COSMETICS

BLOOD-SUCKING INSECTS

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Treatment Against Blood-Sucking Insects Without Skin Irritation

Keywords: anti-louse shampoo, neem oil, DBE-2, skin compatibility, in-vitro, BUS model, MTT, PGE₂

Introduction

In recent times the increasing incidence of head lice infestations (Pediculus humanus capitis, Fig. 1) has resulted in a significant impact on social relationships in children's playgrounds and schoolyards and has affected family activities. As the number of lice infected children has increased, some of the anti-louse products on the German and European markets show reduced activity, and other insecticide-containing products are not effective at all, or provoke painful skin sensations. Thus, the surviving lice give rise to new generations of these unpleasant blood-suckers, that are easily transmitted to heads of other family members or playmates, if, while playing, there is head/hair to head/hair contact. A major problem in increasing the awareness of louse infestations is the mistaken notion that the occurrence of lice on a child's head is the result of unsanitary conditions. This conclusion leads to social isolation of such children and their families, thus producing distinct barriers to interactions in playgrounds and schoolyards. In fact, numerous studies have confirmed that people from all socioeconomic levels are affected. Thus, there is an obvious need to develop anti-louse products with high efficacy rates, with a pleasant aroma, and minimal or no side effects, since louse bites may provoke adverse reactions.

Fig. 1 Light micrograph of a female head louse with an egg – Note the claws at the 6 legs

Summary

In recent times the increasing incidence of head lice infestations (Pediculus humanus capitis) has resulted in a significant impact on social relationships in children's playgrounds and schoolyards and has affected family activities. Many anti-louse shampoos show reduced activity or irritate the skin. They were ineffective or used in low concentrations due to the painful sensations. A newly developed anti-louse shampoo (Wash-Away®) with neem seed extract (Melia azadirachta, 10%) as active ingredient was proved in volunteer studies to be effective and skin compatible. In this in-vitro BUS-study (modified rinse-off conditions, undiluted open application, MTT-assay, Prostaglandin E₂ (PGE₂) - tissue concentration) the skin compatibility of Wash-Away® was compared with a well known brand (Nivea Silky Shine®), Wash-Away® formulation without active ingredient, the pure neem seed extract and the solvent DBE-2.

The skin compatibility of both market products, Nivea Silky Shine®, Wash-Away® were excellent and quite comparable. No statistically significant differences (MTT/ PGE₂) between the two shampoos at any exposure period (0.5h, 1.0h, 5.0h) could be obtained. The active ingredient neem seed extract does not substantially alter the biological properties of the shampoo formulation regarding skin compatibility. The application of pure neem seed extract and the solvent DBE-2 provokes a low skin compatibility. Additionally the application of DBE-2 increased significantly the tissue concentration of PGE₂.

The experimental set up and the study design using the BUS-model allow comparisons of the skin compatibility of active ingredients, solvents, complete formulations and benchmark products under identical experimental conditions.
small lesions and local inflammation due to severe itching. Newly developed anti-
louse products must be formulated for use at least two times within 8–10 days
because none of the available products affect the developing larvae in the egg
(nits) (Fig. 2). These nits are glued to hair shafts in close proximity to the scalp. As
early as 5–8 days after deposition of the eggs by the female, the larvae hatch from the
nit and begin blood sucking activity, reaching maturity within another 10 days
(1–5). Wash-Away-Laus® is a newly developed anti-louse compound with very
good efficacy on head lice.

![Fig. 2 Scanning electron micrograph of an egg (nits) of the head louse. These eggs (nits) are glued to hair shafts in close proximity to the scalp.](image)

According to the relevant EU Council Directive 76/768/EEC (1976) and the 7th
amendment, the producers of cosmetics intended to contact the skin are strictly
obliged to rule out possible damage of the skin. This also includes safety con-
considerations of completely formulated products with active ingredients. Besides
the active ingredients, other substances may be formulated which are able to
penetrate the outermost layer of the skin and may alter the barrier and reservoir
function of the horny layer.

For many years, experimental dermatologi-

cal in-vivo studies for cosmetics on
animals have been prohibited due to
ethical and legal restrictions. Results of
volunteer studies such as episcaneous
tests or general use tests do not match all possible and scientifically related ques-
tions connected with formulated prod-
ucts. In recent years, a great effort was
undertaken to develop animal-free skin
research methods (in-vitro or ex-vivo) to
study penetration and cellular reactions
such as irritation after skin contact.
Without legal and ethical restrictions,
Only undamaged, active mitochondria are able to transform methyl-thiazol-tetrazolium into the water-insoluble formazan complex. The concentration of eicosanoids (ng PGE₂/µg net weight) was determined in order to measure arachidonic acid metabolism activity in the skin tissue (Institute for Pharmacology, Toxicology and Pharmacy, Veterinary University of Hanover). The reliability of the test data was confirmed by Analysis of Variance and a LSD test for multiple comparisons. The MTT values and the PGE₂-tissue concentration were determined for all the exposure periods. The p values were calculated by paired t tests. No adjustment for multiplicity was done, p values ≤ 0.01 are considered as statistically remarkable (significant).

The skin irritation potential of a product was also defined as a comparable numerical quantity by taking the MTT and PGE₂ measurement data, calculating the percentage deviation relative to the data of the untreated skin area (=100%) and using this to obtain a combined, weighted total score (score of untreated skin: 0.0). In order to be predictive for repeated skin contact in humans the score evaluation was combined with three different exposure periods.

The shampoo Wash-Away-Laus® – a medicinal remedy (class 1) produced by the university spin-off company Alpha-Biocare GmbH, Düsseldorf, Germany) which is based on a patented neem-seed extract (Alpha-Biocare GmbH, Düsseldorf) and fine shampoo components was shown to be highly active on head lice (15-16).

Besides aqua, the product contains the following compounds according INCI: Neem-Extract, Polyglyceryl-3- Caprate, Cocamidopropyl Betaine, Dimethyl Glutarate, Glycerin, Dimethyl Adipate, Lauryl Glucoside, Dicaprylyl Ether, Laureyl Alcohol, Benzyl Alcohol, Benzoic Acid, Dimethyl Succinate, Sorbic Acid. No parfum oil mixtures were used.

#### Results

Viability of the model

The viability of the perfused udder tissue was given, the data of the control areas
obtained varied within the historical range. There was no statistically significant difference between control I and control II at any exposure period (Table 3).

Skin compatibility (score evaluation)
On the basis of the results of studies using human volunteers and of epidemiological comparisons and market experience as well, it is possible to consider that, if a total score below 2.5 is obtained in the BUS model, no skin irritation will occur in human beings even after repeated skin contact. The total score of untreated skin is calculated as 0.0. For cosmetic formulations such as skin protection products applied once, the total

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>0.5h [n.s./s.]</th>
<th>1.0h [n.s./s.]</th>
<th>5.0h [n.s./s.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTT</td>
<td>n.s.</td>
<td>n.s.</td>
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</tr>
<tr>
<td>Control I vs</td>
<td>1.03 ± 0.10</td>
<td>1.02 ± 0.10</td>
<td>1.0 ± 0.10</td>
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<td>Control II</td>
<td>1.03 ± 0.09</td>
<td>1.01 ± 0.08</td>
<td>1.0 ± 0.09</td>
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<tr>
<td>PGE₂</td>
<td>n.s.</td>
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<tr>
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<td>0.54 ± 0.02</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>Control II</td>
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<tr>
<td>MTT</td>
<td>n.s.</td>
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<td>[s]</td>
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<tr>
<td>Wash-Away-Laus®</td>
<td>1.03 ± 0.10</td>
<td>1.02 ± 0.10</td>
<td>1.0 ± 0.10</td>
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<td></td>
<td>1.00 ± 0.09</td>
<td>0.96 ± 0.10</td>
<td>0.92 ± 0.09</td>
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<tr>
<td>Wash-Away-Laus®</td>
<td>0.53 ± 0.02</td>
<td>0.56 ± 0.01</td>
<td>0.54 ± 0.02</td>
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<tr>
<td>MTT</td>
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<td>Nivea Silky Shine®</td>
<td>1.00 ± 0.10</td>
<td>0.96 ± 0.09</td>
<td>0.88 ± 0.08</td>
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<td>PGE₂</td>
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<tr>
<td>Nivea Silky Shine®</td>
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<td>0.58 ± 0.01</td>
<td>0.54 ± 0.03</td>
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<td>MTT</td>
<td>[s]</td>
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Table 2: Comparison of the score data (MTT, PGE₂) of all products tested (A=Wash-Away-Laus®, B=Nivea Silky Shine®, C=Wash-Away-Laus® without neem seed extract, D=solvent DBE-2, E=neem seed extract pure) after an exposure period of 0.5h, 1.0h and 5.0h and after one application.

Table 3: The statistical evaluation (mean ± sd; p ≤ 0.01; s = significant, n.s. = not significant) of the control areas I and II and the treated skin samples concerning the parameter cytotoxicity (MTT; μg formazan / μg net weight) and irritancy (PGE₂; ng PGE₂ / μg net weight) at the three exposure periods (0.5h, 1.0h, 5.0h).
score ranges within 0.4 and 0.0 (17). Various skin care products produce a total score up to 1.0 at one of the three exposure periods using the standardized study design (unpublished results). With increasing exposure, cytotoxic cellular reactions are the main features observed (Table 2). After the short exposure period (0.5 h), almost no reaction occurred compared to the untreated skin areas. After the exposure period of 1.0 h slight cytotoxic reactions occurred after DBE-2 or neem seed extract (pure) was applied. The application of Wash-Away-Laus®, Nivea Silky Shine® or the basic solution without neem seed extract induced no significant cellular reactions.

However, higher cytotoxic and inflammatory reactions were induced by application of DBE-2 and neem seed extract pure after the prolonged exposure period of 5.0 h. There is a small difference between the products Wash-Away® and Nivea Silky Shine®. The benchmark product elicited a slightly higher cytotoxic potential when compared with Wash-Away-Laus®.

Comparisons of products, active ingredient, chemicals (statistical evaluation)
Compared with the control area there is no difference between the two products Wash-Away® and Nivea Silky Shine® concerning the skin compatibility regarding cell irritancy (PGE). However concerning the cytotoxic reaction the application of Wash-Away-Laus® induced slightly less reaction (exposure period: 5.0 h) than the application of Nivea Silky Shine® (exposure period: 1.0 h and 5.0 h) (Table 3). Between the products Wash Away® and Nivea Silky shine® no statistically significant difference regarding skin compatibility can be considered if compared directly to each other (Table 3).

Statistically significant deviations from the control were found most frequently after the application of the solvent (DB-2) and the active ingredient neem

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seed extract (pure). After the short exposure period of 0.5h the application of all three test substances induced cytotoxicity and inflammatory reaction (DBE-2) throughout all exposure periods. The tissue concentration of PGE₂ also increased after exposure of 1.0h and 5.0h to the solvent (Table 4).

Discussion
Heavily head louse infested skin shows exudation and itching at the bite sites, which may lead to localized infections. If the anti-louse product used has any capability for skin irritation, these minimal lesions may become sites of painful lesions. The efficacy of the shampoo Wash-Away-Laus® - a medicinal remedy class 1 - was tested in several clinical studies, and was shown to be highly effective on head lice (15-16). During these studies the probands declared that there is support of healing effects on infected louse bites without any burning (Fig. 3). All test persons during efficacy tests had described that the use of Wash-Away-Laus® is very agreeable, since it smoothens the structure of the hair, has no painful effects at the bite sites and has a pleasant aroma. In dermatological tests in concentrations of 1%, 2% and 5% (Epicutaneous test, Project 46/05/07; Dermatest GmbH, Münster, Germany) the product proved to be very compatible on human skin. According to the test results, the rating was considered very good. Therefore, a carefully designed comparison concerning skin compatibility was performed in order to manifest scientifically the described good feeling.

The basic product formulation of Wash-Away-Laus® and the benchmark product Nivea Silky Shine® are similar with the exception that Wash-Away® contains neem seed extract pure as the active ingredient and no perfume oil components. Therefore, it was of interest to compare the skin compatibility of both qualified hair care products. The application of these sort of products intends repeated human skin contact. Using the standardized method of the BUS model, a direct comparison is possible because the epidermal cellular reaction can be biochemically assessed from whole skin biopsies after various exposure periods. The primary exposure period exceeds the usual rinse-off time per-

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<tr>
<td>MTT</td>
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<tr>
<td>Control 1 vs Wash-Away-Laus® without NEEM</td>
<td>1.03 ± 0.10 vs 0.99 ± 0.09</td>
<td>1.02 ± 0.10 vs 0.95 ± 0.08</td>
<td>1.0 ± 0.10 vs 0.91 ± 0.08</td>
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<tr>
<td>PGE₂</td>
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<tr>
<td>MTT</td>
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<tr>
<td>Control 1 vs DBE-2 (solvent)</td>
<td>1.03 ± 0.10 vs 0.96 ± 0.09</td>
<td>1.02 ± 0.10 vs 0.92 ± 0.10</td>
<td>1.0 ± 0.10 vs 0.87 ± 0.08</td>
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<td>PGE₂</td>
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<td>MTT</td>
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<tr>
<td>Control 1 vs Neem seed extract (pure)</td>
<td>1.03 ± 0.10 vs 0.97 ± 0.09</td>
<td>1.02 ± 0.10 vs 0.93 ± 0.08</td>
<td>1.0 ± 0.10 vs 0.85 ± 0.08</td>
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<tr>
<td>PGE₂</td>
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Table 4 The statistical evaluation (mean ± sd; p < 0.001; s = significant, n.s. = not significant) of the control areas and the treated skin samples (product formulation without NEEM, solvent, Neem pure) concerning the parameter cytotoxicity (MTT, µg formazan/µg net weight) and irritancy (PGE₂, ng PGE₂ / µg net weight) at the three exposure periods (0.5h, 1.0h, 5.0h)
BLOOD-SUCKING INSECTS

period, despite wiping away of the surplus after 15 minutes and all substances were applied undiluted. The application of DEB-2 and undiluted neem seed extract provoked the highest cytotoxicity and inflammatory reactions, increasing throughout the exposure periods. So both substances were biologically active within the epidermis and dermis, but no skin irritation can be predicted after repeated skin contact.

For both hair care products already marketed, the results of the score evaluation indicate excellent skin compatibility even after repeated skin contact. This result could be expected in testing a well known and widely used brand as a benchmark. The total score may be also directly compared with other cosmetic products such as skin care products and skin protection formulations [17]. The results of the BUS-skin compatibility test confirmed the results obtained in human patch tests using Wash-Away-Laus\textsuperscript{®}.

In addition to the score evaluation specially designed for the predictive results of repeated skin contacts, the biochemically assessed cellular reactions after a modified rinse-off situation can be used for further differentiation between the products. Despite the very slight differences of Wash-Away-Laus\textsuperscript{®} and Nivea Silky Shine\textsuperscript{®} in comparison to the untreated skin no statistically significant difference was observed in the direct comparison to each other. The degree of excellent skin compatibility is equal for both products even Wash-Away-Laus\textsuperscript{®} a registered medicinal product, contains 'neem seed extract' as the biologically active ingredient.

Literature


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