Parasitol Res (2010) 106:445–449
DOI 10.1007/s00436-009-1683-7

ORIGINAL PAPER

Efficacy of a grapefruit extract on head lice: a clinical trial

Fathy Abdel-Ghaffar · Margit Semmler · Khaled Al-Rasheid · Sven Klimpel · Heinz Mehlhorn

Received: 19 October 2009 / Accepted: 2 November 2009 / Published online: 27 November 2009

Abstract Twenty children aging 2-9 years old—four boys with short hair and 16 girls with long hair—were included in a clinical test on the efficacy of a product against head lice (*Pediculus humanus capitis*). Their hair were exposed to Licatack®, which is a recently developed new anti-louse medicinal product containing extracts of grapefruits besides high quality shampoo components. Prior to this field trial, the product Licatack® was tested dermatologically to be skin safe receiving the grade "very good". The children's mothers combed the kids prior to the start of the test in order to confirm that they were all lice-infested. The obtained lice were used for in vitro tests. All children were heavily infested. After combing and preservation of the living lice, the hair was wet with tap water. Then, 50 ml of the Licatack® shampoo was placed onto the top of each child's head. Then, the mothers distributed the rather fluid product all over the hair thoroughly from their base at the skin until the free end. During this process, a type of massage, the product became foamy and it was easily recognized where the product covered the hair, thus, avoiding untreated spots. The hair of half of the treated children were washed with tap water after 10 min of exposition; while in the other half of the children, the exposition period was prolonged to 20 min before washing. When combing the kids with a metal louse comb after the washing, the lice were found immobile and they did not recover during the following observation period of 4 h. Only two lice from the group with an exposition time of only 10 min showed some slight leg movements after they had been combed off, but they died within the next 2 h. Thus, this new anti-louse medicinal product has a very quick and efficient activity besides its advantages of being non-inflammable, skin safe, and nice smelling. None of the kids claimed any burning at the skin or other side effects, although the skin showed, prior to treatment, lots of scars due to louse bites. The dead lice always appeared considerably shrunken due to drying. The second treatment after 10 days revealed a few dead larval stages since, apparently, some larvae (apparently treated at an early stage of development when treated) had hatched from the extremely numerous nits in the period between first and second treatment. Experiments with cutoff nits, however, showed that the product also kills larval stages inside nits.

F. Abdel-Ghaffar · M. Semmler
Department of Zoology and Parasitology, Cairo University, Giza, Egypt

K. Al-Rasheid
Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia

S. Klimpel · H. Mehlhorn (✉)
Department of Parasitology, Heinrich Heine University, 40225 Düsseldorf, Germany
e-mail: mehlhorn@uni-duesseldorf.de

Introduction

Pediculus humanus capitis, the so-called head louse, is an obligate ectoparasite which is found exclusively on humans. These lice have evolved with mankind and, thus, were distributed all over the world (Aspöck and Walochnik 2007; Burgess 2004; Mehlhorn and Mehlhorn 2009). In our days of intense globalization with migration streams in all directions of the earth, the prevalence of head lice is increasing considerably (Falagas et al. 2008; Mumcuoglu et al. 2009; Burgess 2009; Abdel-Ghaffar et al. 2009). Although in general, head lice do not transmit disease like the body lice which are able to transmit them, especially in

cases where people live closely together, e.g., in refugee camps, in soldiers' camps, or in houses of aged people. Therefore, such a possible transmission cannot become excluded completely as was shown by Robinson et al. (2003) or Sasaki et al. (2006). In any way, the head lice infestation may become a severe health problem (pediculosis) in case of severe inflammation and super-infection of the skin. Furthermore, head louse infestation may result in social embarrassment when infested children and their families become mobbed as "dirty" or "antisocial" (Burgess 2004; Mehlhorn et al. 1995; Mehlhorn 2008; Mehlhorn and Mehlhorn 2009; Toloza et al. 2009).

One of the reasons of the constant spreading of head lice is surely the increasing globalization, leading to a closer contact between many individuals. This is important since the transmission of head lice occurs practically exclusively by close hair-to-hair contact (Takano-Lee et al. 2005). For a fed and gravid female louse, such a contact offers the chance to switch from one head to the other since lice have developed during evolution a so-called positive phototropism, which urges them to run just after a blood meal to the end of the hair and to lure there for a hair-to-hair contact with another host (Martini 1946). If this contact is not given, the females return to the warm scalp's surface and start another blood meal there while gluing eggs at the basis of the hair.

The second reason for the recent constant spreading of lice is the reduction of the efficacy of available anti-louse compounds since many of them had been used for a very long time. This recent lack of efficacy is surely based on a variety of reasons among which are the incorrect use and development of resistances (Picollo et al. 1998, 2000; Vassena et al. 2003; Mogabure Cueta et al. 2008; Toloza et al. 2009; Yang et al. 2004) are probably the most important ones. Some substances such as DDT or lindan have been banned in many countries due to their high toxicity (Mumcuoglu et al. 2009; Mehlhorn 2008; Mehlhorn and Mehlhorn 2009). Other products never reached full activity or are dangerous to use, e.g., they may be highly flammable (Abdel-Ghaffar et al. 2009). Thus, several groups of scientists tried to initiate the use of etheric oils (e.g., cocos oil, anise oil, eucalyptus oil, etc.). These approaches were also not completely successful although many plant extracts exert a certain insecticidal activity (Abdel-Krim and Mehlhorn 2006). Another disadvantage is the fact that many of the etheric oil components may induce unwanted immune reactions leading to allergic skin problems (as it might be the case, e.g., when anise, cocos, etc., are used). Thus, the present scientific problem is to find substances, which are not toxic and are not immunogenic, but which are fully active against all developmental stages of the louse life cycle. Some neem seed extracts had been shown to fulfill these requirements of high efficacy, besides skin safety and non-toxicity (Heukelbach et al. 2006a, b; Abdel-Ghaffar and Semmler 2007). A recently developed extract of grapefruit turned out to be as safe and as efficacious as the neem extracts when studied in several in vitro tests (Abdel-Ghaffar et al. 2009). The present in vivo study with the trade product Licatack® was done to control whether it is also fully effective and could kill, under normal in vivo use conditions, all lice already within an exposition period of only 10 min. Of course, this product was only used on the heads of children after its non-toxicity and its skin safeness had been proven previously in clinical tests in Germany.

Material and methods

Material

Licatack® is a newly developed anti-louse shampoo that contains extracts of grapefruits and high value shampoo components. Previous to the present in vivo study, this product had been tested "very good" with respect to its skin safety and other dermatological aspects by Fa. Dermatest (Münster, Germany). Forty 100-ml tubes were sent to the chief investigator Prof. Dr. Abdel-Ghaffar (Cairo, Egypt) by Fa. Alpha-Biocare (Düsseldorf, Germany) for treating the children twice: on day 1 and day 10). The second treatment was done in order to kill larval stages hatched from eggs that had been produced by female lice just prior to treatment.

Children

The children belonged in principle to five families living in a small village at the desert rim about 1 h from Cairo. The mothers of the children combed, treated, and washed their kids under the supervision of the chief investigator and his co-investigators. Thus, it was guaranteed that the mode of application, the time of treatment, the amount of the product, and the control measurements were standardized according to the protocol. The kids were 2-9 years old. Among the 20 kids, there were four boys with short hair and 16 girls with rather long hair.

Methods

The test was done in a large room at the house of one of the involved families at a room temperature of 30°C. The mothers of the kids combed their kids first by means of a normal comb, and all lice were collected on a white sheet from where they were transferred to plastic Petri dishes with an inlay of white filter paper in order to become used for in vitro tests. After it had been proven that all kids were

strongly infested with all stages of lice (larvae, nits, and adults), the treatment was started. Two groups of the kids were randomly formed. One group of ten children was exposed for 10 min to the product before washing the hair with tap water and subsequent combing. The other group was exposed for 20 min to the product before washing and combing. In order to treat all children, always for the same time within each group, five children were treated always at the same time followed by the other five. After washing, the combed-off lice were collected in a plastic Petri dish (on white filter paper). The inspection, whether the lice were motionless, showed movements of their legs or had intestinal constrictions, was done with the help of a strong magnification glass by two co-investigators and was cross-checked. The collected lice were observed for 4 h in order to detect any signs of recovery.

Previous to the treatment, some hair covered by nits were cut off and placed into a closed, light-impermeable plastic bottle containing a wet filter paper so that a humidity of about 70% was reached. The same was done with hair that had been exposed for 20 min to Licatack® before being washed with tap water. Ten days later, the bottles which had been stored in an incubator of 31°C were opened and the hair was inspected with help of a stereo microscope for hatched larval stages. At day 10, after the first treatment, the treatment with Licatack® was repeated.

Results

All children were combed by their mothers prior to treatment. While the combing of the four boys (with short hair) revealed only 10-20 louse stages, the long-haired girls were visibly infested with many lice stages (up to 50). The children were grouped in two groups of ten. While the hair of the first ten kids were exposed for only 10 min to Licatack®, the hair of the second group was exposed for 20 min. After the exposition, period the hair was rinsed intensively with normal tap water. All this was done by the mothers of the kids (Figs. 1–2). After this washing process, which made the previously gluing hair easy to comb, the mothers used a metal nit comb. They collected all lice on a white sheet, from where they were transferred by help of fine pincers into a plastic Petri dish. There, the louse activity was controlled by means of a strong magnification glass in order to check whether they were immobile or whether they showed leg movements, constrictions (pulsation) of the intestine, or whether they remained without recovering vital activities. All lice remained motionless after treatment—independently whether they had been exposed to the Licatack® shampoo for 10 or 20 min (Fig. 3). Only two of more than 250 lice combed down from the heads of the children exposed for 10 min were seen laying on the back and showing some leg movements and pulsation of the intestine. These movements became constantly slower and ended finally 2 h after the combing. Control lice (e.g., two untreated kids had been combed 20 min after they had been washed with pure tap water) were still alive and mobile when inspecting them 8 h after combing. Ten days after the first treatment, a second treatment was done, which after combing revealed exclusively dead larval stages besides a total of about five dead adult lice from all kids together. Apparently, these adult lice did come from other infested kids. The children claimed that they did not feel any burning or suffered from any other side effect. This clinical test showed the full activity of the Licatack® shampoo in these cases of a very high infestation rate.

Prior to treatment, some hair with numerous nits had been cut off and was placed into a closed plastic bottle

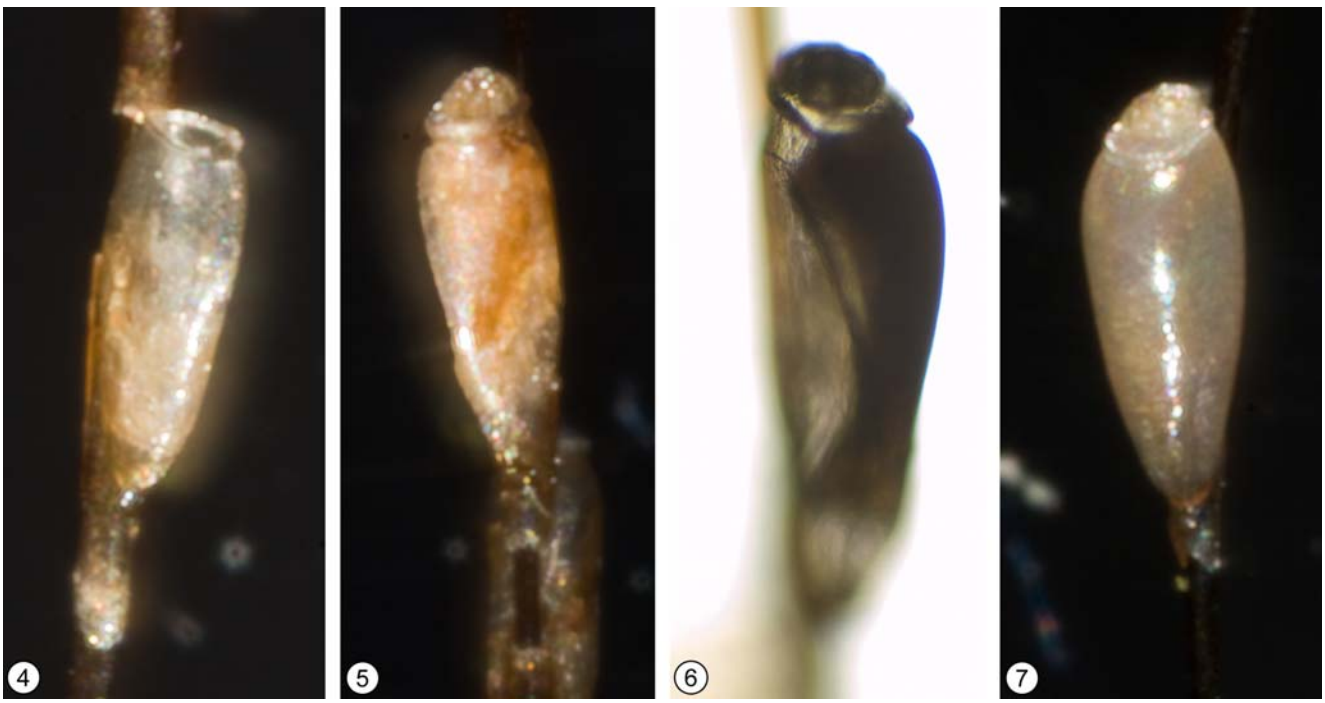
Figs. 1–2 Photographs of the activity of the mothers of the children during the clinical trial of Licatack® in a village close to Cairo

Fig. 3 Petri dish with dead lice from one head after treatment for 10 min

together with a wet filter paper. The same was done with hair after Licatack® treatment for 20 min. Ten days later, the bottles (impermeable for light and being kept in an incubator of 31°C) were opened and controlled for hatched larvae. While such larvae were found in the bottle with untreated hair and the eggs appeared without cover (Fig. 4), larvae was lacking in the bottle with treated hair where nits were found with a closed cover containing a destroyed larval stage (Fig. 5). This indicates that Licatack® has a significant killing effect on the developing larvae inside the nits, which is apparently due to the closing of the openings (= aeropyles) in the covers (= operculum) of the nits (Figs. 5–7). The finding of some dead larvae after the second treatment on day 10 shows that very early stages of larval development (e.g., the early cleavages) are not sufficiently reached within the short treatment.

Discussion

In the present in vivo test, the product Licatack® proved its efficacy on larvae and adult head lice after its efficacy was shown in intense in vitro screening tests (Abdel-Ghaffar et al. 2009). The full efficacy was already given in vivo within a few minutes (10 min) of exposition; while in vitro, it was shown that already a submersion of lice for only 3 min within the undiluted shampoo is sufficient to kill them. The killing effect is apparently due to the penetration of the rather liquid shampoo into the tracheal-respiration system of the lice. At the terminal end of the tracheoles, the active compounds of Licatack® cover the surface and, thus, block the transmission of oxygen via the fluid that protects the cell membranes from drying in this region of the tracheole. Therefore, the mode of action is not chemotherapeutic but physical. The same effect appears apparently in the case of nits where the openings in the operculum were covered during treatment (Fig. 3). Since apparently very early stages of larval development (e.g., morula or blastula stages) are



Figs. 4–7 *4* Micrograph of a nit without cover from untreated hair; *5*, *6* micrographs of closed, but depressed nits being glued at hair. Note the opening (aeropyles) in the cover (operculum); *7* micrograph of a nit on treated hair. Note that the cover is still there, so that the larva died inside

not sufficiently hit within 10 min of exposition, a second treatment after 8-10 days is recommended. That very early cleavage stages are not hit is also known from insecticides. Since Licatack® smoothens the hair, is skin-safe, and smells well, it offers a very efficient and positive alternative to toxic, gluing, flammable, or skin-irritating products found on the market of anti-louse products.

Acknowledgment This trial was in part supported by the Centre of Excellence of the College of Science of the King Saud University at Riyadh, Saudi-Arabia.

References

Amer A-K, Mehlhorn H (2006) Repellency effect of 41 essential oils against *Aedes*, *Anopheles* and *Culex*-mosquitoes. Parasitol Res 99:478–490

Abdel-Ghaffar F, Semmler M (2007) Efficacy of neem seed extract shampoo on head lice of naturally infected human in Egypt. Parasitol Res 100:329–332

Abdel-Ghaffar F, Semmler M, Al-Rasheid K, Mehlhorn H (2009) In-vitro study on the efficacy and safety of 13 anti-louse products. Parasitol Res (in press)

Aspöck H, Walochnik J (2007) Die Parasiten des Menschen aus der Sicht der Ko-Evolution. Denisia 20(60):149–254

Burgess IF (2004) Human lice and their control. Annu Rev Entomol 49:475–481

Burgess IF (2009) Current treatments for *Pediculosis capitis*. Curr Opin Infect Dis 22:131–136

Falagas M, Matthaiou D, Rafailidis P, Panos G, Pappas G (2008) Worldwide prevalence of head lice. Emerg Infect Dis 14:1493–1494

Martini G (1946) Medizinische Entomologie. G. Fischer, Jena

Mehlhorn H (ed) (2008) Encyclopedia of parasitology. 3rd edition Springer, New York

Mehlhorn B, Mehlhorn H (2009) Louse alarm. Düsseldorf University Press, Düsseldorf

Mehlhorn H, Eichenlaub D, Löscher T, Peters W (1995) Diagnosis and therapy of human parasites. G. Fischer, 2. Aufl, Stuttgart

Mogabure Cueta G, Zerba E, Picollo MI (2008) Evidence of pyrethroid resistance in eggs of *Pediculus humanus capitis* (Phthiraptera: Pediculidae) from Argentina. J Med Entomol 45:693–697

Heukelbach J, Speare R, Canyon D (2006a) Natural products and their application to the control of head lice: an evidence-based review. In: Brahmachari G (ed) Chemistry of natural products: recent trends and development. Kerala, India, pp 1–26

Heukelbach J, Oliveira FA, Speare R (2006b) A new shampoo based on neem (*Azadirachta indica*) is highly effective against head lice in-vitro. Parasitol Res 99:353–356

Mumcuoglu K, Gilead L, Ingber A (2009) New insights in pediculosis and scabies. Expert Review Dermatol 4:285–302

Picollo MI, Vassena C, Casadio A, Massimo J, Zerba E (1998) Laboratory studies of susceptibility and resistance to insecticides in *Pediculus capitis* (Anoplura: Pediculidae). J Med Entomol 35:814–817

Picollo MI, Vassena C, Mougabure Cueto G, Vernitti M, Zerba E (2000) Resistance to insecticides and effect of synergists on permethrin toxicity in *Pediculus capitis* (Anoplura: Pediculidae) from Buenos Aires. J Med Entomol 37:721–725

Robinson D, Leo N, Provic P, Barker S (2003) Potential role of *Pediculus humanus capitis,* as vectors of *Rickettsia prowazekii*. Parasitol Res 90:209–211

Sasaki T, Poudel S, Isawa H, Hayashi T, Sekia N, Tomita T, Sawabe K, Kobayashi M (2006) First molecular evidence of *Bartonella quintana* in *Pediculus humanus capitis* (Phthiraptera: Pediculidae), collected from nepalese children. J Med Entomol 43:110–112

Takano-Lee M, Edman J, Mullens B, Clark M (2005) Transmission potential of the human head louse, *Pediculus capitis* (Anoplura: Pediculidae). Int J Dermatol 44:811–816

Toloza A, Lucia A, Zerba E, Masah H, Picollo MI (2009) *Eucalyptus* essential oil toxicity against permethrin resistant *Pediculus humanus capitis.* Parasitol Res (in press)

Vassena CV, Mougabure Cueto G, González Audino P, Alzogaray R, Zerba E, Picollo MI (2003) Prevalence and levels of permethrin resistance in *Pediculus humanus capitis* De Geer (Anoplura: Pediculidae) from Buenos Aires, Argentina. J Med Entomol 40:447–450

Yang Y, Choi H, Choi W, Clark JM, Ahn Y (2004) Ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus capitis* (Anoplura: Pediculidae). J Agric Food Chem 52:2507–2511