Histopathological characterization of induced Paederus dermatitis caused by Egyptian rove beetles (Paederus alfierii)

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Abstract
Outbreaks of Paederus dermatitis (PD) have been reported in Kafr El-Sheikh governorate when Rove Beetles (genus Paederus) are accidentally brushed or crushed on the skin, releasing haemolymph pederin. The aim of this study was to characterize the histopathological changes of induced (PD) caused by Egyptian Rove Beetles (Paederus alfierii). Haemolymph extracts (pederin) of one and three insects were applied on shaved skin of two groups of rats. Skin specimens were collected after 12 h, 3, 7, 14 and 30 days after the extract application and processed for histopathological examination. Gross changes were indicated by erythema, eruptions, marked epidermal necrosis and large bullae formation. Microscopically, there was initial eosinophilic infiltration followed by a rapid destruction of the epidermis associated with exudation. The exudate penetrated the injured area of the epidermis forcing the degenerated cells apart and forming spaces which coalesced to form a vesicle with severe infiltration of the inflammatory cells in the dermis. Vesicles then dried forming crusts followed by repair which took a longer time in case of higher concentration. Conclusion: Reviewing our literature, this is the first time in Egypt to study the histopathological change of (PD) associated with P. alfierii. The induced dermatitis in this study is relatively more severe compared to (PD) of other Rove Beetles occurring in other regions of the world. The degree of dermatitis was directly correlated with the concentration of the toxin. There was no systemic reaction of the topical application of pederin on skin.

1. Introduction
Beetles are a group of insects belonging to the order Coleoptera. There are three families, Oedemeridae, Meloidae and Staphylinidae, with more than 370,000 species. The dermatitis is caused by a vesicant chemical contained in the body fluids of the beetles. The chemical in the body of both Oedemeridae and Meloidae is cantharidin. The third group of blister beetles
belongs to the genus *Paederus* (family Staphylinidae) which does not bite or sting, but when crushed against the skin it releases a potent vesicant and notorious toxin called pederin which results in itching, burning and erythema on human skin (Al-Basheer et al., 2002; Davalos et al., 2002). The toxin is dispersed throughout the body of beetles and is located in their haemolymph (Qadir et al., 2006).

Although (PD) is a specific form of acute irritant contact dermatitis caused by pederin, the mechanisms that lead to these skin reactions are not fully understood. It would appear that some content of the insect body fluid released by the slap of the insect to the skin of the patient which evoked the inflammatory response observed. The causative insect in the reported cases is nocturnal inhabit thus explaining the timing of the bites (Oyedeji et al., 2006).

A major outbreak involving 2000 cases of vesicular dermatitis was reported in Okinawa in 1966 (Armstrong and Winfield, 1969). In Malaysia, cases were reported among 12 medical students who were residents of University Science of Malaysia hostels in Kelantan (Mokhtar et al., 1993). An outbreak of acute vesicating dermatitis was noted among staff members on night shifts and some patients in the open wards of a suburban hospital in Sri Lanka (Kamaladasa et al., 1997). One hundred and fifty-six cases of Paederus dermatitis were also reported among patients attending a dermatology clinic in northern Iran from May to October 2001 (Zagari et al., 2003). Thousands of high rise flat dwellers and dormitory students were affected by a similar outbreak of dermatitis linearis in Penang in 2002 (Rahmah and Norjaiza, 2008). Another outbreak of PD occurred among 36 schoolchildren attending a night tuition class in Malaysia (Rahmah and Norjaiza, 2008). In Egypt, the rove beetle, *Paederus alfieri*, is an active predator of several insect pests attacking a wide variety of cultivated plants which causes necrotizing dermatitis and conjunctivitis when left in contact with the human skin and eye respectively (Morsy et al., 1996).

Students in the campus of Kafr El-Sheikh University complained from skin irritations like burns at morning without any history of exposure to burning agents. Similar complain was also recorded from local people, they mentioned that a marked increase in *P. alfieri* population occurred annually during particular months (January to May) of the year and lesions like burns were noticed on the exposed skin after crushing these insects at the same time of insect population increase then decreased and completely disappeared after June. Symptoms began as itching, erythema, eruption and then vesicles appeared after few hours to one to two days. The vesicles then oozed liquid materials which in infected cases converted to pus for few days then dried and formed crusts. Healing occurred nearly after one month leaving scar in the affected areas. So, the aim of this study was to characterize the histopathological changes of induced Paederus dermatitis (PD) caused by the Egyptian Rove Beetles (*P. alfieri*).

### 2. Materials and methods

#### 2.1. Beetles

Adult *P. alfieri* were collected from several fields in Kafr El-Sheikh governorate for extraction of their haemolymph. Beetles were ground in a small volume of water (30 μl demineralized water/insect). Extraction of the suspension proceeded with 100 μl ethyl acetate and repeated three times. The solvent was removed from the extract in a centrifugal evaporator. The dry sample was again dissolved in 50 μl water and extracted with 100 μl hexane in order to eliminate most of the accumulated lipids, whereas pederin is left in the watery phase (Rupert et al., 1999).

#### 2.2. Experimental animals

Forty five adult albino rats of 80–100 g in weight were obtained from faculty of medicine, Tanta University, Egypt. Rats were housed in wire cages under standard conditions with free access to drinking water and food. The rats were kept in temperature-controlled room with 14 h Light and 10 h dark cycles and were given standard diet and water ad libitum. The rats were left for 2 weeks without any treatment for acclimatization. The rats were further divided into three equal groups after shaving small areas on their skin for application of the insect’s haemolymph extract for only one time. In group (A) fifteen rats were treated with an extract of only one insect, group (B) fifteen rats were treated with extract of three insects and control group (C) fifteen rats were treated with distilled water.

#### 2.3. Histopathology

Skin specimens from three rats of each group were collected after 12 h on 3, 7, 14 and 30 days of the extract application and fixed in 10% neutral buffered formalin. Samples were dehydrated by ascending grades of alcohol, cleared in xylene and embedded in paraffin wax, mounted, sectioned at 5 μm, stained with hematoxylin and eosin (H&E) and then examined by light microscopy (Bancroft and Stevens, 1996).

### 3. Results

#### 3.1. Gross findings

The gross changes appeared after application of the insect’s extracts were the same for both treated groups, however these signs were more severe and appeared earlier in the rats treated with the extract of three insects. A rapid reaction was noticed after 12 h of extracts application in the form of slight erythema with redness at the site of application. After three days, the red areas became elevated and slightly flabby. Moderate to severely swollen skin areas were noticed after seven days of extract application. After 14 days, the swollen areas formed crusts and turned to be small injured to nearly healed after 30 days in the rats of group (A) while in the rats of group (B) there were incomplete healing with crust formation (Figs. 1 & 2).

#### 3.2. Microscopic findings

Twelve hours after the extract application, there was eosinophilic dermatitis which was slight in the rats of group (A) and moderate to severe in the rats of group (B), there were infiltration of eosinophils and polymorphs in the dermis...
(Fig. 3). After three days of application in rats of group (A) there were slight necrotic changes of dermis with increased infiltration of eosinophils, polymorphs and lymphocytes accompanied with slight edematous changes (Fig. 4), but in rats of group (B) there was a perifollicular and intrafollicular inflammatory infiltrate, mainly of lymphocytes accompanied by macrophages and granulocytes forming the image of “swarm of bees” (inflammatory cells around the hair follicle bulbs) in the dermis (Fig. 5). Marked to severe necrotic changes and edema were noticed in the epidermis with superficial perivascular infiltrate of lymphocytes and histiocytes. The exudate had penetrated the epidermis separating the necrosed epithelial cells and forming small chambers which represented the beginning of vesicle formation. Inside the vesicle there was fluid with degenerated epithelial cells “ghosts”. The basal cell layer was intact or indistinct and there was even destruction of the dermo-epidermal junction (Figs. 6 & 7).

After seven days of application in group (A) there was slight necrosis of almost the whole epidermal layers leaving remnants of the cellular nuclei and hyaline membranes accompanied with infiltration of inflammatory cells in the necrotic areas and around the skin appendages with edema, while in rat of group (B) there were complete epidermal necrosis and infiltration of large number of intra-epidermal neutrophils forming a massive layer of necrotic tissue covering the underlying dermis (Fig. 8).

After 14 days of application, in rats of group (A), there was nearly complete healing of skin with formation of few layers of epidermal cells covered with sloughed necrotic scab (Fig. 9), but in rats of group (B) there were incomplete healing of the necrosed epidermis in which the neighboring healthy epidermal cells propagated underneath the necrotic tissue (Fig. 10).
After 30 days of application, the rats of group (A) revealed complete healing with formation of intact epidermis and skin appendages with few numbers of inflammatory cells still present in the dermis in and around dilated blood vessels (Fig. 11). In the other hand, healing in the rats of group (B) was incomplete with formation of young epidermal cells covered with almost freely separated scabs (Fig. 12).

### 4. Discussion

In the present study, local irritation and redness of skin occurred after about 12 h of the insects’ extracts application which then turned to vesicles after three days. This was due to the allergic inflammatory responses of the skin accompanied with release of some neutral serine proteases that led to disruption of desmosomes, detachment of tonofilaments and breakdown the homeostasis of epidermal cells allowing the exudates to enter the cells and form vesicles. This finding was in agreement with Smith et al. (2002) who reported that pederin like cantharidin is absorbed by the lipid layers of epidermal cell membranes and the main pathophysiological changes observed in irritant contact dermatitis were skin-barrier disruption, epidermal cellular changes, and cytokine release mainly from keratinocytes. Meanwhile, Davidson et al. (2009) demonstrated the presence of histamine in the secretion of some genera of beetles. Within a day, vesicles appeared over the erythematous area, ruptured within few days

Fig. 5 – Photomicrograph (group [B] after 3 days) showing perivascular, perifollicular [green arrows] and intrafollicular [black arrows] inflammatory cells infiltrate forming the image of “swarm of bees” (H&E, 400).

Fig. 6 – Photomicrograph (group [B] after 3 days) showing severe necrosis of epidermis [yellow arrow], degenerated epithelial cells “ghosts” scattered in the vesicle [blue arrow] with destructed dermo-epidermal junction [black arrow] (H&E, 400).

Fig. 7 – Photomicrograph (group [B] after 3 days) showing severe necrosis of epidermis [yellow arrow] with massive inflammatory cell infiltration in the dermis [black arrow] and vesicle formation [blue arrow] (H&E, 400).

Fig. 8 – Photomicrograph (group [B] after 7 days) showing severe epidermal necrosis [star] and intense inflammatory cells infiltration forming a massive layer of necrotic tissue covering the underlying dermis [black arrow] (H&E, 400).
forming erosions or crusting or developing secondary infection (Borroni et al., 1991, Lisa Moed, et al., 2001, Cardoso and Haddad, 2005 and Padhi et al., 2007).

We thought that the urticating properties were due to the secretion of an urticating liquid by cells located at the base of hollow hairs and called trichogen cells that is in line with Banney et al. (2000) and Wilkinson and Beck (2004) who claimed that when pederin penetrates the skin, the irritating liquid performs its action.

After the 3–4 days of first appearance of erythema and eruption, vesicle formation was completed and then other changes had followed. During the formation of the vesicle in the epidermis, a more extensive process was found in the dermis. There was a perifollicular and intrafollicular inflammatory infiltrate, mainly of lymphocytes accompanied by macrophages and granulocytes forming the image of “swarm of bees” (inflammatory cells around the hair follicle bulbs). This picture can be considered a pathognomonic feature for the dermatitis caused by this insect. The inflammation led to edema, microvesiculation, necrosis, and the appearance of macrophages in the dermal papillae, parakeratosis and superficial perivascular infiltrate of lymphocytes and histiocytes are also evidence described by Khan et al. (2009).

After seven days, the edema regressed with severe desquamation of the epidermal cells. Such desquamation has been described earlier in a report from northern Iran (Bircher,

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**Fig. 9** – Photomicrograph (group [A] after 14 days) showing nearly complete healing of skin with formation of few layers of immature epidermal cells (arrow) covered with sloughed necrotic scab (star) (H&E, 200).

**Fig. 10** – Photomicrograph (group [B] after 14 days) showing propagation of the neighboring healthy epidermal cells (arrow) underneath the necrotic tissue (star) with infiltration of large number of inflammatory cells (arrow head) (H&E, 200).

**Fig. 11** – Photomicrograph (group [A] after 30 days) showing complete healing: Few inflammatory cell reaction (black arrow) under a thin tightly formed epidermis (blue arrow) in and around dilated dermal blood vessels (orange arrow) (H&E, 400).

**Fig. 12** – Photomicrograph (group [B] after 30 days) showing incomplete healing: Formation of few layers of epidermal cells (arrow) covered with almost freely separated large scab (star) (H&E, 400).
This desquamation and epidermal necrosis were probably attributed to a more potent toxin produced by the local species of *P. alfieri* compared with the other species of *Paederus* beetles in other countries. Higher potency of the toxin in this study is also indicated by the presence of large vesicles and necrosis in the skin of all treated rats, which is a rarely reported finding in *Paederus* dermatitis (Nikbakhtzadeh and Sadeghiani, 1999).

In this study, healing time ranged from 14–30 days in rats treated with an extract of one insect to more than 30 days in rats treated with an extract of three insects. This result was in agreement with Zagari et al. (2003) who noticed that recovery occurred within 8–10 days as the lesions dry and dark scales appeared. After 14 days when the destructive process was at an end, the demarcation between the living and the necrotic epithelium became distinct. The living cells were growing rapidly inward over the floor of the vesicle presented the same characteristics as that growing over any defect. After four weeks the black scales start to peel off, exposing new pink skin. They added that in some cases the scars vanished after six months, in one case scars were still apparent after 12 months. Whereas, Couppie et al. (1992) mentioned that the uncomplicated lesions of *Paederus* dermatitis healed within short time about 7–10 days. Dursteler and Nyquist (2004) also found gradual resolution of the lesions with residual areas of hypo- and hyper-pigmentation in the patients in Pakistan. The acute vesicular lesions were found to undergo crusting within few days and healed completely in 10–12 days (Davalos et al., 2002). This finding can also be explained on the basis of a more severe inflammatory reaction occurred against a more potent toxin produced by the local species of beetles (*P. alfieri*).

It was obvious that the effect of the haemolymph extract from three insects produced more pronounced effect than that of one insect. The same chemical may produce different responses depending upon its concentration, duration of exposure, and individual responses, although; the nature, concentration and duration of contact with a chemical are of primary importance (Qadir et al., 2006).

It is worthy to mention that, there was no internal or systemic involvement due to topical application of pederin throughout the time of the experiment which was unlike the findings reported by Nikbakhtzadeh and Tirgari (2008) who recorded a new finding in only one individual who was suffered from difficulty in breathing and explained that by two possibilities. The first was that the patient was hypersensitive to Pederin and the second that the affected site was the nose and surrounding area leading to dyspnea. Meanwhile, no one has reported any adverse systemic involvement with the topical application of pederin.

**References**


