Safety Evaluation of GP-EGF Lyophilised Powder

ABSTRACT

To clarify the safe risks related to the use of GP-EGF lyophilised powder (GELP), acute toxicity test, dermal allergy test, dermal irritation test, and subchronic toxicological test were evaluated according to the guideline for hygienic standard for cosmetics (2007). In acute toxicity tests using guinea pigs, there was no treatment-related mortality, clinical signs of toxicity, body weight changes and gross findings at a dose level of 2,000 mg·kg⁻¹·d⁻¹. Similarly, dermal allergy test using guinea pigs and dermal irritation test using rabbits revealed no mortality, clinical signs of toxicity and a corrosion reaction on the skin. In the subchronic study, no death or clinical signs, or abnormal haematological, biochemical and histopathological changes were found in rats after receiving, by the concentration in 56 mg·mL⁻¹·d⁻¹, 112 mg·mL⁻¹·d⁻¹ of GELP for 90 days. Our findings indicated that GELP is relatively safe since it did not induce an acute toxicity test, dermal allergy test, dermal irritation test and subchronic toxicological test.

KEYWORDS EGF, GP-EGF lyophilised powder (GELP), acute toxicity test, dermal allergy test, dermal irritation test, subchronic toxicological test

INTRODUCTION

The growth and differentiation of keratinocytes are regulated mainly by a variety of growth factors, of which the members of the epidermal growth factor (EGF) family are the most important for skin wound healing, apoptosis, aged and so on. EGF also known as oligopeptide-1, is the most important members of the EGF family. It is a small polypeptide hormone that modulates proliferation and metabolism in a wide variety of cell types. It is obvious that EGF had strong antioxidant, and could promote cell proliferation, differentiation and to the new cells to replace aging and death.

Safety of cosmetic is not only the basic of product, but also the researchers should focus on and do the best to improve it. Although diverse studies on the biological activities of EGF have been performed by now, no toxicological assessments of percutaneous safety of EGF in genipin (GP) has been reported. In this study, we firstly studied the toxicity, including acute toxicity, sensitisation test, irritation test and subchronic toxicological test.

MATERIALS AND METHODS

Materials

GP-EGF lyophilised powder (GELP) and EGF were made by our laboratory; mannitol was purchased from Guangxi Nanning Chemical Pharmaceutical Co., Ltd (NanNing, Guangxi, People’s Republic of China). 2,4-Dinitrochlorobenzene (DNCB) was purchased from Shanghai Pengshuo Biological Technology Co., Ltd. (Shanghai, People’s Republic of China).

Animals

Male and female guinea pigs, New Zealand rabbit and Sriague–Dawley (SD) rats were obtained from Guangdong Medical Lab Animal center.

Isolation of GELP

The GELP used in this preparation were obtained from EGF, GP and manniol. Subsequently, the mixture was frozen into an ice cube in a refrigerator.
(-4°C) and then dried using a FD-1A-50 lyophiliser with a condenser temperature of -50°C and inside pressure <20 Pa. After 48 h of lyophilisation process, low-density, loosely packed GELP were finally obtained.

**Dermal acute toxicity**

The acute toxicity test was performed according to Hygienic Standard for Cosmetics (2007 edition). Guinea pigs were divided into two sex groups with a dose group and a control group. 24 h before the treatment, the hair (4–6 cm²) was removed on both sides of the back of guinea pigs using a shaver. The denuded skins of animals in the treatment group were treated with 2,000 mg/kg GELP. The control group received isometric PBS solutions. All animals were allowed for food *ad libitum* for 14 days and kept under regular observation for any mortality or behavioural changes (irritation, restlessness, respiratory distress, abnormal locomotion and catalepsy etc.)

**Dermal allergy test**

In accordance with the literature (Hygienic Standard for Cosmetics, 2007 edition), guinea pigs were randomly divided into three groups, consisting of a negative control (NC) group, a treatment group and a positive group, each of which consisted of 10 animals. The hair of guinea pigs was treated in the same way like dermal acute toxicity test. The denuded skins of animals in the treatment group were treated with a concentration of 56 mg/mL GELP. The NC received PBS, and positive controls received 2, 4-dinitrochlorobenzene (DNCB, 0.02%, 0.2 mL). After applying the respective treatments, the treated skin was covered with sterile bandages. After 6 h, the coverings were removed. On days 7 and 14, each group of animals was given the corresponding treatments mentioned above. 14 days after sensitisation, PBS, GELP and DNCB (0.02%, 0.2 mL) were applied to the corresponding group of animals for excitation of skin allergy. After 6 h, the coverings were removed. After 24 and 48 h, the average score was recorded for each group, followed by the evaluation of skin allergic intensity and the ratio of allergic animals in each group was calculated to estimate the allergic intensity in accordance with Table 1. At the same time, animals were closely observed for signs of anaphylaxis i.e., asthma, standing instability, shock or other severe systemic allergic reactions.

**Dermal irritation test**

As described in the literature (Hygienic Standard for Cosmetics, 2007 edition), the hair (5 × 5 cm²) was removed on both sides of the back of rabbits using a shaver. Following that, the treated skin was covered with gauze and cellophane and fixed with bandages. As a control, PBS was applied to the denuded skin on the opposite side. After 6 h, the coverings were removed. Daily once, this treatment was performed and continued for 2 weeks. On day 14, after removal of coverings, any sign of an erythema, edema, pigmentation, or blood oozing at the sites of GELP or PBS treatment were observed and recorded after 1 h. At the same time, erythema and edema symptoms were given scores according to Table 1. The average score was calculated, that is, the total scores of erythema or edema were divided by the number of animals given the same dose of GELP. Subsequently, the irritation intensity of GELP was evaluated according to Table 2.

**Dermal subchronic toxicity**

SD rats were divided into three groups with 2 dose group and 1 control group each (totally three groups and ten isosexual rats in each group); the treated skin was covered with gauze and cellophane and fixed with bandages. The 2 dose groups received GELP of a concentration 56 and 112 mg/mL. The NC was set with PBS treatment. During the exposure time, body weight and food consumption were determined weekly. Food in-taking rate (%) was calculated by (body weight gain)/(food consumption) × 100. After a 90-day successive feeding, the rats were anaesthetised with sodium pentobarbital, and the blood was drawn from the abdominal vein using heparin as an anticoagulant for haematological or blood biochemical examinations, respectively. Serum, used for the blood biochemical examination was obtained by

---

**Table 1** Grading standard for skin allergic or irritation response.

<table>
<thead>
<tr>
<th>Allergic or irritation</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Slight (bare visible)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate (visible)</td>
<td>2</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
</tr>
<tr>
<td>Purple erythema to mild eschar</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Edoema</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Slight (bare visible)</td>
<td>0</td>
</tr>
<tr>
<td>Moderate (apparent swelling edge higher than surrounding skin)</td>
<td>1</td>
</tr>
<tr>
<td>Severe (1 mm of swelling and clear contour)</td>
<td>2</td>
</tr>
<tr>
<td>Extremely severe (over 1 mm of swelling or ulceration)</td>
<td>3</td>
</tr>
<tr>
<td>Highest value</td>
<td>8</td>
</tr>
</tbody>
</table>

**Table 2** Evaluation standard for skin irritation intensity.

<table>
<thead>
<tr>
<th>Average score</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–&lt;0.5</td>
<td>No irritation</td>
</tr>
<tr>
<td>0.5–&lt;2.0</td>
<td>Slight</td>
</tr>
<tr>
<td>2.0–&lt;6.0</td>
<td>Moderate</td>
</tr>
<tr>
<td>6.0–8.0</td>
<td>Severe</td>
</tr>
</tbody>
</table>

---
centrifugation (1,500 r/min, 10 min, 4°C) and the following parameters were determined: blood urea nitrogen levels (BUN, mM), creatinine (CREA, µM), alanine amino-transferase (ALT, U/L), aspartate transaminase (AST, U/L), gamma-glutamyltransferase (γ-GT, U/L), total protein (TP g/L), albumin (ALB, g/L), globulin (GLB, g/L), ratio of albumin to globulin (A/G) and total bilirubin (TBIL, µM). At autopsy, the treated skin, liver, spleen and kidney were removed and weighed. The blood biochemical examination was processed at the Guangzhou Overseas Chinese Hospital and determined by an automatic blood biochemical analyser (ACE, USA).

**Statistical analyses**

The data were statistically analysed using the SPSS 19.0 software package by one-way ANOVA. The differences were considered to be statistically significant if P < 0.05.

### RESULTS AND DISCUSSION

#### Dermal acute toxicity

Guinea pigs (both male and female) administered with GELP did not induce any clinical signs of toxicity either immediately or during the post-treatment period, even at dose up to 2,000 mg·kg⁻¹·d⁻¹, indicating that GELP was a practically non-toxic substance. Changes in body weight during the administration were shown in Table 3 and Fig. 1.

#### Dermal allergy test

After 1, 7, 14, 21 and 28 days of the exposure to GELP with guinea pigs, no erythema, edema or other signs of anaphylaxis were observed, while erythema and edema symptoms were observed in the positive group of animals, given DNCB (Table 4).

### Table 3 Changes of body weight in acute toxicity (x ± s, n = 10).

<table>
<thead>
<tr>
<th>Gender</th>
<th>Administration</th>
<th>Dose (mg/kg)</th>
<th>0 day (g)</th>
<th>7 days (g)</th>
<th>14 days (g)</th>
<th>Symptoms of poisoning</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>♂</td>
<td>Percutaneous</td>
<td>2,000</td>
<td>300 ± 15</td>
<td>316 ± 25</td>
<td>345 ± 25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>♀</td>
<td>Percutaneous</td>
<td>2,000</td>
<td>298 ± 16</td>
<td>312 ± 36</td>
<td>351 ± 30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Fig. 1** Dermal acute toxicity of GELP in guinea pigs (A: 0 day, B: 7 days, C: 14 days).
Dermal irritation test

In the dermal irritation test, no treatment-related mortality, clinical signs of toxicity and body weight changes were observed in any of the animals treated with GELP during the study period (data not shown). As shown in Fig. 2, there were no erythema, eschar and edema formation from 1 to 14 days after exposure to GELP.

Dermal subchronic toxicological test

Values for all dose groups in both sexes were comparable to those of the control group and no significant differences were recorded throughout the administration period. During the experimental period, no significant abnormality in food intake was observed (data not shown). The serum biochemistry and organ coefficient results after administration of 90 days were shown in Tables 5 and 6, respectively.

For the GELP-treated groups, after 90 days of exposure, the upper stratum corneum showing a basket weave-like pattern, and with a higher exhibiting of accumulated keratinised cells on skin (Fig. 3). However, no visible differences were found in liver, heart, spleen, kidney, and pancreatic samples (Fig. 4).

DISCUSSION

Recently, a wide range of cosmetic products has been appearing in our daily life. However, more and more reports about safety could be found frequently. As safety is the basic of cosmetic, researchers should focus on and do the best to improve it.

GELP is a whitening and rejuvenating cosmetic product that could be painted, sparged or by the similar ways spread on the skin. It consists of EGF, GP, mannitol and so on. The safety evaluation of EGF and GP in cells and animals were varied existence, recently. However, risk assessment of GELP following dermal exposure is lacking. To assess the dermal safety of GWLP, a systematic...
Safety evaluation of GP-EGF lyophilised powder

EGF is a small polypeptide hormone, which modulates and metabolism in a wide variety of cell types.\textsuperscript{16,17} GP is an aglycon derived from geniposide, an iridoid glycoside extracted from \textit{Gardenia jasminoides} Ellis. It is generally categorised as a non-toxic constituent, and could not receive a LD\textsubscript{50}. To assess the dermal acute toxicity of GELP, the maximum dose method was observed in guinea pigs. In addition, no treatment-related effects on clinical signs of toxicity, mortality, and changes in body weight were observed in any treated male and female guinea pigs at a dose level of 2,000 mg/kg. This suggested that GELP did not induce an appreciable dermal acute toxicity in guinea pigs.

In dermal subchronic toxicity, all dose groups in both sexes were comparable to those of the control group and no significant differences were recorded throughout the administration period. During the experimental period, no significant abnormality in food intake was observed. This indicated that little or no accumulation of GELP was observed in rats.

Irritations and sensitisation effects on skin are the reactions caused by cosmetics. To assess the irritations and sensitisation effects on the skin by GELP, dermal allergy and irritation test were generated. In allergy test, after the days of the exposure to GELP with guinea pigs, no erythema, edema or other signs of anaphylaxis were observed. The results were similar to the skin of New Zealand rabbit in the dermal irritation test. Totally, GELP did not induce irritation in rabbits and sensitive in guinea pigs.

CONCLUSION

The previous finding of our studies indicated that EGF and GP did not exhibit acute toxicity and dermal sensitivity in guinea pigs\textsuperscript{18–24}, dermal irritation in rabbits\textsuperscript{11,25,26} or subchronic toxicity in rats\textsuperscript{27,28}, which corroborates with the results of this study. These results demonstrate the absence of acute toxicity as a consequence of the percutaneous treatment with GELP suggesting that their percutaneous LD\textsubscript{50} is much higher than 2,000 mg/kg. The data obtained in these studies are relevant as they provide for the use of a species of great economic and medical importance. However, other studies based on elaborating by regulatory agencies should be performed (such as genotoxic, reproductive toxicity and so on) to evaluate the total safety of this plan.
REFERENCES


