



# Reduction of Melanin in Melanocytes, Anti-inflammatory Effect on Macrophages, Soothing Effect on Mast Cells and AGEs clearance in bovine serum cells of Moutan Extract

LITTLE DREAM GARDEN

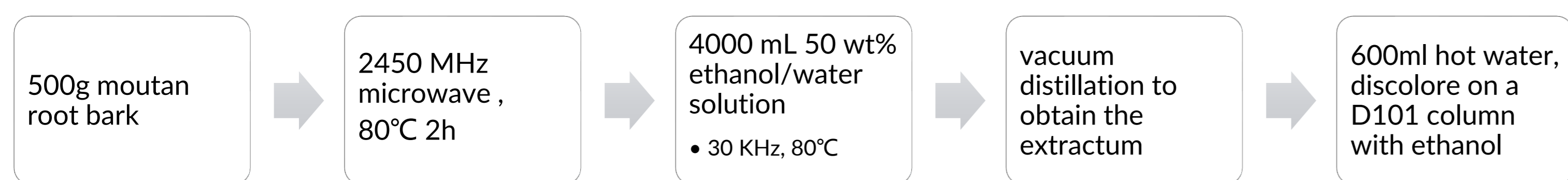
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## Introduction:

Moutan, considered as the "national flower" of China since ancient times, prefers a warm, cool, dry and sunny environment. China is particularly rich in moutan resources, with various varieties of moutan distributed throughout the country. The cultivation area of moutan is the largest and most concentrated in Heze, Luoyang, Beijing, Linxia, Pengzhou, Tongling, etc. The main chemical components in moutan bark are mainly galanyl glucose, benzoic acid, paeonol, paeoniflorin and flavonoids, among which the main active components are paeoniflorin, paeonol and flavonoids. Traditional Chinese medicine describes moutan as having the function of clearing heat, cooling blood, promoting blood circulation and removing blood stasis. Modern studies show that moutan root peel has antibacterial, anti-inflammatory, anti-allergy, anti-tumor, hemostasis, dispelling blood stasis, clearing heat and detoxification, sedation, analgesia, spasmolysis and other activities, but also can promote monocyte phagocytosis, improve the body specific immune function, increase the weight of immune organs. The current research on moutan root bark is mostly focused on its application in medicine, but less on its role in the cosmetics industry. In order to explore whether moutan root bark has some skin care effects, a series of studies have been carried out.

## Materials & Methods:

### EXTRACTION PROCESS



### CELL TESTS

1. Cytotoxicity test  
MTT, micro plates spectrophotometer.
2. Mast cell-based soothing test  
C48/80, IPP.
3. Measurement of melanin content in melanocytes  
405 nm, absorbance value.
4. Expression of Rac1, Cdc42, gp100 and TRP1 in melanocytes  
RNAiso, PCR.
5. Anti-inflammatory experiment of macrophages  
IL-6, TNF- $\alpha$ , NO, ELISA kit.
6. Inhibition rate of non-enzymatic glycosylation  
370 nm, 440 nm, fluorescence microplate analyzer.

## Results & Discussion:

### 1. Mast cell-based soothing test

As can be seen from Fig.-2, the concentration range of 0.3125-0.625% moutan extract can effectively reduce the degranulation rate of mast cells induced by C48/80 stimulation, and the lowest degranulation rate of mast cells is 25.86% when the concentration of moutan root bark extract is 0.625%.

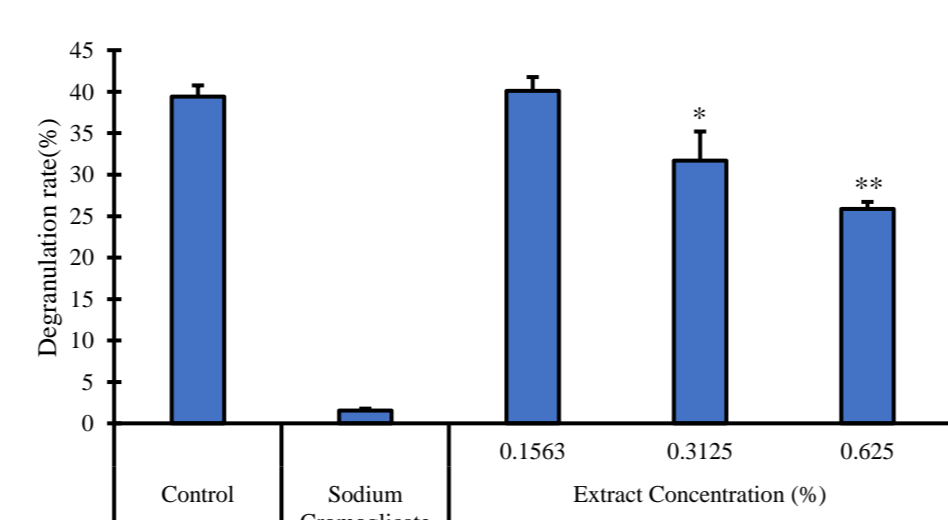


Fig.-2 Effects of sodium cromoglicate and different concentrations of moutan root bark extract on the degranulation rate of mast cells

## Results & Discussion:

### 2. Effects on melanocytes

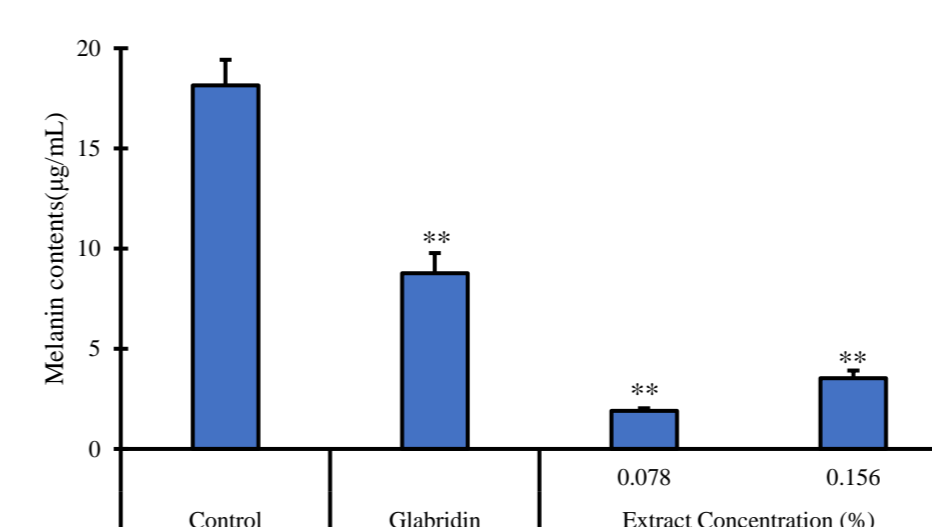


Fig.-5 Decreasing effect of glabridin and different concentration of moutan root bark extract on melanin content

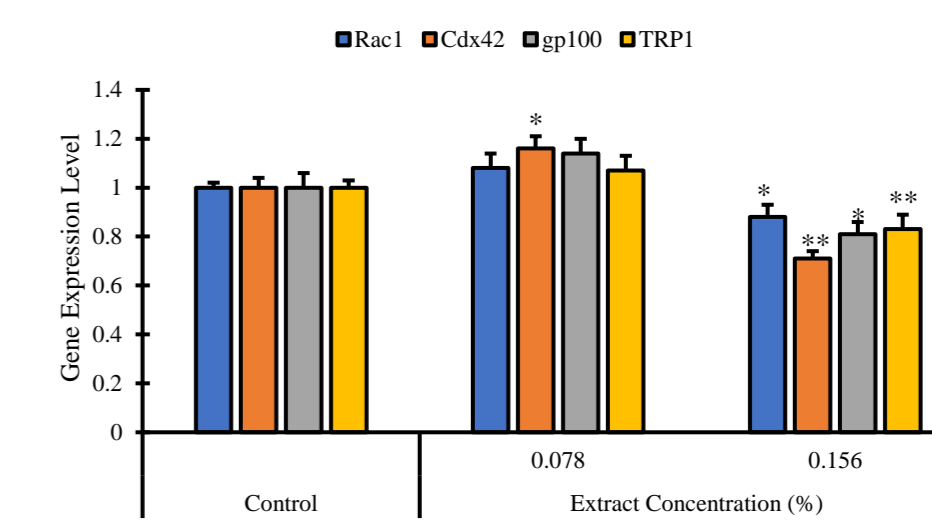


Fig.-6 Effect of moutan root bark extract on Rac1, Cdc42, gp100 and TRP1 expression level in melanocytes.

It is found that 0.156% moutan root bark extract can decrease the gene expression levels of Rac1, Cdc42, GP100 and TRP1, while 0.078% moutan extract has no significant effect.

### 3. Anti-inflammatory experiment of macrophages

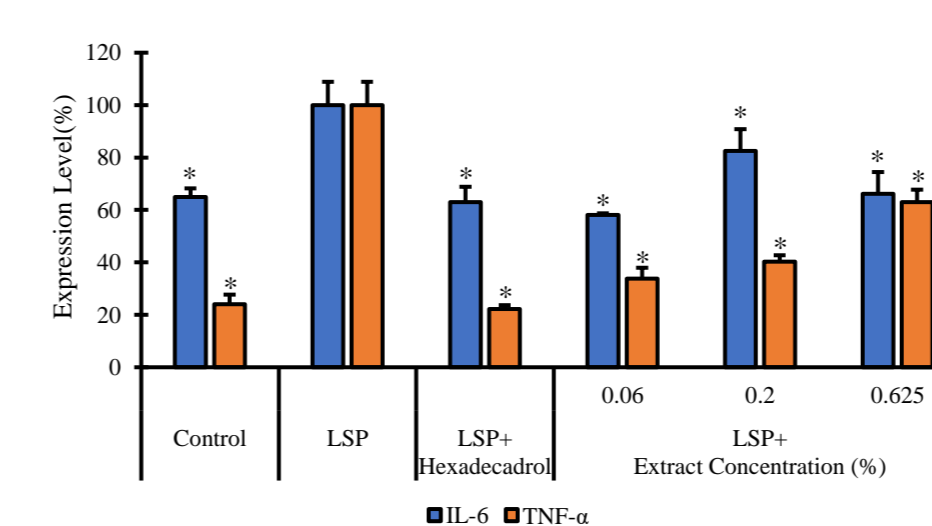


Fig.-7 Relationship between moutan root bark extract concentration and macrophage activity

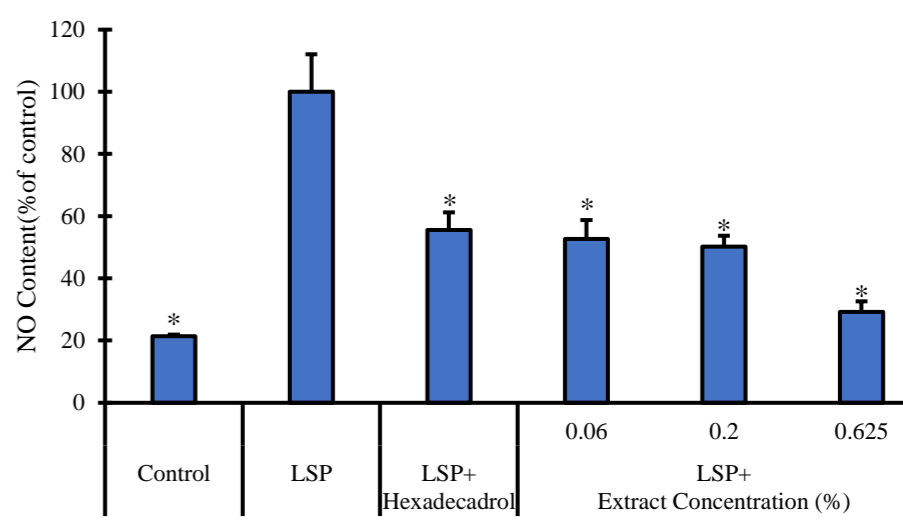


Fig.-8 The expression levels of inflammatory cytokines IL-6 and TNF- $\alpha$  in macrophages are affected by LSP, LSP+hexadecadro and LSP+moutan root bark extract.

Macrophages stimulated by 1  $\mu$ g/mL LSP produce inflammatory cytokines, including IL-6 and TNF- $\alpha$ , and release NO. However, moutan root bark extract can reduce the production of inflammatory factors after LSP stimulation, and the expression levels of IL-6 and TNF- $\alpha$  at 0.06% concentration are only 58.09% and 33.89%, respectively. At the same time, moutan root bark extract can reduce the release of NO, and the higher the concentration, the lower the release of NO. After using 0.625% moutan root bark extract, the release rate of NO in macrophages is only 29.24%.

### 4. Anti-inflammatory experiment of macrophages

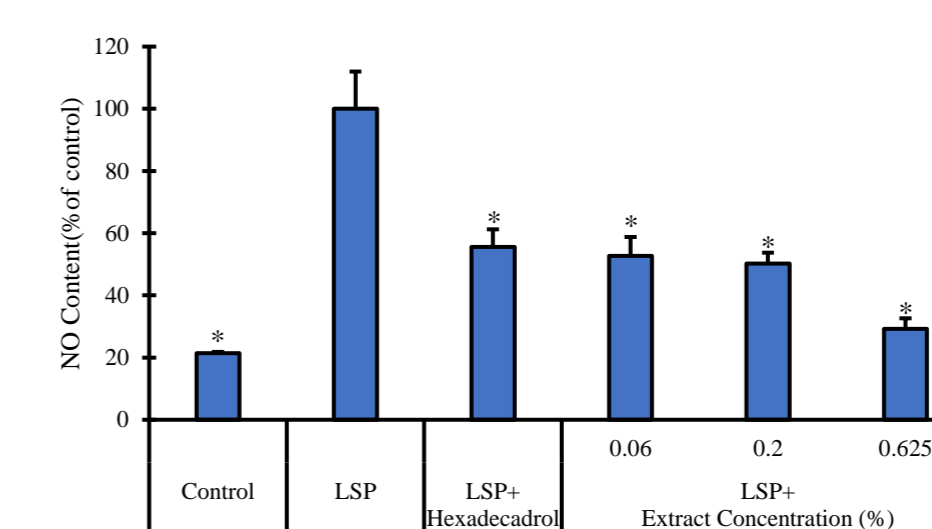


Fig.-9 The NO content in macrophages are affected by LSP, LSP+hexadecadro and LSP+moutan root bark extract

Fig.-9 shows that moutan root bark extract and PBS can inhibit AGEs in bovine serum protein. The inhibitory effect is positively correlated with the concentration of extract. When the concentration of the extract is 10%, the inhibition rate is 52.76%.

## Conclusions:

In conclusion, the extract formed after alcohol extraction, D101 decolorization and concentration of moutan root bark contains substances with the ability to relieve allergic reaction, dilute melanin in cells, reduce the production of inflammatory factors after stimulation, remove AGEs and even anti-aging. These effects are completed by regulating cell function or related protein expression. This suggests that if used in a reasonable range, the extract of peony root bark can be used as a raw material in skin care products with relevant effects.

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