EFFECTS OF THE PELOID CREAM FROM THE MONTENEGRIN ADRIATIC COAST ON SKIN HUMIDITY, TRANSEPIDERMAL WATER LOSS AND ERYTHEMA INDEX, EXAMINED WITH SKIN BIOENGINEERING IN VIVO METHODS

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Abstract

The healing properties of mineral mud (peloid) have been used for centuries for medicinal and cosmetic purposes. In this study there were studied in vivo effects of locally applied peloid from the Montenegrin Adriatic coast on the skin humidity-hydration level of the stratum corneum (SCH), transepidermal water loss (TEWL) and erythema index (EI).

Examination of the effects of peloid in the form of 1% of cream (P cream) on SCH, TEWL and EI was carried out at the Department of Pharmaceutical Technology and Cosmetology, Faculty of Pharmacy in Belgrade, using modern skin bioengineering in vivo methods. Short-term (one hour) and long-term (one month) in vivo studies on 20 healthy volunteers aged 24 ± 1.2 years on the volar part of the forearm were conducted. One-way ANOVA and post-hoc Tukey test were used for statistical analysis of data.

There was noticed a statistically significant difference (p<0.05) in stratum corneum humidity, expressed as arbitrary units in the group treated with P cream for each observation period, in comparison to the baseline values, as well as with the control group. Percentage of the change in TEWL value after a month of using P cream was not statistically significant (p>0.05). Statistically, EI values were not significantly increased (p>0.05) in any time point for the tested P cream, while there was an increase of this parameter in the case of untreated control.

In conclusion, P cream has a beneficial hydrating effect, it doesn’t lead to the breaking of the skin barrier, and irritation potential during single use, as well as continuous usage for skin care was not manifested.

Keywords: peloid, skin hydration, moisture loss, erythema index

Rezumat


Studiile au fost efectuate pe 20 de voluntari sănătoși (24 ± 1,2 ani), în cadrul Departamentului de Tehnologie Farmaceutică și Cosmetologie, Facultatea de Farmacie din Belgrad. Pentru analiza statistică a datelor experimentale au fost utilizate testele Anova și Tukey.

Keywords: peloid, skin hydration, moisture loss, erythema index
Introduction

In recent years high attention in medicine and pharmacy was dedicated to the study of physical and chemical factors of the environment or natural resources and their application in pharmacotherapy. One of the natural resources whose therapeutic effects are scientifically confirmed and application proven in medicine is the mineral mud-peloid. As defined by International Society of Medical Hydrology, peloid is a mixture of mineral water with organic and inorganic substances, and occurs as a result of geological and biological processes.

Information about peloid use was found on papyrus written 4000 years ago, which shows that the Egyptians used peloid when treating burns, gynecological diseases and joint problems. King Herod and Queen Cleopatra used mud and water from the Dead Sea for medicinal and cosmetic purposes[11].

With their physical and chemical composition, peloids are complex heterogeneous systems consisting of two solid and one liquid phase. Solid phase consists of crystalline skeleton (determines the mechanical structure) and colloidal skeleton (defines thermal properties, and consistency), and liquid phase is the peloid solution which is the carrier of the therapeutic effect [10,14].

Because of its characteristics and qualities that enable its appliance in the medical-balneological purposes, mineral mud from the Montenegrin coast was the subject of interest of scientists for centuries. In our study we used the peloid from the site of Ulcinj coast of the Adriatic Sea, located in the southeast of Montenegro, which covers an area of 1468ha, at a depth of 1.5 m below the surface, and its thickness is up to 10m [19]. Physico-chemical analysis of the content of macro and microelements in the obtained peloid was performed using method of microwave digestion with sequential extraction in three phases at the Institute for Chemistry, Technology and Metallurgy (ICTM) in Belgrade. The content of macro and microelements which were present in the peloid is shown in Figures 1 and 2 [20].

![Figure 1](image)

Histogram display of macroelements in peloid from the site of Ulcinj coast [20]
As the histogram shows, investigated peloid mostly contains sodium, aluminium and iron. It is well-known that sodium detoxifies the skin and chelated iron compounds protect the skin from radiation. It is added to the preparations for the sun protection of the skin [21]. In the first two phases there is only a small proportion of total aluminium, which means that it cannot be mobilized to a greater extent. For this reason, its influence on the observed skin parameters is probably small. Magnesium is essential for cell metabolism, it affects the maturation of the skin in patients with psoriasis and acts as an anti-allergic agent.

![Figure 2](image)

Histogram display of microelements in peloid from the site of Ulcinj coast [20]

As the histogram shows, chromium is the most important microelement in investigated peloid. According to Tântaru G et al. (2011) [24], this biometal is known as a redox catalyst, an oxygen transporter and as Cr(III) an essential nutritive agent. Along with zinc and vitamin A is used to treat seborrheic skin and acne.

The therapeutic efficiency of peloid is the result of interaction of its mechanical, physical and chemical effects. Numerous scientific and professional medical studies, books and manuals were written about therapeutic effects of peloid in treating gastrointestinal illnesses, rheumatic diseases, neurological diseases, in dentistry, and especially interesting for us, the effects of peloid on various dermatological diseases, as well as its use in skin care preparations and in cosmetics. Considering dermatological diseases, the effects of peloid were investigated in treating psoriasis [9, 23], psoriatic arthritis [8], rosacea and atopic dermatitis [2]. Also, it was used in preparations for sun protection and skin photo aging [17].

The water content of *stratum corneum* is an important factor affecting the biophysical properties of skin, so the preservation of moisture level is important both from an aesthetic (cosmetic), and the clinical aspects [3, 12]. Moisture content in the skin is also an important parameter for dermatological
preparations, since it was established that the moisture content in the *stratum corneum* (SCH) affects the barrier function of skin, penetration of active substances, and the mechanical properties of skin. In addition, by measuring transepidermal water loss, together with SCH, we are getting the data indicating the state of skin barrier and its ability to retain water and perform its barrier function [27,25]. Using different cosmetic products may cause irritation (redness, itching, tightening or peeling of the skin), but also some skin reactions (allergic reactions, irritant contact dermatitis, etc.). It has been proved that the presence of certain scents and preservatives, particularly substances of natural (plant) origin in cosmetic products, can cause these reactions. The appearance of redness after the use of cosmetic product is one of the milder, but also the first indicators of irritation, which is why determining the erythema index (as a criteria of discoloration/redness of the skin) is very significant parameter in terms of estimation of irritation potential of the preparation or its ingredients [7,1,16].

The aim of this study was to examine, using *in vivo* bioengineering methods, the effects of locally applied peloid from Ulcinj coast of the Adriatic Sea to the following skin parameters:
- skin moisture - the degree of *stratum corneum* (SC) hydration (SCH)
- transepidermal water loss (TEWL) and
- erythema index (EI).

**Materials and Methods**

**Investigated preparation**
Preparation is formulated for local application to the skin in the form of cream (hereinafter referred to as: P cream), with the peloid at a concentration of 1% as the pharmacologically active substance. It has a hydrophilic outer aqueus phase as the continuous phase and belongs to the group of oil in water creams U/V. Peloid is processed by the special technique (heated with water at T=75ºC on magnetic mixer, mixed until a homogeneous mass, and than filtered and cooled. Than it was incorporated into the ambiphilic basis (Galsana, manufacturer - Galenika AD, Belgrade) with the addition of water and oil phase (Oleum jojoba, *Simmondsia chinensis*). Recent research conducted in Israel showed that jojoba oil successfully protects against sunburn after sun [26] and accelerates faster penetration into the skin and acts as an inhensera [13].

**Place and time of examinations**
The tests were performed at the Department of Pharmaceutical Technology and Cosmetology, Faculty of Pharmacy in Belgrade, during the period from October 2010 to January 2011.
The population of examinees

To assess the impact of P cream on skin hydration - hydration level of the stratum corneum (SCH), transepidermal water loss (TEWL) and erythema index (EI), we have carried out short-term (one hour) and long-term (one month) in vivo studies in 20 human, healthy volunteers, aged 24 ± 1.2 years in average, using modern objective skin bioengineering techniques.

After obtaining the written consent for voluntary participation in the trial, following the detailed information about the study protocol, the P cream was locally applied to examinees, to the volar part of the forearm. The control group (C group; n = 20) consisted of the same examinees whose skin parameters, measured on the volar part of forearm of the other (untreated) hand, were observed.

Design of examination

Measurements of the degree of SCH, TEWL and EI were carried out using non-invasive skin bioengineering techniques as follows:
- the initial measurements of mentioned skin parameters were carried out on pre-defined areas (basal values), after which the application of P cream was performed;
- one hour after application of P cream the measurement of examined parameters was performed; after that, examinees were obligated to apply the product to a particular skin area of the volar part of the forearm two times a day (morning and evening) for a period of one month;
- after a month of daily use of the cream, the control measurement of examined parameters was performed (without applying cream on the morning before the last measurement).

All measurements were carried out after the half-hour acclimation to environmental conditions: temperature 20-23°C and humidity 33-36% - in line with the EEMCO guidance [4,15,5,22].

Examinees were required not to apply other cosmetic products for skin care and protection to the examined area during the study period (one month), and they were advised to maintain their own personal hygiene normally.

Measurement of moisture content in the skin/stratum corneum hydration (SCH) was conducted with the corneometer integrated into cutometer (Cutometer® MPA 580, Courage + Khazaka, Germany). The Probe is registering the capacity of capacitor locally build by the skin surface and the probe, which is a criterion of SCH. The high dielectric constant of water relative to other components of the skin allows the device a very accurate assessment of humidity at a depth of 60-100µm in the surface layers of the skin, and the results are expressed in arbitrary units.
Transepidermal water loss (TEWL) was measured using a device with an open type probe (Tewameter® TM210, Courage + Khazaka, Germany). The probe, a small hollow cylinder with two hydro and two thermal sensors, with a small distance between them, is measuring a vapor voltage near the skin surface, and was kept on the skin until the establishment of a stable TEWL value (~1 min). Results are expressed in g/m²/h.

Measuring the degree of irritation/erythema index (EI) was carried out with the device of the calorimeter (Mexameter® MX18, Courage + Khazaka, Germany) type. EI is calculated by the intensity of the absorbed and reflected light at 568 and 660 nm (the wavelength of hemoglobin), and results are expressed in arbitrary units.

The applied methods represent modern noninvasive techniques used to assess the effects of dermopharmaceutical and cosmetic preparations on human skin.

**Statistical analysis**

All results are presented as an average ± standard deviation (SD), for each time point (basal values, after 1 hour, and after one month). One-way ANOVA and post-hoc Tukey test were applied whenever the results allowed it, and in cases when conditions for performing parameter statistics were not met, one-way analysis of variance (Kruskal-Wallis analysis) was conducted. Values obtained after 1h and after one month were compared with basal values, and comparison of the changes in measured parameters between the surface of the skin treated with P cream and control (untreated skin) was also performed.

**Results and Discussion**

Tables I-III show average values of measured parameters at different time points (baseline values, the values obtained by measuring 1 hour after application of P cream and after one month's use of P cream) with a mark of statistically significant changes compared to baseline values - (*) and in relation the untreated control - (#).

<table>
<thead>
<tr>
<th>Table I</th>
<th>Stratum corneum humidity (SCH) in examinees treated with P cream (P group) compared with untreated control (C group) at basal conditions, after 1 hour and 1 month after the application of P cream, in arbitrary units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal values</td>
</tr>
<tr>
<td>P group</td>
<td>40.2±5.9</td>
</tr>
<tr>
<td>C group</td>
<td>40.1±6.0</td>
</tr>
</tbody>
</table>

* p<0.05

It's noticeable from Table I that there was a statistically significant difference (p<0.05) in the SCH expressed in arbitrary units, in the group
treated with P cream in both observation periods, compared to basal values, as well as in comparison with control group.

Figure 3 shows the percentage of the change of obtained arbitrary values in examinees treated with P cream (P group) compared with untreated control (Group C) in basal conditions and after a month of applying the cream.

![Figure 3](image)

*Stratum corneum* humidity (SCH) in examinees treated with P cream (P group) compared with untreated control (group C) in basal conditions and after a month of applying cream, in the percentage of change (%)

From the Figure 3 it can be noted that a statistically significant increase in SCH, compared to the basal values after one month, has occurred in the group treated with P cream, while the percentage of change in SCH after a month in the control group remained statistically unchanged compared to basal values.

The obtained results indicate a satisfactory hydrating effect of P cream, both in terms of current hydration (after a single application), and the prolonged effect of increasing the humidity of the surface layers of skin with a continuous use of cream (twice daily) during the study period of 1 month.

**Table II**

Transepidermal water loss (TEWL) in examinees treated with P cream (P group) compared with untreated control (C group) at basal conditions, after 1 hour and 1 month after the application of cream, in g/m²/h

<table>
<thead>
<tr>
<th></th>
<th>Basal values</th>
<th>After 1 hour</th>
<th>After one month</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P group</strong></td>
<td>1.8±1.5</td>
<td>5.8±2.3*</td>
<td>2.8±1.9</td>
</tr>
<tr>
<td><strong>C group</strong></td>
<td>1.7±1.4</td>
<td>3.8±1.3</td>
<td>4.5±3.7*</td>
</tr>
</tbody>
</table>

*p<0.05*
The increase of the water content probably resulted in an increase of TEWL after 1 hour of P cream application (p <0.05), which was expected if we take into account the results presented in the previous table, where there was an increase of SCH in the group treated with the examined cream. Because of this, there was probably an increased evaporation of the water from the skin surface, i.e. from the film that occurs after application and "absorption" of the cream, so the statistically significant increase of TEWL that has occurred does not indicate a violation of the skin barrier, caused by the application of the tested P cream.

After an hour, measured TEWL values were lower in control group compared with the P group, but after a month the opposite results were obtained. After a month of the P cream application, TEWL remained unchanged statistically, compared to basal values, but also in comparison to untreated control (p>0.05), while it was increased significantly in statistical sense in untreated group, compared to the basal values (p <0.05).

![Figure 4](image)

**Figure 4**

Transepidermal water loss (TEWL) in patients treated with P cream (P group) compared with untreated control (C group) in basal conditions and after a month of applying cream, in the percentage of change (%)

It is noticeable from the Figure 4 that the percentage of change in TEWL value after a month of P cream use was not statistically significant, which coincides with the results in Table II. From the standpoint of long-term application of the tested preparation, we can conclude that P cream does not lead to statistically significant improvement of the skin barrier condition, but its continued use prevents any eventual violation of the skin barrier, which (often) results from the use of bath and shower cosmetic products.
Table III

<table>
<thead>
<tr>
<th></th>
<th>Basal values</th>
<th>After 1 hour</th>
<th>After one month</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P group</strong></td>
<td>187±48</td>
<td>186±33</td>
<td>196±37</td>
</tr>
<tr>
<td><strong>C group</strong></td>
<td>186±39</td>
<td>195±36</td>
<td>214±23*</td>
</tr>
</tbody>
</table>

*p<0.05

**Figure 5**

Erythema index (EI) in patients treated with P cream (P group) compared with untreated control (group C) in basal conditions and after one month of applying cream, in the percentage of change (%)

From Table III and Figure 5, we conclude that the EI values were not significantly increased (p>0.05) in statistical sense in any time point for the tested P cream, while in the case of untreated controls there was an increase of this parameter that reached statistical significance after a month (p <0.05) of measuring. This finding suggests that P cream does not result in normal healthy skin irritation after a single, but also with the continuous long-term use of preparation during the test period of 30 days.

Similar results in improving skin barrier function and preventing premature aging of skin were obtained with the peloid from the Blue Lagoon site [6], as well as with the peloid from the Dead Sea site [18].

**Conclusion**

Based on the results of our study and taking into consideration the participation of macro- and microelements in the cream with peloid and their effects on the skin, it can be concluded that this cream has a favorable hydrating effect on the skin, and does not violate the condition of the skin.
barrier and its ability to retain water. Also, these obtained results confirm that the examined peloid cream performs its barrier function and does not show the irritation potential on the healthy skin of volunteers during the study period.

References
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