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ORIGINAL PAPER

Comparative in vitro tests on the efficacy and safety of 13 anti-head-lice products

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Abstract Head lice are an emerging social problem, not only in economically poor countries but also in practically all other societies. Several of the common anti-louse products have lost—at least in part—their efficacy due to increasing resistance of lice against insecticides such as permethrin or allethrin. Other compounds, like lindan, were redrawn or banned due to high toxicity. Some recently developed products are based on dimethicones or cyclomethicones and turned out to be easily inflammable. Other styled medicinal products are based on plant extracts—some were proven of high efficacy—others of ineffectivity. The present study investigated in in vitro tests the anti-head louse efficacy of 13 products, the contents of which are used worldwide: Aesculo®-Gel L, EtoPril®, Goldgeist® Forte, InfectoPedicul®, Jacutin® Pedicul Fluid, K.Laus®, Liberalice®, Licatack®, Mosquito® Läuse-Shampoo, Nyda®, Paranix®, Picksan® Louse Stop, and Wash Away Louse®. It turned out that several of them are easily inflammable, and therefore, they endanger users (InfectoPedicul®, Paranix®, EtoPril®, Nyda®, Goldgeist® Forte, and K.Laus®, see Table 1). Others have to remain for many hours on the hair in order to reach efficacy (Table 4). During such long periods, highly dosed oils may become inhaled and thus may become dangerous for the user's lung epithelia by covering them. When incubating the lice for 3 or 10 min in vitro, only the following products killed all lice exposed to these products: InfectoPedicul®, Paranix®, Jacutin® Pedicul Fluid, Nyda®, K.Laus®, Picksan® Louse Stop, Licatack®, and Wash Away Louse®. When evaluating the possible dangers for the users, the last three products, especially, derived from plant extracts, are safe and highly effective at the same time. Furthermore, they had been tested dermatologically as "very good".

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Introduction

Head lice (*Pediculus humanus capitis*) occur worldwide and go with humans in all corners of the world (Aspöck and Walochnik 2007; Falagas et al. 2008). Although, in general, head lice do not transmit agents of disease as it is known from body lice (*Pediculus humanus corporis*), they may have considerable impact on health of humans. Their bites, especially in cases of huge infestations, may lead to enormous pruritus, skin inflammation, urticaria, exudations, lymph node swellings, eczema, scars, hair glue-up to "plica polonica", ending in pain and restlessness especially in children (Burgess 2004, 2009; Mumcuoglu and Rufli 1983; Mumucuoglu et al. 2009; Mehlhorn et al. 1995; Mehlhorn 2008; Mehlhorn and Mehlhorn 2009). All these symptoms are described as a disease called "pediculosis" which also includes psychological damages of infested children and their caring mothers. These effects occur since infestation with lice is often considered to have its origin in dirtiness of the infested persons and their families. This wrong belief ends often in "mobbing" of children infested with lice. Then, the children refuse to go to the child yard or to school. This reaction disturbs considerably the normal run

of the daily family life. In consideration of these physical and psychological consequences of an infestation with head lice, many countries made some anti-louse products prescribable by physicians, and thus, these products are given for free to the patients. Since all family members must be treated at once—they may invisibly be infested—the additional treatment doses must be paid from the income of the family. Therefore, many products worldwide are on the market claiming efficacy. However, many of them (under different trade names) are dangerous for users due to their inflammability or by covering the surfaces of lung epithelia. Others are ineffective, leaving surviving lice that start another series of reproduction affording further treatments and thus producing inevitable and considerable

Table 1 Composition and inflammability of different formulations, shampoos, lotions, etc. against head lice (*Pediculus humanus capitis*)

Number	Trade name	Company/address	Charge number	Active compound noted	Inflammability
1	Infecto Pedicul® medical solution (50 ml)	Infectopharm Arzneimittel Von-Humboldt-Str. 1 D-64646 Heppenheim, Germany	WO 60819.1	Permethrin	Yes
2	Paranix® Medical solution (60 ml)	Teva Pharmaceutical Industries Ltd. P.O. Box 3190 Petach–Tikva Israel	WC 8 E 28-51	*Cocos nucifera* oil, *Illicium verum* (anise) oil, *Cananga odorata* oil	Yes
3	Mosquito® Läuse Shampoo (100 ml)	WEPA-Apothekenbedarf GmbH & Co. KG Am Fichtenstrauch 6-10 D-56204 Hillscheid, Germany	08337-F 197	Cocoamido-propylbetaine, *Glycine soja*	No
4	Jacutin® Pedicul Fluid medical solution (100 ml)	Almirall Herman GmbH Scholtzstraße 3 D-21465 Reinbeck, Germany	837442	Dimethicone	No
5	EtoPril® Medical solution (100 ml)	Dr. August Wolff GmbH & Co. KG Arzneimittel D-33532 Bielefeld, Germany	VM 49	Dimethicone Cyclomethicon 5	Yes
6	Liberalice® Duo LP-Pro medical solution (150 ml)	Duhot S.A. Avenue de Nivelles 7, 1300 Limal Belgium Licence of Terra Santé, France Delivery: Trommsdorf GmbH & Co, KG Arzneimittel 52475 Alsdorf, Germany	08197	Oxypthirine	No
7	Nyda® Spray (50 ml)	G. Pohl-Boskamp GmbH & Co. KG Kieler Straße 11 D-25551 Hohenlockstedt, Germany	204716	Dimethicones of high and low viscosity	Yes
8	Goldgeist® Forte medical solution (75 ml)	E. Gerlach GmbH D-32292 Lübbecke, Germany	030219	Pyrethrum from blossom of *Chrysanthemum cinerarieafolium*	Yes, for a short period only
9	K.Laus® medical solution (100 ml)	SSL International plcVenus 1 Old Park Lane, Trafford Park Manchester, M 417HA, UK Distribution: SSL Healthcare GmbH & Co. KG D-63477 Maintal, Germany	P 712954	Cyclomethicon, isopropylmyristate	Yes
10	Aesculo® Gel "L" medical gel (100 ml)	Medice Arzneimittel Pütter GmbH & Co, KG Kuhloweg 37 D-58638 Iserlohn, Germany	82008/1	*Cocos nucifera* oil	No
11	Licatack shampoo (100 ml)	PM Consumer Products NL 1184 VW Oudekerk a/d Amstel, The Netherlands	Pre-production sample	Grapefruit extract	No
12	Wash Away Laus shampoo (100 ml)	Alpha-Biocare GmbH Merowinger Platz 1 a D-40225 Düsseldorf, Germany	K-05-01	MelAza-extract	No
13	Picksan® Louse Stop shampoo (100 ml)	OTC Pharma International BV Postbus 816 4200 AV Gorinchem, The Netherlands	20702633	MelAza-extract	No

costs especially in families with several children (Abdel-Ghaffar and Semmler 2007; Heukelbach et al. 2006a, b). The present in vitro study aimed to compare the efficacy and possible inflammability of 13 European products, which either contained insecticides (permethrin, pyrethrum extract), silicon-derivates, or plant extracts. The selected exposition periods (3 and 10 min) were rather short. This was done since, in practical use, the procedure of an anti-lice action should be as short as possible. Such a short time will bother the families less and will let them better avoid possible side effects due to the effective compounds in the anti-lice products.

Materials and methods

Materials

In the present in vitro test (carried out in Cairo, Egypt), the 13 products that were used are listed in Table 1. The head lice were obtained by combing 20 children that had been included in an in vivo test of the newly developed anti-louse product "Licatack®" during the same period in October 2009 (Fig. 1).

Methods

Efficacy test

Always ten adult = large lice were placed into small baskets (Fig. 2), the bottom of which consisted of metal net. These baskets were placed into plastic petri dishes, the bottoms of which were covered with white filter paper (Fig. 3). Then, the lice were completely covered by with one of the 13 products listed in Table 1. The lice were either incubated for 3 or 10 min within the different compounds. After the incubation period, the baskets were taken out of the petri dishes, and the lice were washed three times for 30 s with tap water until they were completely free from any remnants of the compounds. Then, they were placed onto fresh white filter paper in another petri dish. After 10, 20, 30, 60 min, 2, 3, 4 h, the lice were inspected with the help of a strong magnification glass that allowed to see even eventual leg movements or constrictions of the intestine. The findings were noted and are documented in Table 2.

Fig. 2 Small basket with a metal net as bottom for incubation of lice in different compounds

Inflammability test

Hair from human heads was collected from local barbershops. For testing the inflammability of different products against human head lice, defined portions of hairs, weighing from 1.1 to 1.5 g, were assorted into small heaps measuring about 5 cm in diameter and about 0.5–1 cm in height. Then, and for each preparation separately, these hair portions were treated with the products to be tested in accordance to the consumers instructions. In all cases, the hair was made fully wet by the

Fig. 1 Obtaining lice by combing from infested head

Fig. 3 Partial aspect of the products and vials at the beginning of the test

preparations and then immediately put on a white glazed tile (14.8×14.8 cm). The tile with the treated hair was placed into a closed laboratory drain (digestorium). Then, the flame from a piezoelectric lighter was brought into contact with the treated hair. The results of each test series were documented by a digital motion picture camera. As soon as an individual test series was ended, the glazed tile was thoroughly cleaned before another test was started. As control, untreated hair was used for inflammation tests.

Results

Inflammability test

The results of this test are documented in Tables 1 and 3. It turned out that some products, even if their active compounds are not inflammable without contact to hair, may become inflamed when there is a mixture consisting of hair and anti-lice compound. This makes it unpredictable when a danger is given for the user of the product. It must not be open fire that lights up treated hair. In practice, it had been shown that already the starting flesh of a fan may inflame hair (report shown by the German television broadcaster WDR=Westdeutscher Rundfunk as well as by several newspapers in Austria and The Netherlands; Mehlhorn and Mehlhorn 2009).

The inflammable products showed the following burning features. While untreated, lit-up hair was melting, the inflammable products initiated the following reactions:

1. InfectoPedicul® (No. 1): treated hair produced flames up to 4–7 cm high, persisted constantly inflamed, and wandered along the hair.

Table 2 In vitro test of 13 different anti-louse products after an exposure of ten lice for 3 min or 10 min and after 3×30-s washing the lice with tap water

Product number (names in Table1)	10min after washing	20min after washing	30min after washing	1h after washing	2h after washing	3h after washing	4h after washing
1[a]	2, m	2, m	1, m	1, m	1, m	1, m	1, m
1[b]	nm	nm	nm	nm	nm	nm	nm
2[a]	nm	nm	nm	nm	nm	nm	nm
2[b]	nm	nm	nm	nm	nm	nm	nm
3[a]	5, m	all m	all m	all m	all m	all m	all m
3[b]	all m	all m	all m	all m	all m	all m	all m
4[a]	nm	nm	nm	nm	nm	nm	nm
4[b]	nm	nm	nm	nm	nm	nm	nm
5[a]	nm	1, m	nm	nm	nm	nm	nm
5[b]	nm	nm	nm	nm	nm	nm	nm
6[a]	4, m	all m	all m	all m	all m	all m	all m
6[b]	all m	all m	all m	all m	all m	all m	all m
7[a]	nm	nm	nm	nm	nm	nm	nm
7[b]	nm	nm	nm	nm	nm	nm	nm
8[a]	2, m	2, m	2, m	2, m	2, m	1, m	1, m
8[b]	nm	2, m	2, m	2, m	3, m	3, m	3, m
9[a]	nm	nm	nm	nm	nm	nm	nm
9[b]	nm	nm	nm	nm	nm	nm	nm
10[a]	3, m	2, m	2, m	2, m	3, m	3, m	2, m
10[b]	3, m	3, m	3, m	3, m	nm	nm	nm
11[a]	nm	nm	nm	nm	nm	nm	nm
11[b]	nm	nm	nm	nm	nm	nm	nm
12[a]	nm	nm	nm	nm	nm	nm	nm
12[b]	nm	nm	nm	nm	nm	nm	nm
13[a]	nm	nm	nm	nm	nm	nm	nm
13[b]	nm	nm	nm	nm	nm	nm	nm

M mobile, *nm* non-mobile/dead; lice, *n*=10 per vial

[a] Exposed for 3 min

[b] Exposed for 10 min

Table 3 Declarations given directly on the bottle, which is used during treatment

Number	Trade name	Effective compounds declared	Signs of inflammability	Age of children given	Mode of use	Hints for safety
1	InfectoPedicul®	Yes	No, but written information	No	In parts	No
2	Paranix®	No	No, although inflammable	No	No	Yes
3	Mosquito® Läuse Shampoo	Yes	No	From 3 years (GB), 3 months (Germany)	Yes	No
4	Jacutin Pedicul® Fluid	Yes	No	No	No	No
5	EtoPril®	Yes	No, although inflammable	From 6 months	No	Yes
6	Liberalice®	No	No	No	Yes	Yes
7	Nyda®	No	Yes	No	No	No
8	Goldgeist Forte®	Yes	No, although inflammable	No	No	Yes
9	K.Laus®	Yes	No, although inflammable	No	No	Yes
10	Aesculo® Gel L	Yes	No	No	Yes	No
11	Licatack®	Yes	No	Yes	Yes	Yes
12	Wash Away Louse®	Yes	No	No	Yes	Yes
13	Picksan® Louse Stop	Yes	No	No	Yes	Yes

2. Paranix® (No. 2) initiated similar effects like Infecto-Pedicul®.
3. EtoPril® (No. 5) initiated flames of 4–7 cm in height, which persisted and wandered.
4. Nyda® (No. 7) reacted like EtroPril®.
5. Goldgeist Forte® (No. 8) initiated only a short burning of 3–6 s, and the flames did not wander.
6. K.Laus® (No. 9) initiated the same flames like EtoPril® (No. 5) or Nyda® (No. 7).

Untreated hair and hair treated with Mosquito®, Jacutin® Pedicul Fluid, Liberalice®, Aesculo® Gel L, Licatack®, Wash Away Louse®, and Picksan® did not burn but melted, when coming in contact with open fire.

Efficacy test

When looking at Table 2, which summarizes the results of the whole in vitro trial, it is clearly seen that only the products Paranix®, Jacutin® Pedicul Fluid, EtoPril®, Nyda®, K.Laus®, Licatack®, Wash Away Louse®, and Picksan® Louse Stop are able to kill/immobilize all lice within 3 min of exposure (without any recovery within 4 h). The product InfectoPedicul® left some short-term survivors, who died after 5 h. On the other hand, the products like Mosquito® Läuse-Shampoo, Liberalice®, and Aesculo® Gel L let all lice survive after an exposure period of 3 min. When exposing the lice for 10 min (series b in Table 1), the products InfectoPedicul®, Paranix®, Jacutin®

Table 4 Recommended period of treatment and elimination of the compound from hair

Number	Trade name	Producer's recommended time for treatment	Elimination of the product from hair after treatment
1	InfectoPedicul®	30–45 min	Use of tap water
2	Paranix®	15 min	Use of shampoo
3	Mosquito® Läuse Shampoo	As normal shampoo, then 2×30 min	Use of tap water
4	Jacutin Pedicul® Fluid	10 min	Use of a shampoo
5	EtoPril®	At least 8 h	Use of a shampoo
6	Liberalice®	Overnight and hair cover	Use of a shampoo for at least 10 min
7	Nyda®	At least 8 h	Use of shampoo
8	Goldgeist Forte®	30–45 min	Use of tap water
9	K.Laus®	10 min	Use of a shampoo
10	Aesculo® Gel L	60 min	Use of a shampoo
11	Licatack®	10 min	Use of tap water
12	Wash Away Louse®	10–20 min	Use of tap water
13	Picksan® Louse Stop	10–20 min	Use of tap water

Pedicul Fluid, EtoPril®, Nyda®, K.Laus®, Licatack®, Wash Away Louse®, and Picksan® Louse Stop were 100% successful in killing the exposed lice.

Untreated controls survived for up to 16–24 h when combed down from hair and placed onto white filter paper in a closed plastic petri dish.

Discussion

This comparative in vitro study has revealed significant results, which give more clear insights in the efficacy range of a certain products than do in vivo tests, since the latter are always endangered by wrong use or by non-contact of lice to the active compounds (the lice may crawl away quickly from the products) when it is brought into hair. It was shown that several products are inflammable and thus represent a danger, especially in cases of lack of warning on the bottles (Table 3). Such a sign was missing in four of six inflammable products. The design of this in vitro study yielded those products needing the shortest time until the lice were killed (Tables 2, 4). In case there is a full activity, the shortest time for treatment is the best, since that way the families are less bothered by the anti-lice action than in cases when these actions need eight or more hours, plus an intensive procedure to eliminate the often gluing product from the hair. Furthermore, a very short period of treatment also reduces the possibility that some components of the product may be inhaled and covers the lung epithelia or may initiate allergic skin reactions. Some products, such as EtoPril®, Liberalice®, and Nyda® afford at least 8 h on the head (Table 4). However, the present in vitro tests showed that they kill the lice in a few minutes, too. Therefore, there must be a problem in bringing these products in vivo on all regions of hair when it is applied. This failure is probably due to the gluing consistency of these products when mingled with hair, since Oliveira et al. (2007) had also found an in vitro activity within 5 min.

Cyclo- and dimethicones are commonly used in skin care products, e.g., conditioners. However, the products contained, in general, 1% of these compounds and rarely up to 15% (Nair 2003). Reports cited in the latter paper claimed that short-term inhalation of dimethicones did not initiate adverse effects in rodents. However, the use of these methicones as "repair conditioners" which cover the surface of hair make it understandable that they may also cover lung epithelia, if they are inhaled while using it in concentrations of more than 50% for many hours. Although the Cosmetic Ingredient Expert Panel considered it unlikely that any of these polymers would be significantly absorbed into the skin, there is a cited case where a cream formulation with 1% dimethicone had severe adverse effects (Nair 2003). This shows that those compounds may induce harm if they are transported by a carrier into the skin. Similar hints are given in a website—www.nohomis.at—that the weakening compounds in silicones may be harmful. Therefore, it cannot be fully excluded that silicon-based anti-lice products do not endanger users, especially children in cases of very high concentrations, very long exposure periods, and numerous application schemes at short intervals. Thus, in times of increasing resistance of lice (Vassena et al. 2003; Picollo et al. 2000; Mogabure Cueto et al. 2008) against pyrethroids and after banning of compounds like lindan there is a need for

(a) quick, easy to use, and fully effective compounds,
(b) skin-safe compounds,
(c) non-inflammable compounds, and
(d) availability at reasonable prices.

Conclusions

The present study clearly shows that the three plant-derived products Licatack®, Picksan® Louse Stop, and Wash Away Louse® have many advantages with respect to other products:

(a) they are not inflammable (Table 1),
(b) their contents are not toxic and are tested "very good" by a professional dermatological clinical group,
(c) they do not contain components (as do silicon-derivates or Paranix® with their volatile oils) that may harm the surface of lung epithelia, especially as it might be the case, when these components are included in the products at concentrations higher than 50%.
(d) the period of treatment is very short (10–20 min) compared with other products which need at least 30 min up to 8 h (Table 4) and peculiar methods to get them out of the hair after treatment.
(e) The declarations on the bottle itself are clear and allow safe use, while in other products, important information is only given on the information sheet in the package.

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