Regular Article

The Reparative Effect of *Dendrobium officinale* Protocorms against Photodamage Caused by UV-Irradiation in Hairless Mice

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*Dendrobium officinale* protocorms (DOPs) are a specific developmental stage of *Dendrobium officinale* Kimura et Migo, which is used in folk medicine to ease skin issues, such as wrinkles and erythema. The purpose of the current study was to evaluate the effect of DOPs on UV irradiation-induced skin damage in bc_nu hairless mice, using matrixyl as a positive control. Hairless mice were randomly separated into 6 groups (8 mice per group). The normal control group received solvent and was not exposed to UV irradiation, while the model control group received solvent and was exposed to UV irradiation. The positive control group was subjected to UV irradiation and then received a 10 mg/mL formulation of matrixyl. The DOPs-treated groups received a transdermal application of a DOPs formulation after 4 weeks of UV irradiation. Relevant indicators, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), thiobarbituric acid reactive substances (TBARS) and matrix metalloproteinases (MMPs), were then used to evaluate the ability of DOPs to repair photodamage. The results indicated that DOPs significantly reduced erythema and protected the skin from dryness and therefore exhibits a significant anti-photoaging effect. In addition, the expression of CAT, SOD, and GSH-Px increased while TBARS and MMPs levels decreased in DOPs-treated mice. This demonstrated that DOPs can inhibit photodamage in the skin of hairless mice. DOPs could be used as a potential therapeutic agent to protect the skin against UV-induced photoaging.

**Key words** *Dendrobium officinale* protocorm; skin photoaging; repair effect; chemical profile

INTRODUCTION

UV irradiation refers mostly to UVA and UVB irradiation, because UVC can be blocked by the ozone layer. Exposure to UV can result in a series of oxidation products and lipid components in vivo, ultimately leading to suppression of the immune system and skin diseases, including skin cancer and premature skin aging.1) UV irradiation has been identified as the ultimate cause of erythema and dryness, and skin barrier destruction, which are widely known as symptoms of photoaging in the skin.2)

Photoaging is a pathological phenomenon, with symptoms such as wrinkles and pigmentations which develop earlier in sun-exposed skin than in unexposed skin. It is believed that the mechanism of photoaging involves UV irradiation, which induces the proliferation of keratinocytes and epidermal hyperplasia, while reducing the production of type I procollagen, leading to the loss of collagen. Consequently, wrinkle formation and skin thickness are increased, while skin elasticity is reduced.3)

Pharmacological studies have revealed that photoaging involves matrix metalloproteinases (MMPs) and several antioxidant indicators. Altering the expression of these factors may represent a reasonable approach to prevent photoaging. MMPs are reported to increase in activity over wide areas of mouse skin during UV exposure and contribute to wrinkle formation via destruction of the basement membrane structure, followed by degradation of extracellular matrix components, such as collagen fibers.4) Moreover, cellular oxidation inevitably produces oxides, including peroxide and superoxide. The excessive production of oxides is implicated in various pathological processes.5) Most aerobic organisms possess oxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), which contribute to the decomposition of peroxide and superoxide.6) Thiobarbituric acid reactive substances (TBARS) are formed as a byproduct of lipid peroxidation, which can be detected by the TBARS assay using thiobarbituric acid as a reagent. One of the frequently mentioned TBARS is malonaldehyde (MDA). The formation of MDA is widely regarded as a crucial factor in the aging progress because it is toxic, carcinogenic, and mutagenic. Furthermore, MDA can easily react with proteins and nucleic acids to cause damage to biopolymers. Therefore, TBARS levels are regarded as an excellent index of the degree of lipid oxidation.7)

*Dendrobium officinale* Kimura et Migo serves as a tonic herb in traditional Chinese medicine and also as a functional food.8) Modern pharmacological studies have shown that polysaccharides contained in *D. officinale* possesses several pharmacological properties, such as anti-inflammatory and anti-oxidant effects, and its ability to protect mucous membranes, *D. officinale* is also considered to be a useful skin care product.13) *Dendrobium officinale* protocorms (DOPs) are light yellow and consist of conical embryonic tissues formed after seed germination. DOPs have been used in folk medicine as a skin care agent for the treatment of skin issues such as sunburn, erythema, and wrinkles. However, to date, there have been no investigations of the effect of DOPs in the repair of photoaging.

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Matrixyl, used as a positive control substance in the current study, is known as an eminent anti-aging substance and can improve the penetration of molecules through the lipid structures of the skin. Moreover, matrixyl is a type of matrikine, a family of messenger peptides capable of regulating cell activities by interacting with their specific receptors. Matrixikines activate certain genes involved in extracellular matrix renewal and cell proliferation. By activating the neo-synthesis of extracellular matrix macromolecules, matrixyl exhibits a potent anti-wrinkle effect.

The objective of the present study was to assess the skin-care effects of DOPs in attenuating UV irradiation-induced skin damage. The effects of DOPs in reducing skin photoaging were investigated by examining histological changes in the dorsal skin of hairless mice and by determining levels of antioxidants in the skin.

MATERIALS AND METHODS

Materials and Treatments  Fresh DOPs, which were yellow-greenish globular embryos, were collected from the Guangzhou University of Chinese Medicine Tissue Culture Laboratory, freeze-dried and finely ground, and then stored at −20°C. The use of DOPs in this research was in alignment with its traditional use. In order to determine the optimum dose, the fluidity and ductility of different preparations were tested. The optimized dose of DOPs was determined to be 50 mg/mL, which was created by dissolving 0.25 g of finely ground DOPs powder in 5 mL of deionized water to make a suspension. We also prepared two additional formulations, which were 50 and 20% of the optimized concentration, respectively. We therefore had 3 different DOPs concentrations available for testing (10, 25 and 50 mg/mL), each being treated with a transdermal application of DOPs at different concentrations. DOPs formulations were applied directly to the dorsal skin of hairless mice by smearing.

Animals and Treatment  Pathogen-free male bc-nu hairless mice (7 weeks of age) were purchased from the Guangzhou University of Chinese Medicine Laboratory Animal Center. These mice were individually housed in plastic cages with stainless steel wire roofs and had ad libitum access to rodent chow (purchased from the Guangzhou University of Chinese Medicine Laboratory Animal Center) and drinking water. The room was maintained at 25°C with 65% atmospheric humidity and a 12-h light/dark cycle.

After 1-week of acclimatization, the mice were randomly separated into 6 groups, including a normal control group, a model group, a positive control group, and 3 DOPs-treated groups (10 mg/mL treated group, 25 mg/mL treated group, 50 mg/mL treated group), with 8 mice per group. The normal control group received solvent and was not irradiated with UV. The model group received solvent and was subjected to UV irradiation. The positive control group was subjected to UV irradiation and then received a formulation of matrixyl. The DOPs-treated groups were subjected to UV irradiation and then received a transdermal application of DOPs at different concentrations. DOPs formulations were applied directly to the dorsal skin of hairless mice by smearing. The area of embrocation was marked by methylrosanilinium chloride solution. The application were performed every other day a week, start from Monday and 4 times in total, from the fifth to the tenth week after UV irradiation.

This research was conducted in accordance with the Laboratory Animal Welfare and Ethics Committee of the Chinese Association for Laboratory Animal Sciences.

UV Irradiation  Mice were irradiated twice a week using 3 UVB lamps (15 W SANKYO DENKI G15T8E) and 3 UVA lamps (15 W PHILIPS ACTINIC BL) cross fixed in a 1.3-m-high shelf and covered using a blind curtain to protect them from extraneous light. UV irradiation was measured using a radiometer (UV340B XINBAORUI Instrument Co., Ltd., Shenzhen, China) immobilized at 100 mJ/cm². UV irradiation was lasted 4 weeks (from the first to the forth week), which was proceeded by exposing hairless mice to UV light every Tuesday and Thursday, 1 h at a time.

Tissue Collection and Microscopic Skin Examination  Twelve hours after the last treatment, the hairless mice were anesthetized using an intraperitoneal injection of pentobarbital (50 mg/kg). We removed the eyeballs from each mouse and then collected blood samples. The dorsal skin was then photographed using a digital camera. Then, the mice were sacrificed by cervical dislocation and their dorsal skin was quickly removed.

Harvested skin tissue (100 mg) was washed in a 0.15 M solution of NaCl and cut into two small pieces. Histoicte ly sate (0.5 mL) and potassium phosphate buffer (pH 7.0, 1.5 mL)
were added to one of the skin pieces and the mixture was homogenized. The tissue homogenate, and blood samples, were centrifuged at 3000 × g for 10 min at −4°C to obtain supernatants, which were subsequently stored at −80°C for the measurement of antioxidant indicators. The other piece of skin tissue was fixed using 4% paraformaldehyde and stained using hematoxylin-eosin for histological examination.

**Measurement of Weight Changes, Dryness, and Erythema Induced by UV Irradiation** All experimental animals were weighed once a week at a fixed time. Hydration of the stratum corneum was used to measure the degree of skin dryness; this was analyzed using a Corneometer (KEONBASO KBS-315) fitted with a multiprobe adapter. When the probe was pressed onto the skin and held for 1 s, the horny layer came into the scatter range of the condenser field. Different capacitance changes were then converted into a measured digital value, which was proportional to skin humidity. Skin erythema was assessed visually.

**Statistical Analyses** All statistical analyses were performed using GraphPad Prism 7.0. The Student’s t-test was used to assess differences between the 3 DOPs-treated groups and the model group. Significant differences were evaluated via one-way ANOVA. All data were two-sided at the 5% significance level and are reported as means ± standard deviation (S.D.) of triplicate experiments.

**RESULTS**

**Macroscopic and Microcosmic Observations** UV irradiation caused a significant amount of erythema (p < 0.05) and severe dryness on the dorsal skin of the animals, compared with the normal control group (Fig. 1). Mice in the UV-treated groups suffered from weight loss after 4 weeks of UV irradiation, indicating that the UV irradiation caused severe physical damage. However, the body weight of these mice recovered gradually after the UV irradiation ceased and after they began medicinal treatment (Fig. 2).

The skin moisture of UV-treated mice was at least 1.6% lower than that of mice in the normal control group. However, this symptom was eased by the transdermal application of DOPs and matrixyl. Better skin moisture was observed in mice from the 25 mg/mL DOPs treated group and 50 mg/mL DOPs treated groups, compared with those in the positive control group.
Control group; moisture content for these mice was 8.8 and 3.8% higher than the positive control, respectively (Fig. 3A). Despite this, the differences in skin moisture between the groups were not statistically significant. The extent of erythema was significantly reduced in the DOPs treated groups compared with the positive control group ($p < 0.05$). This demonstrated that the formation of erythema was categorically depressed by DOPs formulations (Fig. 3B). However, the extent of erythema in the 3 DOPs-treated groups were similar, revealing that the ability of DOPs to inhibit erythema was not dose-dependent. This result concurred with the macroscopic appearance of the skin, as shown in Fig. 1.

Histological analysis of the model group (Fig. 4) revealed a thickened epidermal layer, disordered collagen fibers and sebaceous hyperplasia after UV irradiation, which is consistent with a previous study.\textsuperscript{19} Compared with the model group, the DOPs-treated groups showed several significant improvements. Firstly, a reduction in epidermal thickness and sebaceous hyperplasia on the dorsal skin. Secondly, cell shape in the epidermal layer was more regular, and not shrunken. Thirdly, collagen fiber density in the dermis increased significantly. Furthermore, we quantified sebaceous hyperplasia and epidermis thickness (Figs. 4A, B); this data indicated that UV irradiation caused severe effects upon the skin in terms of epidermal thickening and deterioration of sebaceous hyperplasia. However, the extent of sebaceous hyperplasia was decreased, and epidermis thickness was similar to the normal group, normal group following the application of matrixyl and DOPs. There was also an improved photoaging repair effect in the hairless mice as the concentration of DOPs increased. Moreover, the reparative effect of the 50 mg/mL DOPs formulation was almost equivalent to that of the matrixyl.

Examination of Antioxidant Indicators The expression of CAT was depressed in the model group mice, compared with those in the normal control group but was expressed at much higher levels following the transdermal application of matrixyl and DOPs. There was an enhancement in CAT production in the DOPs-treated groups compared with the model group. Indeed, the expression of CAT in the 50 mg/mL treated group was 200% higher than that of the model group,
The normal control was not irradiated with UV and received solvent. The positive was subjected to UV irradiation and received matrixyl formulation. The 10 mg/mL treated group was subjected to UV irradiation and received DOPs formulation transdermal application. The 25 mg/mL treated group was subjected to UV irradiation and received DOPs formulation transdermal application. The 50 mg/mL treated group was subjected to UV irradiation and received DOPs formulation transdermal application. Different letters above mean symbols (a, b) indicate that the mean bars were statistically different compared to other values with the same symbols ($p < 0.05$). Each bar represents mean ± S.D. of quadruplicate determinations.

As a result of UV irradiation, the model control produced a significant amount of TBARS compared to the normal control, while TBARS levels were significantly reduced following the application of matrixyl. These results suggested that matrixyl could inhibit the production of TBARS. DOPs played a similar role as matrixyl in terms of preventing the production of TBARS. DOPs depressed the production of TBARS in all 3 doses groups and acted in a dose-dependent manner. The
concentration of TBARS in mice from the 50 mg/mL group (4.98 µM) was significantly lower than that of the model group (11.88 µM) and the normal group (10.48 µM). This suggests that DOPs could potentially depress the production of TBARS, and that the 50 mg/mL DOPs formulation exhibited the strongest inhibition of TBARS (Fig. 5D).

Inhibitory Effects on the Production of MMPs  UV irradiation in hairless mice substantially increased the expression of MMP-1 and MMP-3 in epithelial tissues by 0.51 and 40.0%, respectively, compared with the normal group. The expression of MMPs in the DOPs-treated group was reduced (Fig. 6). There were significant differences between the DOPs-treated groups and the model group in terms of the expression of MMP-3. In addition, the 25 and 50 mg/mL formulations demonstrated better MMPs suppressive activity compared with the 10 mg/mL formulation. The capability of the 25 and 50 mg/mL formulations to suppress MMPs were slightly different, and both resulted in levels of MMPs expression that were similar to the positive control group. These results showed that the UV irradiation-induced increase in the expression of MMPs could be improved by DOPs.

Chemical Profiling of DOPs  We identified a range of chemical compounds, such as amino acids, polysaccharides and long-chain lipids in the DOPs (data not shown). It is these chemical compounds that are likely to be responsible for the reparative effects against skin aging. To investigate this, DOPs extracts were subjected to GC-MS analysis. A total of 28 compounds were identified in these samples (Fig. 7). These

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Table 1. Fat-Soluble Compounds in DOPs Identified by GC-MS

<table>
<thead>
<tr>
<th>Categories</th>
<th>No.</th>
<th>Compound</th>
<th>Molecular formula</th>
<th>RT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>C11</td>
<td>Phenol, 2,2’-methylenebis 6-(1,1-dimethylethyl)-4-methyl-</td>
<td>C12H26O2</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>C22</td>
<td>Vitamin E</td>
<td>C29H50O2</td>
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<tr>
<td>Steroids</td>
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<td>Stigmasterol</td>
<td>C29H48O</td>
<td>26.8</td>
</tr>
<tr>
<td></td>
<td>C24</td>
<td>Sitosterol</td>
<td>C29H50O</td>
<td>27.7</td>
</tr>
<tr>
<td></td>
<td>C25</td>
<td>Stigmastanol</td>
<td>C29H52O</td>
<td>27.9</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>C26</td>
<td>Spirost-8-en-11-one, 3-hydroxy-</td>
<td>C30H50O</td>
<td>28.0</td>
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<tr>
<td></td>
<td>C27</td>
<td>Lupeol</td>
<td>C30H50O</td>
<td>28.9</td>
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<tr>
<td>Long chains of hydrocarbons</td>
<td>C1</td>
<td>3-Hexen-1-ol, 2,5-dimethyl-, formate, (Z)-</td>
<td>C12H18O2</td>
<td>6.2</td>
</tr>
<tr>
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<td>C2</td>
<td>Neophytadiene</td>
<td>C13H26O2</td>
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<tr>
<td></td>
<td>C3</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
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<td></td>
<td>C4</td>
<td>Methyl palmitate</td>
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<td>Oleic Acid</td>
<td>C17H32O2</td>
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<td></td>
<td>C6</td>
<td>11,14-Octadecadienoic acid, methyl ester</td>
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<td></td>
<td>C7</td>
<td>Methyl 12,13-tetradecadienoate</td>
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<td></td>
<td>C8</td>
<td>Phytol</td>
<td>C20H38</td>
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<td></td>
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<td>C21H40O2</td>
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<tr>
<td></td>
<td>C10</td>
<td>9,12-Octadecadienoic acid (ZZ)</td>
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<td></td>
<td>C12</td>
<td>1,2-15,16-Diepoxyhexadecane</td>
<td>C23H28O2</td>
<td>17.4</td>
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<td></td>
<td>C13</td>
<td>Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester</td>
<td>C24H30O2</td>
<td>17.5</td>
</tr>
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<td>C14</td>
<td>9,12-Octadecadienoic acid (ZZ), 2-hydroxy-1-(hydroxymethyl)ethyl ester</td>
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<td>2-Methylhexacosane</td>
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<td>20.0</td>
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<tr>
<td></td>
<td>C16</td>
<td>9,12-Octadecadienoyl chloride, (Z, Z)</td>
<td>C27H50O4</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>C17</td>
<td>Heptacosane</td>
<td>C28H50O4</td>
<td>20.4</td>
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<tr>
<td></td>
<td>C18</td>
<td>9-Octadecenamide, (Z)</td>
<td>C29H50O4</td>
<td>21.1</td>
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<tr>
<td></td>
<td>C19</td>
<td>Squalene</td>
<td>C30H50O2</td>
<td>21.8</td>
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<td></td>
<td>C20</td>
<td>Desacylcedran-1-methanol, acetate (ester)</td>
<td>C31H50O4</td>
<td>23.8</td>
</tr>
<tr>
<td></td>
<td>C21</td>
<td>Batilol</td>
<td>C32H50O2</td>
<td>24.9</td>
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</table>
compounds were classified into four categories, including phenols, steroids, and triterpenes and long-chain hydrocarbons (Table 1). Some of the compounds identified are well known for their ability to protect skin, for example, squalene, vitamin E, lupeol, fenretinide and oleic acid. Many of these compounds may also contribute to the moisturizer-like effect exhibited by DOPs.

**DISCUSSION**

In the present study, we investigated the anti-photoaging activity of DOPs using a hairless mouse model of UV-irradiation-induced skin damage. DOPs treatment was compared with the administration of matrixyl. This study was performed by exposing the dorsal skin of bc-nu hairless mice to UV irradiation for 4 weeks, and then treating these groups of mice with transdermal DOPs and matrixyl for 6 weeks (4 times per week).

Photographs and microcosmic images of the dorsal skin were obtained after 6 weeks of DOPs and matrixyl application. Ulcers appeared on the skin of the non-DOPs-treated mice, which was regarded as erythema. This may indicate that UV irradiation was not the only cause of erythema. The innate low immunity of hairless mice may also have contributed to the formation of erythema. Of the non-DOPs-treated groups, the level of erythema in the model group was much greater than that in the other two groups; this erythema was improved following the transdermal application of matrixyl. Strikingly, there was no erythema in DOPs-treated mice.

UV irradiation damaged both epidermal and dermal skin and resulted in photoaging. As the epidermis is the protective barrier of the skin, epidermal thickening is considered to be an adaptive response to UV irradiation, and provides protection from photoaging. MMPs also initiate photoaging in the skin moisturizing and anti-aging in daily use. However, the dose–effect relationship was unclear for lacking of available literature. Further robust investigations are now needed to evaluate the anti-photoaging mechanism underlying the action of DOPs, and to explore the relationship between the chemical components of DOPs and their relative abilities to repair photodamage.

**CONCLUSION**

This is the first report to show that the transdermal application of DOPs prevents UV-induced skin photoaging. The DOPs formulation was prepared in-house by mixing a fine powder of DOPs and deionized water to create a homogeneous suspension. The transdermal application of DOPs protected skin against UV-induced damage in a mouse model. Collectively, our results suggest that DOPs could be used as a therapeutic agent to protect the skin against UV-induced photoaging. Further studies are now in progress to investigate the active substances involved in this photoaging repair effect.

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**Conflict of Interest** The authors declare no conflict of interest.
REFERENCES


