly decreased after one week. In the present study, we found that serum concentrations of IgA and IgG in the Apios intervention groups were lower than those in the model group, which also accounted for the alleviation of inflammation following Apios treatment.

Fourthly, uterine tissue reconstructed following Apios treatment was associated with some peptides responsible for tissue repair. TGF-β is a pleiotropic cytokine belonging to the TGF-β superfamily that includes three different isoforms (TGF-β1–3) (O'kane and Ferguson, 1997). TGF-β1 and TGF-β3 are important in stimulating wound contraction. It was reported that skin wounds without scars exhibited high concentrations of TGF-β3 and low concentrations of

TGF-β1 (Lin et al., 1995; Soo et al., 2003), while an increased ratio of TGF-β1/β3 reduced scarring (O'kane and Ferguson, 1997). Our results also showed that TGF-β1 and TGF-β3 were important biological factors involved in the uterine involution process following Apios intervention.

Finally, Apios regulated the levels of MMPs and inhibitors in the post-partum uterus. After parturition, the involuting uterus goes through a rapid reduction in size which requires remodeling of the ECM, mainly the collagen (Curry and Osteen, 2003). MMPs are Zn-dependent endopeptidases that can degrade most components of the ECM at neutral pH (Nagase et al., 2006; Murphy and Nagase, 2008). In addition to various types of collagens, MMP2 and MMP9 can cleave the gelation into small peptides (Shimizu et al., 1983). These appear to increase on the day of labor, and then decrease 4 or 5 d after parturition (Manase et al., 2006; Nguyen et al., 2016). Our results showed that Apios treatment accelerated the reduction in MMP2 and MMP9 content in post-partum uteri.

The activity of rat MMP7 was first identified in the post-partum uterus. MMP7 can degrade a wide range of gelatins, proteoglycans, and glycoproteins of the matrix (Woessner, 1996). It was shown to reach a peak at 1–2 d after parturition, and then decrease (Sellers and Woessner, 1980). The level of MMP7 was lower in the Apios treatment groups than in the model group (Fig. 5d). Collagenase activity reaches a maximum within 24 h and decreases slowly to low values at 5 d post-partum (Woessner, 1979). In the rat uteri, we also detected MMP13, which is known to be orthologous to human interstitial collagenase-1 (Wolf et al., 1996). We found that Apios accelerated the decline of MMP13. MMP14 causes the degradation of collagen types I, IV, and V, and is likely the activator of proMMP2 (Gaide Chevronnay et al., 2012). Our results also showed that trends of proMMP2 content were similar to those of MMP14. In contrast to MMPs, TIMP-1 binds to almost all MMP active sites (Umenishi et al., 1991; Willenbrock and Murphy, 1994), preferring to bind to proMMP9 (Hulboy et al., 1997). Apios treatment elevated the levels of TIMP-1 to inhibit all the MMPs (Fig. 5g).

One limitation of the current study was that the addition of Apios to the diet may have led to an imbalance of energy or protein content in Apios groups. Though functional components of Apios helped uterine involution, protein availability or energy may play a role in the process.

5 Conclusions

In summary, we have verified our hypothesis that post-partum recovery, particularly uterine involution, was improved by Apios treatment. We investigated hormone levels, morphological changes, and levels of various cytokines and enzymes, finding that Apios accelerated several processes related to uterine involution. Understanding the influence of Apios on the uterine healing process can provide direct evidence that it may be a potential functional food for post-partum females. Results from the current study suggest that the active ingredient in Apios is heat-stable. Hence, identifying the active ingredient and its properties would be important, and may provide clues as to its nature.

Contributors

Zi-huan ZHENG performed the experimental research and data analysis, wrote and edited the manuscript. Ying HAN contributed to the experimental research and data analysis. Shi-ying YOU and Zuo CHEN contributed to the experimental research. Xiao-dong ZHENG contributed to the study design and editing of the manuscript. All authors read and approved the final manuscript and, therefore, had full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Zi-huan ZHENG, Ying HAN, Shi-ying YOU, Zuo CHEN, and Xiao-dong ZHENG declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：美国豆芋加快产后大鼠的子宫修复

目　的：研究美国豆芋（*Apios americana*）对产后恢复过程，特别是子宫复旧的影响。

创新点：开发针对产后恢复的新食品。

方　法：将雌雄大鼠配种交配，对照组除外（没有交配的雌性大鼠，基础日粮，*n*=6）。分娩后，将雌性大鼠分为五组：基础饮食（模型组，*n*=6）；基础饮食+灌胃给药 5.4 g/kg 的产妇康颗粒（阳性组，*n*=6）；含 10%豆芋粉末的基础日粮（低剂量组，*n*=6）；含 20%豆芋粉末的基础日粮（中剂量组，*n*=6）；含 40%豆芋粉末的基础日粮（高剂量组，*n*=6）。实验结束后，称量子宫和脾脏质量，计算子宫和脾脏指数。并测量血清内激素和细胞因子水平，研究子宫的组织病理学变化，检测基质金属蛋白酶和抑制剂在子宫中的表达。

结　论：豆芋干预减少了产后子宫指数并调节了激素浓度，加速了子宫形态复旧的过程，减轻了分娩引起的炎症反应。同时发现，转化生长因子 β 也受豆芋调节。豆芋干预使得基质金属蛋白酶显著下调，其抑制剂则显著上调，这表明豆芋增加了胶原清除过程的速度。

关键词：美国豆芋；子宫复旧；细胞因子；基质金属蛋白酶