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Plumbagin, a Sterilant for *Musca domestica* Males. A Light and Electron Microscopic Study of its Effect on Testis*

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Musca domestica L. adult males and larvae subjected to the topical applications of plumbagin in concentrations ranging from 0.02 to 10 μg resulted in an absolute mortality in 10 μg treatment given to the adults. The number of pupae and adults emerging from the treated larvae was reduced and a very high incidence of sterility was induced in 2 μg treated adult males.

Histologically, within 72 hr of plumbagin treatment, the testis of *M. domestica* lost its compactness due to disappearance of a large number of spermatocysts. The cell divisions were delayed and the sperm bundles became agglutinated. The secretory material in the calyx lumen lost its denseness. Ultrastructurally, in the plumbagin-affected testis of *M. domestica* the spermatocytes became extremely vacuolized with disintegrated organelles. In the spermatids the axonemal microtubules were highly susceptible to the effect of the compound. In the calyx the epithelial cells turned phagocytic and ingested the entrapped sperm.

Key Words : ***Musca domestica*, Plumbagin, Testis, Histology, Ultrastructure**

Introduction

Plumbagin, a naphthoquinone, is toxic, antifeedant, ecdysis inhibitor, causes growth retardation and displays a wide range of morphogenetic activities (Kubo et al. 1980, 1993, Chadha et al. 1986, Joshi et al. 1988, Gujar & Mehrotra 1988). It has a low inhibitory effect on chitin synthetase, disrupts the activity of ecdysone 20-monoxygenase, brings about a suppression of ecdysteroidal titres and affects the culticular proteins, the neurosecretory cells and the corpora allata volume (Kubo et al. 1983, Mitchell & Smith 1988, Joshi & Sehnal 1989, Krishnayya & Rao 1995). Plumbagin treatment results in a steep decline in the number of haemocytes and their destruction (Saxena & Tikku 1988, 1990, Tikku et al. 1992). It also induces testicular abnormalities due to its effect on the somatic tissue, the spermatocytes and the mature sperm (Saxena

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& Tikku 1988). Taking into view these diverse effects of the molecule, it was tested against a disease-carrier dipteran, the house fly *Musca domestica* L., for its possible control. The reason behind the resultant male sterility was investigated in the adult testis of the house fly by light and electron microscopy.

Materials and Methods

Plumbagin was dissolved in acetone and applied topically along with the controls (acetone-treated), in concentrations of 0.001-0.5%, to the freshly emerged (± 3-4 hr) adult males of *Musca domestica* L. (reared at 26-28°C and 75% RH; dose applied: 2 µl/fly/concentration; number of replicates: 10 × 10), which were provided with untreated virgin females of the same age. Mortality of treated adult males was noted till 72 hr (after which oviposition took place in the controls).

The wandering larvae (prior to pupation) were also subjected to topical applications of 0.05, 0.1, and 0.5% (dose : 2 µl/larva; number of replicates : 40 × 10). The percentage of pupation and adult emergence from treated larvae was recorded and freshly ecdysed affected males given normal virgin females.

The number of eggs laid by the females, mated with topically treated males, as also with the affected males obtained from treated larvae, were counted and hatching recorded.

Spermathecae of some of the adult females (single-mated) were examined for the presence of sperm bundles (Smith et al. 1988), 48-72 hr after mating with untreated and topically treated adult males.

For electron microscopy, the testes of plumbagin-treated (2 µl of 0.1% concentration) males were dissected out 72 hr after treatment (i.e. just prior to first oviposition in the controls and when highly effected cells are discernible in the testis of the treated males) and processed for embedding in Epon (Tikku & Saxena 1985). The semi-thin sections were stained in toluidine blue and ultrathin contrasted by Daddow's method (1983). Grids were viewed under the electron microscope (JEOL 100 CX-II).

Results

The highest concentration (i.e. 2 µl of 0.5% solution = 10 µg) of plumbagin applied topically on the adult males of *M. domestica* was lethal as 80-90% of the flies died within 24-48 hr (table 1) and the rest died in 4-5 days time. The lower concentrations were ineffective in causing death, except for a very low mortality (10%) in 2 µg application.

In wandering larvae the percentage of pupation was reduced in 10 µg concentration whereas the emergence of adults was effected in both 10 as well as 2µg (table 2). Almost all the concentrations used for adult and larval treatments were excellent sterilants, especially for the former, as evident by the fecundity and fertility data (tables 1 and 3). No oviposition was recorded in the females mated with 2µg topically treated adult males whereas a large number of eggs were deposited by the females crossed with the males obtained from treated larvae at this concentration and the hatching percentage of these eggs was almost equal to that of the controls. In adult treatments, concentrations lower than 2µg were very effective in reducing the egg laying and totally preventing their hatch (except in 0.02 µg).

The examination of spermathecae brought forth the contrast between the controls and

Table 1 *Percentage mortality and the fecundity and fertility of plumbagin-treated adult males of M. domestica × untreated females*

Concentration	% Mortality* in			Number of eggs laid (by 10 pairs)	% Hatching
	24 hr	48 hr	72 hr		
10 µg	80.0 ± 7.78	90.0 ± 8.02	90.0 ± 8.02	-	-
2 µg	10.1 ± 1.75	10.1 ± 1.75	10.1 ± 1.75	000.00 ± 00.00	-
1 µg	00.0 ± 0.00	00.0 ± 0.00	00.0 ± 0.00	149.10 ± 12.29	00.00 ± 0.00
0.2 µg	00.0 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	178.20 ± 26.84	00.00 ± 0.00
0.1 µg	00.0 ± 0.00	00.0 ± 0.00	00.0 ± 0.00	218.30 ± 20.21	00.00 ± 0.00
0.02 µg	00.0 ± 0.00	00.0 ± 0.00	00.0 ± 0.00	221.10 ± 11.32	40.80 ± 6.17
Control	00.0 ± 0.00	00.0 ± 0.00	00.0 ± 0.00	622.70 ± 23.99	83.70 ± 6.75

Control = acetone; *Number of insects taken/concentration : 10 × 10

Table 2 *Percentage pupation and adult emergence from plumbagin-treated wandering larvae of M. domestica*

Concentration	Dupation	% Adult emergence
10 µg	76.6 ± 7.80*	47.7 ± 6.72
2 µg	98.9 ± 1.64	77.2 ± 7.88
1 µg	99.1 ± 1.51	99.0 ± 1.54
Control	99.3 ± 1.26	99.3 ± 1.48

Control = acetone

Number of larvae taken/concentration = 40 × 10

*15% dead as prepupae and 6% as half-pupae

Table 3 *Fecundity and fertility of affected males of M. domestica (obtained from plumbagin-treated wandering larvae) × untreated females*

Concentration	Number of eggs laid (by 10 pairs)	% Hatching
10 µg	454.80 ± 16.80	91.50 ± 9.31
2 µg	555.20 ± 16.76	97.80 ± 5.94
1 µg	713.90 ± 10.17	92.10 ± 7.98
Control	787.50 ± 11.88	98.20 ± 3.84

Control = acetone

the treatments. While the former were full of ripe sperm bundles, in the latter a dose-dependent presence of such bundles was observed i.e. in the females mated with 2µg treated males the spermathecae were almost empty and in lower concentrations it contained only a few sperm bundles. This indicated a hindrance in the sperm production, which necessitated a histological study of the testis of treated males to pinpoint the cause.

Plumbagin effect was apparent in the semi-thin sections of *M. domestica*. The testicular compartment was devoid of a large number of spermatocysts, resulting in a loss of compactness and emptied areas within the follicle (figure A). Pycnosis had set in

most of the cells, especially in the primary and secondary spermatogonia in which pycnotic nuclei were well marked (figure B). A delay in the cell divisions could be observed in the region close to the central core of the follicle because instead of spermatids occupying the major portion, the main components of the area were the pycnotic spermatogonia and spermatocytes (figure C). Most of the sperm bundles in the testis had become melanized, agglutinated and were accompanied by the pycnotic material (figure D). In the sperm storage organ *viz.* the calyx, these bundles had lost their cohesiveness i.e. the spermatozoa were loosened and diffused (figures A, E and F). The secretion in the lumen of the calyx too had lost its dense viscocity and exhibited porosity (figures E and F).

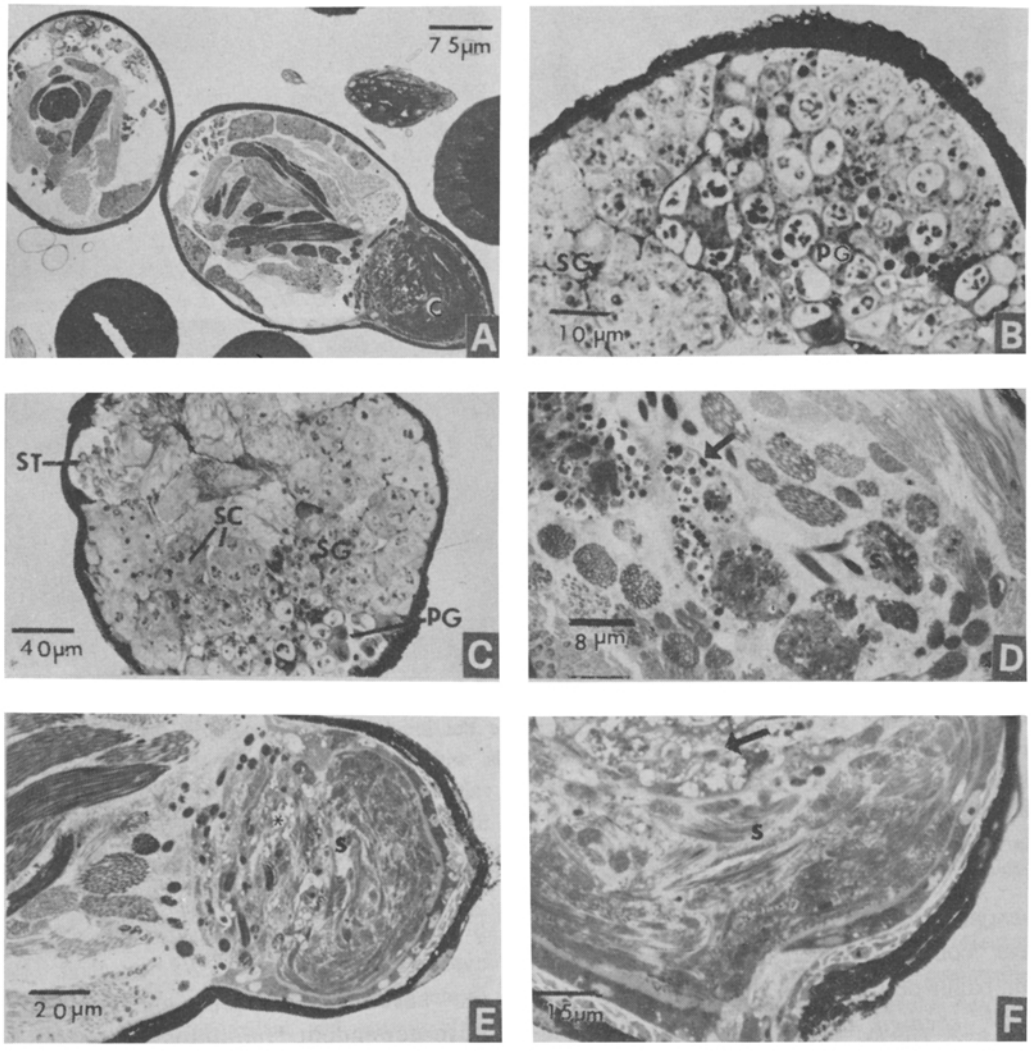
In ultrathin sections of the testis the developing spermatocytes and spermatids were seen to be highly susceptible to the effect of plumbagin. Degeneration was very prominent in the spermatocytes which began with the appearance of numerous vacuoles in the cytoplasm leading to a complete disintegration of the organelles and in extreme cases there remained only electron-dense remnants of the cytoplasm in the cells (figure G). In most of the spermatids the axonemal microtubules had lost their orderly arrangement and had become diffused (figure H). In the calyx lumen, the sperm appeared to be disintegrating in a secretory fluid of very thin consistency (figure I). A very interesting observation was the phagocytotic activity of the epithelial cells lining the lumen of the calyx, as apparent by the ingestion and lysis of sperm within their cytoplasmic vacuoles (figure J).

Discussion

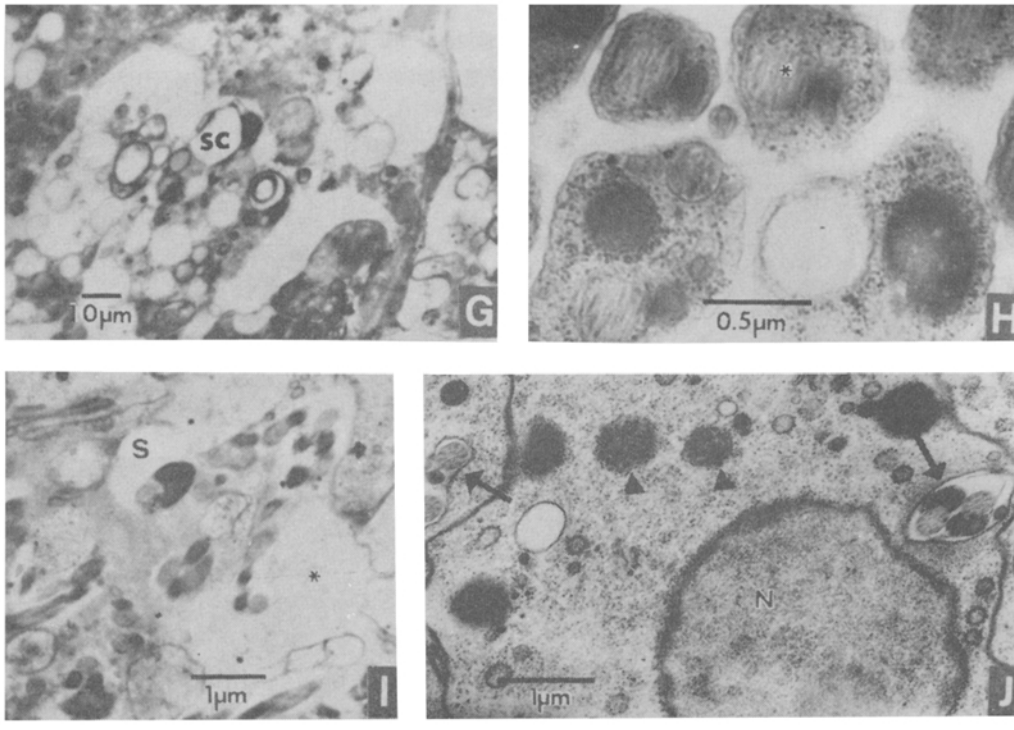
Plumbagin is reported to effect the growth and development (Joshi et al. 1988) but in *M. domestica* wandering larvae the development was not prolonged, only pupation and adult emergence was effected. The most important effect was the dose-dependent induction of sterility i.e. the adults turning infecund in 2μg and depositing sterile eggs upto 0.1μg concentration applied topically on males.

Histological and ultrastructural studies of the tesis confirmed the sperm production to be hampered due to distortion of cells. In the normal untreated house fly, the central core of the testicular compartment carries spermatids and sperm (Tikku & Saxena 1995). In the present studies this area is occupied by the earlier stages of spermatogenesis i.e. the spermatogonia and spermatocytes, pointing thereby to a delay in the cell divisions. In chick embryo fibroblasts plumbagin is reported to arrest the cell growth, decrease the mitotic rate and cause chromosomal aberration, cytoplasmic vacuolization and disintegration (Santha Kumari et al. 1980).

On the basis of this study it was concluded that at lower concentrations the compound behaves like the spindle poison colchicine by effecting the cell mitosis and in higher quantities it exhibits nucleotoxic and cytotoxic effects. In *M. domestica* too perhaps it is the decrease in the mitotic divisions that causes a delay in the spermatogenesis cycle. Vacuolation, the other predominant effect of plumbagin in the house fly testis, has been reported for the spermatocytes of *Dysdercus koenigii* (Saxena & Tikku 1988). However, the severity of the effect was more prominent on the spermatids and sperm of the hosue fly, since in *D.*



Figures A-F Light micrographs of the testis of plumbagin-treated ***M. domestica*** : **A**, L S of an affected testis continuing posteriorly into the calyx (C). Another testicular follicle cut transversely is lying close to it. Absence of a number of spermatocysts has left many empty areas in both the follicles. The stored sperm bundles in the calyx have loosened; **B**, Pycnotic nuclei in primary (PG) and secondary (SG) spermatogonia; **C**, The region near the central core of the testicular follicle occupied by the pycnotic primary spermatogonia (PG), the secondary spermatogonia (SG) and spermatocytes (SC). A few spermatids (ST) can also be seen; **D**, The melanized and clumped sperm bundles (S) in the testis. Arrow points to the pycnotic material; **E**, The diffused sperm bundles (S) in the calyx lumen. Note the porous secretion (*); and **F**, An enlarged view of figure E. Note the loose sperm bundles (S) and affected secretory material (arrow)



Figures G-J Transmission electron micrographs of the testis of plumbagin-treated ***M. domestica:*** **G**, Degenerating spermatocytes (SC) of testis. Note the innumerable vacuoles in the electron-dense cytoplasmic remnants of the cells; **H**, Spermatids with diffused axoneme (*); **I**, Disintegrating sperm (S) and thinned secretory fluid (*) in the calyx lumen; and **J**, Phagocytosis of sperm by the epithelial cells lining the calyx lumen. Note the ingested sperm within cytoplasmic vacuole (arrow) and resorbed spermatozoa (arrow-heads). N, nucleus of the epithelial cell

koenigii some of the axonemal microtubules were countable whereas in *M. domestica* plumbagin destroyed all the tubular elements. In *D. koenigii* haemocytes also, the compound displayed a specific effect on the microtubules of the structured granules (Tikku et al. 1992). In house fly a physical measure like the temperature stress results in sterile spermatozoa in which the regular alignment of the axonemal microtubules is altered (Tikku & Saxena 1992). Unlike the thermal effect, plumbagin aims at microtubular destruction and not in their alteration. The apparent chemical action of plumbagin therefore corroborates the statement of Santha Kumari et al. (1980) about the dual activity of the compound i.e. anti-mitotic in low and cytotoxic in high doses. Wagner et al. (1985) too have reported plumbagin to exert a dose-dependent immunostimulatory and cytotoxic action. The reported inhibition of two enzymes *viz.* ecdysone 20-monooxygenase (Mitchell & Smith 1988) and chitin synthetase (Kubo et al. 1983) by plumbagin in some of the insect sps. indicates a hormonal imbalance to be responsible for the derailment of spermatogenesis. But the ultimate cell dealth is brought about by cytotoxicity, which prevails over all other effects, including the disturbance in the hormonal system.

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