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Effect of plumbagin on haemocytes of *Dysdercus koenigii* F.

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Abstract. Effect of plumbagin, a phytochemical, on the haemocytes of *Dysdercus koenigii* was studied after topical application. Scanning electron microscopical studies showed deformity in surface morphology in almost all the 5 types of haemocytes categorized in the bug, especially that of granular haemocytes and plasmatocytes, which are devoid of their filopods in the treated insects. The fat droplets of adipohaemocytes shrink while oenocytoid is affected to a lesser degree. Plasma membrane of all the cells becomes fragile leading to a gradual loss of cytoplasm and ultimately only a few interconnected cytoplasmic strands are left. Ultrathin sections revealed a highly vacuolized condition and disintegrating organelles which pass out of the cells due to ruptures of very thin plasma membrane. Total and differential haemocyte counts performed after 24 and 48 h of treatment showed a drastic reduction of all the types i.e. the prohaemocytes disappear from blood, the number of granular haemocytes and plasmatocytes declines while oenocytoids and adipohaemocytes comprise the major part of counts. Because of the damages caused to haemocytes and the suppression of filopodial elongations of plasmatocytes and granular haemocytes (the types that are active in defense mechanism), it can be inferred that cellular defense reactions of *Dysdercus koenigii* are reduced after plumbagin treatment.

Keywords. Plumbagin; haemocytes; *Dysdercus koenigii*; electron microscopy; cellular reactions.

1. Introduction

Insect haemocytes are known to respond to biological agents as well as toxins by exhibiting various reactions like phagocytosis, encapsulation, disintegration or distortion in their contours, in accordance with the nature of substance by which they have been challenged (Feir 1979; Ratcliffe and Rowley 1979). Observation of gradual deaths in diseased condition within a period of 5–10 days after emergence in *Dysdercus koenigii* adults, exposed to a plant derivative plumbagin, led to a speculation that the compound might be interfering with the defense mechanism of the bug by increasing its susceptibility to diseases. Literature survey also reveals plumbagin to retard growth and cause mortality of nymphs in *D. koenigii* as well as *D. cingulatus* (Chadha *et al* 1986; Joshi *et al* 1988), in addition to inhibiting ecdysis of some lepidopterous larvae (Kubo *et al* 1983). Keeping this in view, the effect of plumbagin on the haemocytes of *D. koenigii* has been studied.

2. Materials and methods

D. koenigii adults reared under standard conditions (Saxena and Srivastava 1972), were given a topical application of 0·1% plumbagin (source: *Plumbago zeylanica*: pure compound) in acetone (2 μl/individual) and total (THC) and differential (DHC) haemocyte counts taken (after 24 and 48 h) with simultaneous controls of pure acetone. Scanning and transmission electron microscopical studies were conducted according to the methods of Sharma *et al* (1986) and Saxena *et al* (1988).

3. Results

D. koenigii contains 5 types of haemocytes as identified by light microscopy (unpublished results) viz. prohaemocytes, plasmatocytes, granular haemocytes, oenocytoids and adipohaemocytes. Plumbagin application showed a significant reduction in almost all the types (table 1). Figures 1–12 show the deformity in shapes of these cells. The haemocytes which generally act for defense i.e. plasmatocytes and granular haemocytes, mainly bear the effect because most of them either lyse, change their contour or become fragile and are on the verge of collapse. There is loss of pseudopods in plasmatocytes and a shrinkage in their cytoplasm (figure 4) while, due to filopodial retraction, the geometry of granular haemocytes is altered (figure 5) and in extreme forms only reminiscent of a cell, having interconnected cytoplasmic strands, is left (figure 9). The adipohaemocytes retain their fat droplets but on account of shrinking in the size of such droplets, a bizarre bunch-like appearance is imparted to the cell (figure 8). The least distorted type is oenocytoid,

Table 1. The DHC and THC before and after plumbagin treatment.

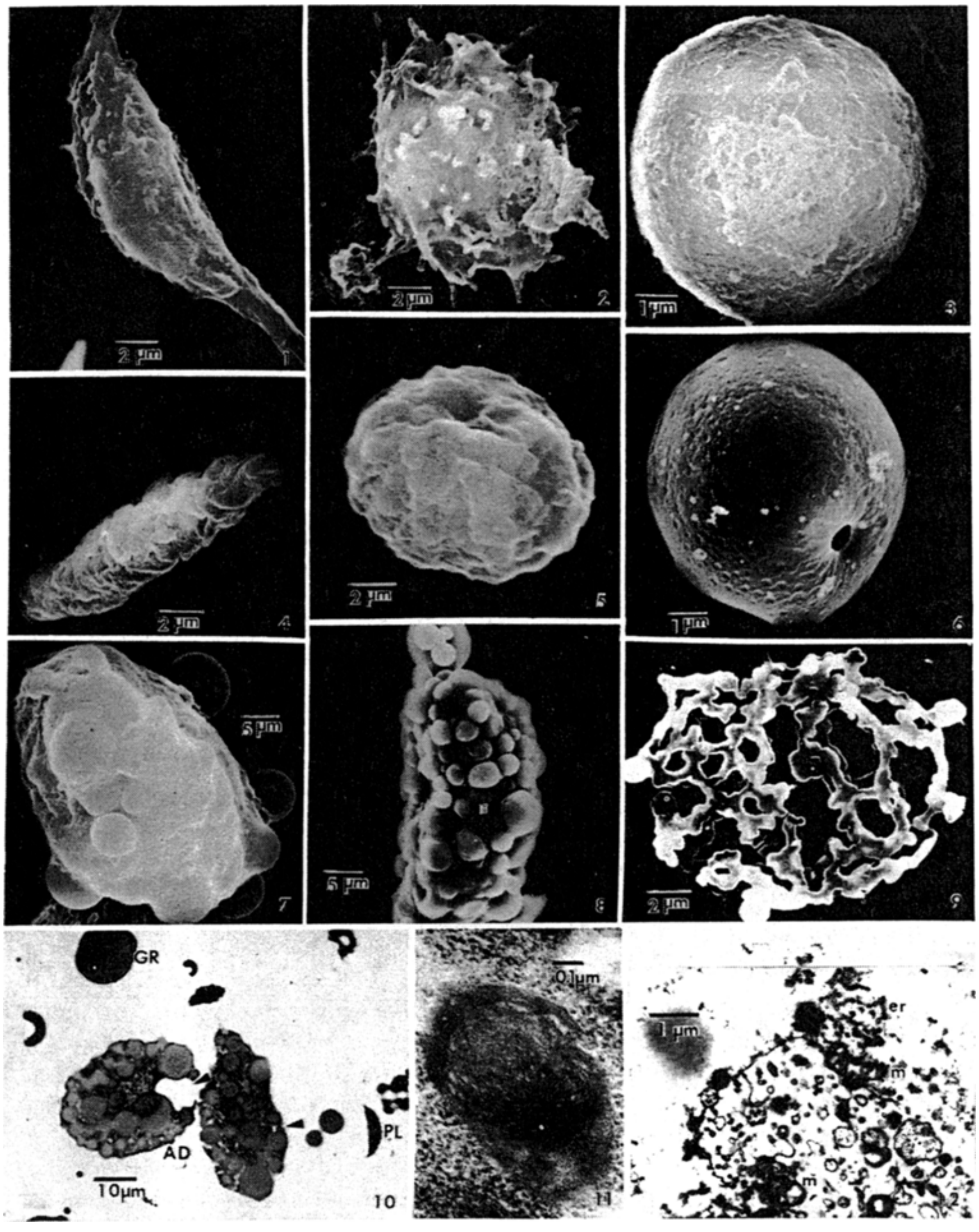
	24 h		48 h	
	Control* DHC	Treated DHC	Control* DHC	Treated DHC
Prohaemocytes (%)	11·60 ± 2·45	0·00 ± 0·00	6·10 ± 2·42	0·00 ± 0·00
Granular haemocytes (%)	36·90 ± 6·13	23·10 ± 5·36	37·00 ± 5·56	14·00 ± 5·92
Plasmatocytes (%)	31·80 ± 2·93	15·70 ± 5·17	33·50 ± 7·97	10·70 ± 5·79
Oenocytoids (%)	12·20 ± 5·57	30·00 ± 6·09	15·20 ± 3·96	37·80 ± 8·37
Adipohaemocytes (%)	7·50 ± 3·95	34·10 ± 4·53	10·10 ± 6·33	36·80 ± 6·16
Number of haemocytes per cubic mm (THC)	7000·00 ± 608·27	4360·00 ± 517·68	5860·00 ± 403·73	2780·00 ± 649·61

N = 10; % = number of particular haemocyte out of 100 haemocytes counted for each reading.
*Pure acetone.

Figures 1–12. Haemocytes of normal and plumbagin treated *D. koenigii*. **1–3 and 7.** Normal haemocytes. **1.** Spindle shaped plasmatocyte with extended pseudopods. **2.** A granular haemocyte with cytoplasmic projections and filopods emanating from the body. **3.** An oval oenocytoid with an almost smooth outline. **7.** Large fat droplets protruding from the body of an adipohaemocyte. **4–6, 8 and 9.** Plumbagin affected haemocytes. **4.** A shrunken plasmatocyte. **5.** A granular haemocyte with absence of all processes. **6.** A punctured oenocytoid. **8.** An adipohaemocyte with diminutive fat droplets. **9.** Remanants of a granular haemocyte. **10.** A toluidineblue stained semithin section showing some of affected haemocytes, note the degranulation of a granular haemocyte (GR) and an enhancement of vacuole formation (arrows) in adipohaemocytes (AD) (PL, plasmatocyte). **11.** The structured granule of an affected haemocyte in the process of losing its structured compactness. **12.** A haemocyte with ruptured boundary and disorganised organelles, (m, mitochondria; er, endoplasmic reticulum). (Magnifications: **1 and 4** × 3,000; **2, 3, 5 and 6,** × 6,000; **7 and 8,** × 2,000; **9,** × 4,000; **10,** × 800; **11,** × 58,000; **12,** × 10,000).

even in this cell the plasma membrane is punctured or dissolved because of extreme fragility (figure 6).

In addition to topographical changes, the internal organisation of cells is disturbed beginning with an intensive vacuolization of cytoplasm (figure 10) to be followed by loss of compactness of structured granules (figure 11) and finally totally disintegrated cells containing organelles (e.g. mitochondria and endoplasmic reticulum) bound by disrupted membrane, are left. Later, because of thinning of



Figures 1–12.

plasma membrane there remains no restraint on these organelles and they ultimately pass out of the cell (figure 12).

4. Discussion

The occurrence of haemocytic reactions to foreign bodies or microorganisms has been studied in many insects and phagocytosis or encapsulation or nodule formation etc. are usually the resultant phenomena (Francois 1975; Ratcliffe and Rowley 1979; Wago and Ichikawa 1979; Ratcliffe and Walters 1983; Walters and Ratcliffe 1983; Wago and Kitano 1985). In *Pieris rapae crucivora* and *Bombyx mori* scanning electron microscopical studies have shown that plasmatocytes or granular haemocytes are active participants in such processes by spontaneously extending their lamellipods or filopods in recognition of foreignness (Wago 1980, 1983; Wago and Kitano 1985). Similar observations are true for *Spodoptera litura* where filopods entrap ruptured cells (Saxena *et al* 1988). The effect of many insecticides and biological agents has been studied on insect haemocytes but majority of such details pertain either to light microscopy or transmission electron microscopy (Zaidi and Khan 1977; Feir 1979; Ratcliffe and Rowley 1979). Since an analysis of the effect of any phytochemical on surface morphology of haemocytes is needed, the present investigations on *Dysdercus* haemocytes by the topical application of plumbagin are important. The difference between the blood of an untreated and treated bug was obvious by almost a complete absence of the elongation processes i.e. filopods in affected granular haemocytes as well as plasmatocytes. These two types of cells normally provide protection against any invading microorganism and the suppression of their cytoplasmic processes indicates clearly their inability to cope with the situation created in the body by the compound. Also, the plasma membrane of cells becomes fragile and this fragility leads to punctures (as in oenocytoid) or dissolution which makes the outer membrane incapable of keeping a hold over the cytoplasm whose gradual oozing leaves behind a cell having a skeletal interconnection of cytoplasmic strands (as in granular haemocyte). The abundance of fat droplets shields the adipohaemocyte but, as compared to a normal healthy cell, it presents a shrivelled up appearance in the scanning micrograph.

The transmission microscopy used as a sequel to scanning electron microscopy confirmed the cells disintegration which, starting with vacuolization and loss of compactness of organelles, ends with an emptied cytoplasm which is devoid of its components and covered by a ruptured membrane. Such a haemocyte is ultimately lost from the haemolymph—a fact corroborated by differential and total counts. Shapiro (1979) has dealt at length with the changes in haemocyte populations in response to particulate and non-particulate materials, poisons and diseases. In *Galleria mellonella* injection of the bacterium *Bacillus cereus* into haemocoel rapidly depletes the number of circulating plasmatocytes (Chain and Anderson 1983). An alteration in haemocyte counts is induced upon injection of yeast cells in *Poecilocerus pictus* haemocoel (Sharma *et al* 1986). Unlike all this injected foreign matter, plumbagin is a plant product whose topical application to the body of *D. koenigii* causes a rapid decline of almost all the cell types. The prohaemocytes are totally eliminated from the blood while granular haemocytes and plasmatocytes decline continuously from 24–48 h of treatment. The fall in counts of these major representative types of haemocytes in treated insects increases the percentage of oenocytoids and adipohaemocytes in DHC (table 1). However the THC clearly project the overall total reduction in the counts in comparison with those of the controls.

With the present work, it is difficult to state conclusively the reason for changes in haemocyte morphologies and the steep decline in their numbers. The question whether the cells are affected directly or via some physiological or endocrinological pathway is yet to be answered in spite of the report that in *D. cingulatus* retardation of growth by plumbagin is on account of its effect on endocrine system (Joshi *et al* 1988).

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