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A new shampoo based on neem (*Azadirachta indica*) is highly effective against head lice in vitro

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Abstract Because topical compounds based on insecticidal chemicals are the mainstay of head lice treatment, but resistance is increasing, alternatives, such as herbs and oils are being sold to treat head lice. To test a commercial shampoo based on seed extract of *Azadirachta indica* (neem tree) for its in vitro effect, head lice ($n=17$) were collected from school children in Australia and immersed in Wash-Away Louse™ shampoo (Alpha-Biocare GmbH, Germany). Vitality was evaluated for more than 3 h by examination under a dissecting microscope. Positive and negative controls were a commercially available head lice treatment containing permethrin 1% ($n=19$) and no treatment ($n=14$). All lice treated with the neem shampoo did not show any vital signs from the initial examination after immersion at 5–30 min; after 3 h, only a single louse showed minor signs of life, indicated by gut movements, a mortality of 94%. In the permethrin group, mortality was 20% at 5 min, 50% at 15 min, and 74% after 3 h. All 14 head lice of the negative control group survived during the observation period. Our data show that Wash-Away Louse™ is highly effective in vitro against head lice. The neem shampoo was more effective than the permethrin-based product. We speculate that complex plant-based compounds will replace the well-defined chemical pediculicides if resistance to the commonly used products further increases.

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Introduction

Prevalence of head lice (caused by infection with *Pediculus capitis*) has been increasing worldwide (Burgess 2004). Topical insecticides based on insecticidal chemicals are still the mainstay of head lice treatment (Heukelbach and Feldmeier 2004), and resistance to the common over-the-counter pediculicides has been reported repeatedly by clinicians and confirmed by in vitro studies (Burkhart et al. 1998; Burkhart and Burkhart 2006; Downs et al. 2002; Elston 2003; Hunter and Barker 2003; Mumcuoglu et al. 1995; Picollo et al. 2000; Yoon et al. 2003). Resistance is particularly frequent in countries where head lice are common and where chemical insecticides are extensively used, such as in the US, UK, Israel, and Australia. Because the prevalence and degree of insecticide resistance is expected to increase, alternative topical therapies for head lice infestations are needed.

We tested the shampoo “Wash-Away Louse™” based on seeds of *Azadirachta indica* (neem tree) for its effect on head lice in vitro. The results show that the product is highly effective against *P. capitis*.

Materials and methods

Head lice were collected from two school children and their mothers in Townsville, Australia on two occasions separated by a month. After collection, lice were pooled and held on human hair in 5 cm diameter plastic Petri dishes at 27°C and 50% relative humidity. In vitro tests were started within 1 h after collection of lice, or when this was not possible, lice were fed on the dorsum of the hand of one of the investigators and used for testing within 1 h after feeding.

A commercially available head lice shampoo based on extracts of neem seeds (Wash-Away Louse™; Alpha-Biocare GmbH, Düsseldorf, Germany) was tested.

Wash-Away Louse™ contains a patented oily extract from seeds of the plant *A. indica*, formulated with the following cosmetic ingredients: distilled water, polyglyceryl-3-

caprate, cocamidopropylbetaine, glycerol, lauryl glucoside, dicaprylyl ether, and as preservation components benzyl alcohol, benzoic acid, and sorbic acid.

As a positive control, a 1%-permethrin-based over-the-counter compound was used (Quellada Head Lice Treatment, Glaxo Smith Kline).

For the tests, lice clasping hair strands were immersed completely in the product for 1 min, then placed with hairs onto Whatman filter paper in Petri dishes. To prevent lice from desiccation, the filter paper had been previously moistened by 200 μ l tap water. After lice were placed on the filter paper, obvious pools of the liquid product rests adherent to the body of lice were wiped from the lice by a jeweller's forceps directed under a dissecting microscope. Negative control lice were placed directly on the filter paper without any treatment. To simulate treatment on an infested host, head lice were washed in tap water after 20 min and placed into a new Petri dish.

The number of lice was 17, 19, and 14 in the neem group, the permethrin group, and the control group, respectively. Lice were tested in batches of seven to 14 and the results were then pooled.

Lice on the filter paper were examined under a dissecting microscope by a single observer in all cases to prevent interobserver variation.

The criteria used for survival of lice were extremely strict: if any even minor signs of life, such as internal movements, or movements of antennae or minimal leg movements were observed (with or without stimulation by a forceps), the lice were categorized as alive. The lice were judged as dead if there were no vital signs at all (complete physical death). These data were compared to the usual criteria for "mortality": the inability to walk in a progres-

sive fashion or no righting reflex when rolled onto the back (Hunter and Barker 2003; Mougabure et al. 2002).

Examinations were performed after 5, 10, 15, 20, 30, 45, 60, 90, and 180 min. All lice were maintained at 27°C during the tests.

Results

Using the strict criteria for mortality, all lice treated with the Wash-Away Louse™ shampoo did not show any vital signs after 5 min (Fig. 1). After 30 min, in two lice minimal internal gut movements were observed, giving a mortality of 88.2%. After 3 h, mortality was 94.1% with one louse showing persistent gut movements. All 14 head lice in the negative control group survived during the observation period. In the permethrin group, the number of vital lice decreased steadily from 20% at 5 min to 73.7% at 3 h (Fig. 1). Interestingly, the activity of two lice remained normal throughout the 3-h observation period.

"Mortality" as defined by the usual criteria of loss of righting reflex was 100% in the Wash-Away Louse™ shampoo group during the observation period and ranged between 68 and 90% in the permethrin group (Fig. 2).

Discussion

The usual criteria for "mortality" in head and body lice is not physical death, but a lesser state judged by the inability of the insects to walk in a progressive fashion (Mougabure et al. 2002), to show a righting reflex when rolled onto its back (Hunter and Barker 2003) or when movement has ceased and appendages fail to move when touched with a

Fig. 1 Mortality of head lice treated with Wash-Away Louse™ shampoo and control groups at different points in time, using highly strict criteria for mortality (no external or internal vital signs)

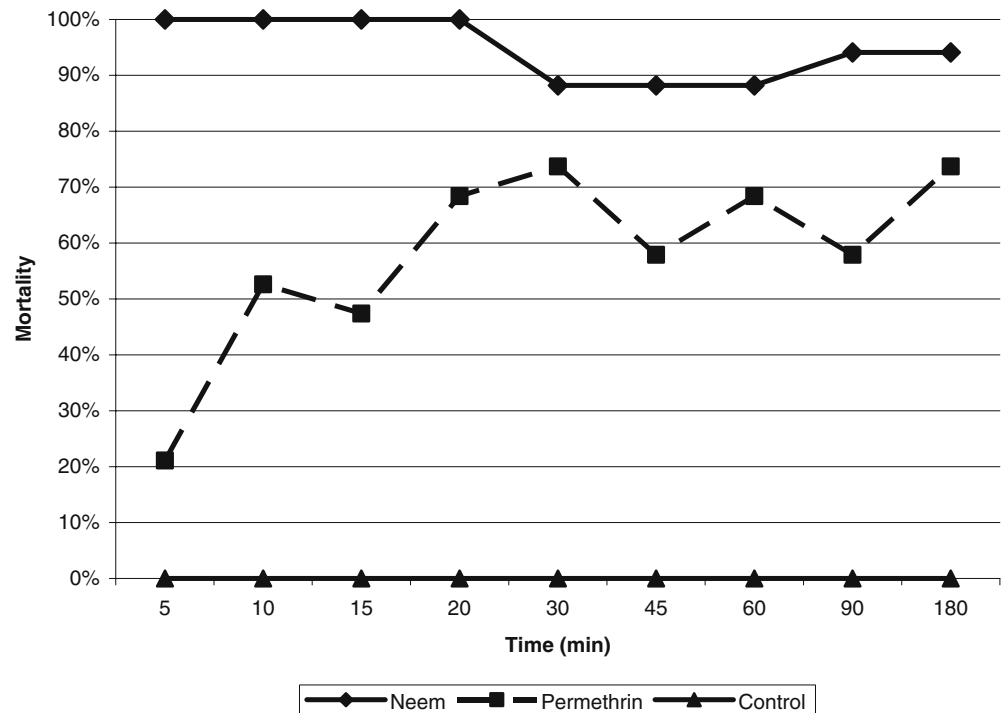
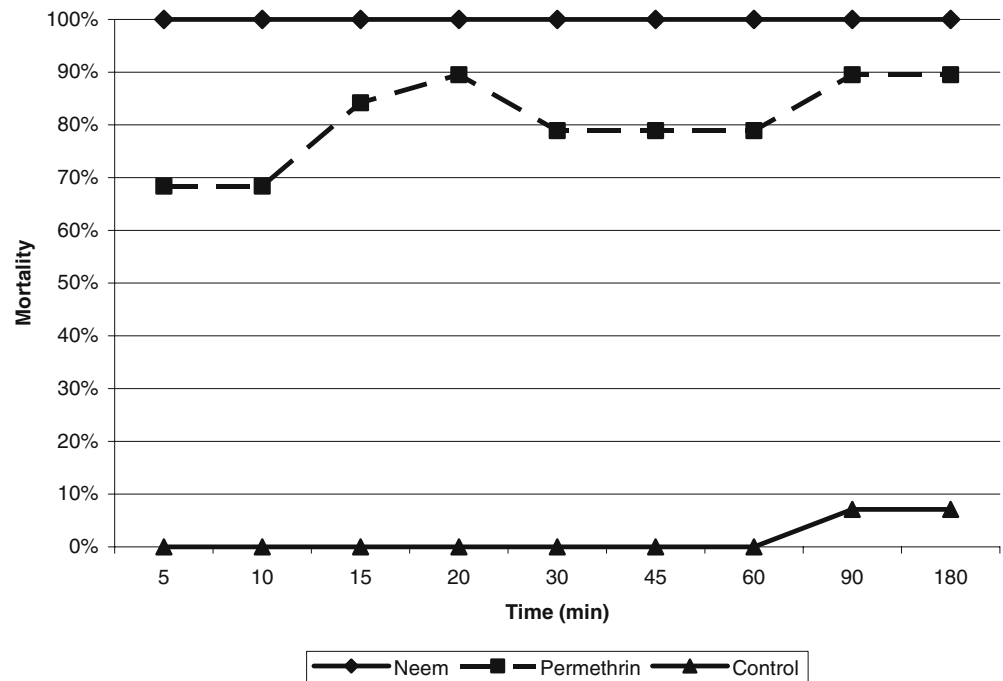


Fig. 2 “Mortality” of head lice treated with Wash-Away Louse™ shampoo and control groups at different points in time, as defined by usual criteria for survival (inability to walk in a progressive fashion, no righting reflex when rolled onto back)



needle (Oladimeji et al. 2000). However, the use of strict criteria (complete physical death of the insects) is particularly important, as it is known that head lice can resurrect within a short period of time (Burkhart and Burkhart 2006). Using usual and also strict criteria for mortality, our data show that Wash-Away Louse™ shampoo is highly effective in vitro against head lice collected in Australia, an area where resistance to commonly used pediculicides is prevalent. After 5 min, all head lice did not show any vital signs and did not recover after 3 h. The effect of the shampoo was better than a permethrin-based product, which is considered the first line treatment.

First data of a field study from Egypt indicate that Wash-Away Louse™ is also highly effective for treatment of individuals heavily infested with head lice (Ghaffar and Semmler 2006).

The neem tree has the widest spectrum of use of all natural products. The first known use of neem by the Harrappa culture in ancient India dates back 4,500 years (Dasgupta et al. 2004). Today, neem extracts are used to treat various skin diseases, as an antiseptic substance, against endo- and ectoparasites, or simply as hair conditioner (Tross et al. 1998).

Anecdotal clinical data suggested a good efficacy of shampoos based on neem oil against pediculosis (Knust 1998; Tross et al. 1998). Neem extract has also an excellent effect as a nontoxic repellent, insecticide, and pesticide (Li et al. 2003; Mulla and Su 1999). The Ayurvedic medicine uses the neem tree as one of the main ingredients of its pharmacy.

It is possible that in the long run, plant extracts will substitute chemical compounds. For example, products based on cinnamon, eucalyptus, marjoram, pennyroyal, *Lippia multiflora*, and rosemary oils were effective against head lice in vitro (Oladimeji et al. 2000; Yang et al. 2004a;

Yang et al. 2004b; Yang et al. 2005). A citronella formulation has been shown to be efficacious as a repellent against head lice (Mumcuoglu et al. 2004). In clinical studies, a combination of paw paw, thymol, and tea tree oil, a combination of coconut oil, anise, and ylang ylang, and a preparation from *Annona squamosa* seed extract were highly effective against head lice infestations (McCage et al. 2002; Mumcuoglu et al. 2002; Tiangda et al. 2000).

In conclusion, it can be speculated that the tested shampoo and similar products will substitute chemical compounds on the market, if resistance to the commonly used pediculicides further increases.

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