Biomedicine & Pharmacotherapy 89 (2017) 438–446

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Original article

# Hair growth promoting effect of white wax and policosanol from white wax on the mouse model of testosterone-induced hair loss

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ARTICLE INFO

*Article history:*
Received 26 October 2016
Received in revised form 1 February 2017
Accepted 7 February 2017

*Keywords:*
White wax
Policosanol from white wax
Androgenetic alopecia
Dermal papilla cells
Hair follicles
5α- reductase

ABSTRACT

White wax (WW) has been traditionally used to treat hair loss in China. However there has been no reporter WW and its extract responsible for hair growth-promoting effect on androgenetic alopecia. In this paper, we examined the hair growth-promoting effects of WW and policosanol of white wax (WWP) on model animal of androgenetic alopecia and the potential target cell of WW and WWP. WW (1, 10 and 20%) and WWP (0.5, 1 and 2%) were applied topically to the backs of mice. Finasteride (2%) was applied topically as a positive control. MTS assays were performed to evaluate cell proliferation in culture human follicle dermal papilla cells (HFDPCs). The inhibition of WW and WWP for 5α- reductase were tested in Vitro. Results showed more lost hairs were clearly seen in mice treated with TP only and TP plus vehicle. Mice which received TP plus WW and WWP showed less hair loss. WW and WWP showed an outstanding hair growth-promoting activity as reflected by the follicular length, follicular density, A/T ratio, and hair bulb diameter. The optimal treatment effect was observed at 10% WW and 1% WWP, which were better than 2% finasteride treatment. MTS assay results suggested that WW and WWP remarkably increased the proliferation of HFDPCs. Inhibitor assay of 5α- reductase showed that WW and WWP inhibited significantly the conversion of testosterone to dihydrotesterone, and the $IC_{50}$ values of WW and WWP were higher than that of finasteride. In Conclusion, WW and WWP could act against testosterone-induced alopecia in mice, and they promoted hair growth by inhibiting 5α-reductase activity and HFDPCs proliferation. DPCs is the target cell of WW and WWP.



## 1. Introduction

Androgenetic alopecia (AGA) is a hereditary condition related to androgen levels, and its occurrence is therefore skewed toward males. Its pathogenesis involves the conversion of testosterone to dihydrotestosterone (DHT) catalyzed by 5α-reductase, and then DHT binds to the androgen receptor in dermal papilla cells (DPCs) of sensitive hair follicles, inhibiting the proliferation of DPCs and extending the telogen phase, and then hair is lost before the formation of new hair [1]. Therefore, DPCs is vital cell for hair growth. At present, finasteride and minoxidil are widely used to treat AGA. Minoxidil promotes hair growth by promoting the survival of DPCs to extending the anagenic phase [2]. Finasteride promotes hair growth by inhibiting the activity of 5α-reductase [3]. However, the two drugs have some adverse effects. For example, minoxidil leads to acute anteroseptal, myocardial infarction and anorexia [4,5], while finasteride is associated with adverse sexual effects [6]. Recently, natural products, which are safe and promote hair growth, have become widespread in the hair care industry [7,8].

White wax (WW) is a secondary metabolite of the male larva of the white wax scale *Ericerus pela* Chavannes (Hemiptera: Coccoidea) [9]. The main components of WW are large wax esters consisting of monobasic saturated fatty alcohol and monobasic saturated fatty acids, which account for about 93–95% of its constituents [10]. Hexacosanoic acid hexacosyl ester and octocosoic acid dioctadecyl ester are the major wax esters. Policosanol from white wax (WWP) is a mixture of saturate monohydric alcohols, which can be prepared from WW with $LiAlH_4$ and saponification. Its main components are hexacosanol (45–56%) and

*Abbreviations:* WW, white wax; WWP, policosanol from white wax; HFDPCs, human follicle dermal papilla cells; DPCs, dermal papilla cells; AGA, androgenetic alopecia; DHT, dihydrotestosterone; A/T, anagen/telogen; TP, testosterone propionate.

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http://dx.doi.org/10.1016/j.biopha.2017.02.036
0753-3322/

octacosanol (34–38%) [11]. In China, WW is used to treat hair loss. Ancient texts [12] also reported that WW promoted hair growth over time when administered topically to the head where hair loss had occurred. However there has been no reporter WW and WWP responsible for hair growth-promoting effect on androgenetic alopecia. The present study was carried out to evaluate the effects of WW and WWP on testosterone-induced hair loss in vitro and in vivo, and to elucidate its potential target site of WW and WWP.

## 2. Materials and methods

### 2.1. Drugs and reagents

Testosterone propionate (TP) injections (25 mg/mL, lot: 150813) were purchased from Hangzhou Animal Medicine Factory (Hangzhou, China). TP (5 mg/mL) was diluted in sterile soybean oil. Finasteride (99.9%) was purchased from Dalian Meilun Biotech Co., Ltd, (lot: M1122A; Dalian, China). 2% finasteride solution as a positive control was diluted in the vehicle. A CellTiter 96® AQueous One Solution Cell Proliferation Assay was obtained from Promega Corporation. (Madison, WI, USA).

WW were purchased from the institute of white wax emei in Sichuan, China. WWP was prepared from WW by reduction method of lithium aluminium hydride [11]. In brief, white wax and lithium aluminium hydride were started to react in a round-bottom flasks when tetrahydrofuran was dripped slowly at 60 °Cand ultrasound, and then tetrahydrofuran was neutralized with acid, washing with hot water and drying. Finally, the solid was recrystallized by chloroform: ethanol (v: v 3:1) and drying. The yield of WWP was 95.00%.WWP was analysed by Acme 6000 Gas Chromatographic system-FID detector and was equipped with BD-17 column (30 m × 0.32 mm × 0.25 μm) using a 1:10 split ratio of nitrogen carrier gas injection at 300 °C.The injection volume was 0.5 μL.The initial column temperature was held at 250 °C for 2 min, and then increased to 290 °C with the rate of 5 °C/min and held for 6 min. Melissyl ester as internal standard, Data indicated that content of tetracosanol, hexacosanol, and octacosanol was 11.49%, 54.15% and 27.64% respectively (Fig. 1). WW and WWP were prepared as a suspension in a vehicle (absolute ethyl alcohol/1, 2-propylene glycol, 80:20). According to preliminary experiment, the hair of animals treated with 10%WW and 1%WWP were more smooth and lustrous than those of animals treated with other concentrations by visual. So we selected 10% WW and 1% WWP as median concentrations. The applied concentrations of WW and WWP were 1, 10, or 20% and 0.5, 1, or 2% respectively.

**Table 1**
Treatments for each group.

| groups | Name | Treatments |
|---|---|---|
| control | CK | Untreated |
| | model | TP only |
| | vehicle | TP + vehicle |
| | positive | TP + 2% finasteride |
| treatment | 1% WW | TP + 1% WW |
| | 10% WW | TP + 10% WW |
| | 20% WW | TP + 20% WW |
| | 0.5% WWP | TP + 0.5% WWP |
| | 1.0% WWP | TP + 1.0% WWP |
| | 2.0% WWP | TP + 2.0% WWP |

TP, testosterone propionate; WW, white wax, WWP, policosanol from white wax.

### 2.2. Animals

Five-week-old male Kunming mice [Animal permit number: 3cdk (chuan) 2013-24, Lot: 0015129] were supplied by Chengdu Dossy Experimental Animals Co., Ltd. (Chengdu, China). The experimental protocol was approved by the Ethic Committee of the Research Institute of Insect Resources of the Chinese Academy of Forestry (Kunming, China) and the animal experiments were performed in accordance with the guide for Care and Use of Experimental Animals. The mice were housed in cages at ambient temperature (25 ± 2 °C) and were controlled under light/dark cycles 12 h/12 h and fed standard mouse chow and water *ad libitum*. They were acclimated to the laboratory environment for 1 week.

### 2.3. Treatments

Treatments were performed as described previously [13] with slight modification. In brief, animals were randomly divided in 10 groups of eight mice each (Table 1). Mice in all groups, except the CK group, were subcutaneously administered (sc) 0.1 mL of 5 mg/mL TP for 28 d, and once daily. WW, WWP, and finasteride were prepared at the required concentrations. Approximately 0.5 mL of each solution or vehicle was applied topically on the back skin (2 × 3 cm) once daily. Differences in hair growth, color and loss in

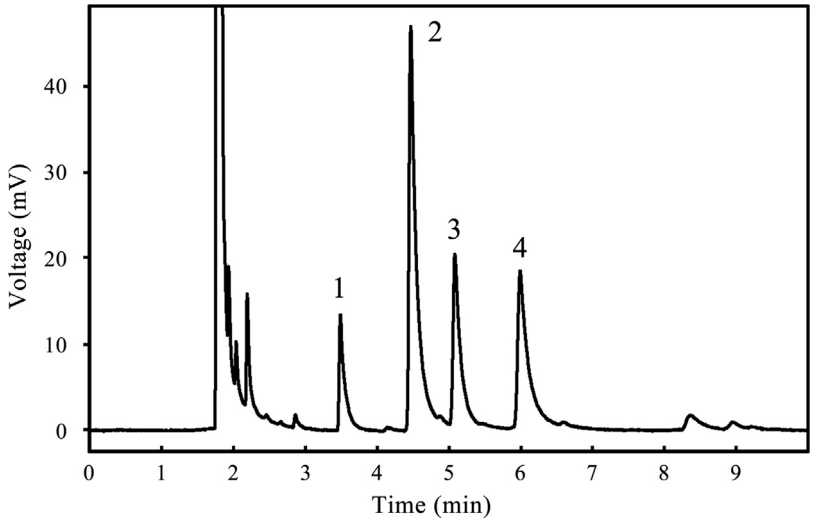


**Fig. 1.** GC chromatogram of WWP. GC chromatogram of WWP was analysed by GC-FID using 1:10 split ratio of nitrogen carrier gas injection at 300 °C. (1) tetracosanol (2) hexacosanol (3) internal standard (4) octacosanol.