

# HISTOLOGICAL CHANGES IN *LOCUSTA MIGRATORIA* TREATED WITH D-ISO ASCORBIC ACID

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## INTRODUCTION

Ascorbic acid analogues in the diet of insects may hamper growth and produce all the syndrome accompanying ascorbic acid lack (Hornig (1974); Kramer *et al.*, (1978) and Navon *et al.*, (1985)). Moreover, ascorbic acid analogues are of value in the dissociation of specific stages in the development of structural and connective tissue of insects particularly with respect to membrane constituents associated with smaller organs required for reproduction (Navon *et al.*, 1983; Eid and Moursy, 1992). The activity of D-iso ascorbic acid on insects antimetabolite suggested by Salem *et al.* (1992), Moursy (1995) and Elsayed and Moursy (1998) exhibited many discrepancies in food utilization, weight gain, growth rate, sclerotization, phenoloxidase activity, and deposition of exoskeleton strata. Ascorbic acid may take part in the melanization and sclerotization processes in *Schistocerca gregaria* (Dadd, 1960); *Laspereysia pomonella* (Bounias, 1980); *Manduca Sexta* (Navon *et al.*, 1985); and in *Spodoptera littoralis* (Navon *et al.*, 1985).

The objective of this study was to investigate the influence of D-isoascorbic acid on changing some histological structure of *Locusta migratoria* tissues, which can be used as an insect control measure.

## MATERIAL AND METHODS

Egg masses of *L. migratoria* were incubated in a closed jar at 33°C, until eclosion. Newly hatched nymphs were maintained on fresh wheat seedlings and wheat bran at 30°C, 50-70% relative humidity and on a 12:12 L/D cycle. Five individuals from the first and fifth instar nymphs were reared in glass jars capped with metal grids.

Wheat seedlings impregnated in D-iso ascorbic acid (0.25%) were supplied as food. Wheat seedlings impregnated in distilled water only was supplied in the control. Individuals of the first and fifth instars were collected after two days and four days from treatment and control jars, respectively. Longitudinal sections were made in thoracic and abdominal regions after fixing in aqueous buin and then drying in ascending alcohol concentrations. The sections were then double embedded in celloidin and paraffin wax. The  $6\mu$  sections were stained with Relish's heamatoxylin for staining the nucleus and Eosin for staining the cytoplasm.

## RESULTS AND DISCUSSION

### Effect of D-iso ascorbic acid on the thorax integument, muscles and fat bodies

Data presented in Table (1) show that the mean thickness of the thorax integument in treated first and fifth instar nymphs were 11.4 and 28.6 $\mu$ , respectively while, it was 39.9 and 57.2 in the untreated nymphs, respectively. But the mean thickness of the muscles under the integument was not affected by D-iso ascorbic acid. The mean thickness of the fat body was lower in the treated nymphs than in the untreated nymphs.

Microscopic examinations showed curling in the cuticular layer of the integument of the treated first instar nymphs. However, in the fifth instar nymphs, it had been curled and twisted (Figure 1). Also, the hypodermal cells were diminished in size and the hypodermis layer was consequently thin. Microscopic examination showed many vacuoles in fat bodies of treated nymphs.

TABLE (1)

Average values (in microns) of the integument, muscles, fat body and mid gut thickness for the treated first and fifth instar nymphs of *Locusta migratoria* by the D-iso ascorbic acid.

Treatment	Thorax integument		Muscles		Fat body		Midgut cell	
	1 <sup>st</sup> instar	5 <sup>th</sup> instar	1 <sup>st</sup> instar	5 <sup>th</sup> instar	1 <sup>st</sup> instar	5 <sup>th</sup> instar	1 <sup>st</sup> instar	5 <sup>th</sup> instar
114.4	39.9	57.2	28.6	48.8	85.8	171.6	87.4	114.4
Treated nymphs	11.4	28.6	28.6	48.6	28.6	85.8	28.6	45.6

Each figure is the average of three replicates (each replicate has 10 reading).

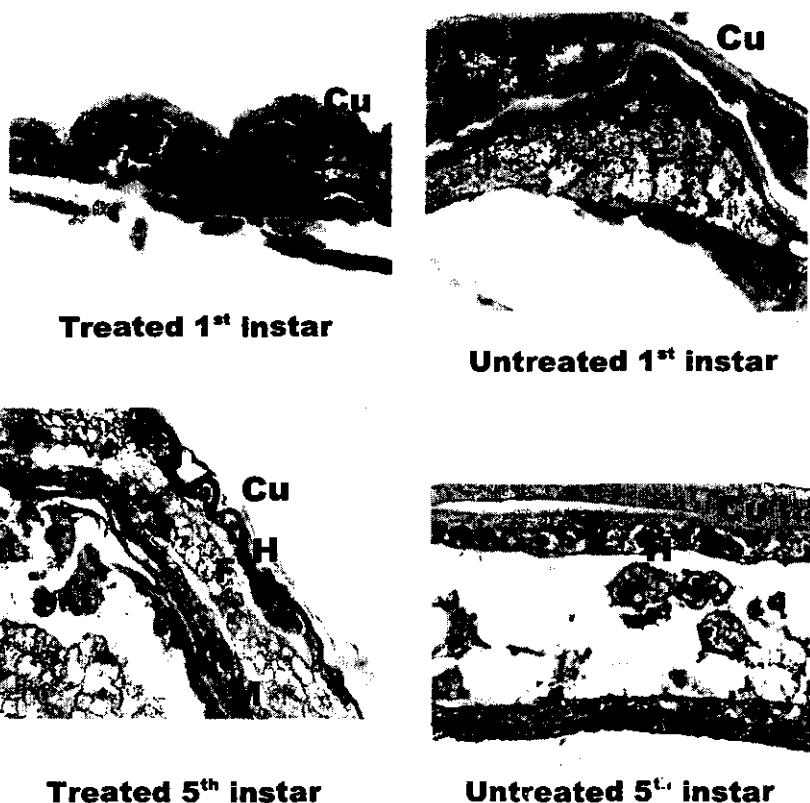


Figure (1): Longitudinal section in the integument of the first and fifth instar nymphs. Cu.= Cuticle, H.= Hypodermis, F.= Fat body, M.= Muscles

#### **Effect of D-iso ascorbic acid on the mid gut**

The mean thickness of mid gut in treated first and fifth instar nymphs was  $28.6 \mu$  and  $45.6 \mu$ , respectively (Table 1). The columnar and goblet cells (secretion cells) of the first instar nymphs fed on wheat seedlings treated by D-iso ascorbic acid were elongated and reduced in diameter. The secretory cells in fifth instar nymphs were destroyed. The fat bodies were poorly deposited around the mid gut of treated nymphs (figure 2).

#### **Effect of D-iso ascorbic acid on the nerve cord**

The mean length and diameter of the nervous ganglion, in the fifth instar nymphs fed on untreated wheat seedlings were  $257.4 \mu$  and  $85.8 \mu$ , respectively. The

corresponding values for the treated nymphs were 85.8  $\mu$  and 57.2  $\mu$ , respectively. Figure (3) shows the effect of D-iso ascorbic acid on nerve ganglions of the fifth instar nymph, the nerve ganglia was malformed, the nerve cells in the ganglion was curled with very small nuclei, the nerve cord became absent and the ganglion sheath (ganglion lamella) was hypertrophied.

Decrease of the hypodermal cells in size together with thin cuticle layer in the treated nymphs by D-iso ascorbic acid treatment may be due to that the D-iso ascorbic acid inhibited the growth of the hypodermal cells; therefore the synthesis of cuticle was reduced. The integument of the fifth instar nymphs treated with D-iso ascorbic acid had been curled and cuticle layer twisted. It may be suggested that the physiological activity of this compound was increased in the later stages. This finding is in agreement with, Elsayed and Moursy (1998), after treating the different stages of *Locusta migratoria* with D-iso and D-gluco ascorbic acid. Moreover, Navon *et al.*, (1983) suggested that, the abnormal sclerotization in the L-ascorbic acid deficient insects could blink with the value of tyrosine. Tyrosine content in the third and fifth instar nymphs of *L. migratoria* was reduced by D-iso ascorbic acid (Elsayed and Moursy, 1998).

The results of fat body in the nymphs fed on wheat seedlings treated by D-iso ascorbic acid indicate that, this compound has an effect on the metabolism of this insect. Finding of many cavities in the fat body may be due to that, the fat body became devoid of glycogen or injured. This result is in agreement with that of Martoja *et al.* (1985) after treating *L. migratoria* by cadmium and mercury.

Changes in the mid gut cells in treated nymphs may be a result of weakness of longitudinal and circular muscles around the mid gut. Mid gut of fifth instar nymphs was destroyed by D-iso ascorbic acid. This may be attributed to the weakness of the connective tissues. This finding is in agreement with that of Nabil and Elsayed (1997) after treating the adult females of *Euphranta connexa* plorans by lead nitrate; and also with Binder and Bower (1994) for larvae of *Helicoverpa zea* treated with precocene II.

The histological results of nerve ganglion changes in treated nymphs may indicate that, the D-iso ascorbic acid had a hormonal effect on *L. migratoria*.

In conclusion this study reveals that, D-iso ascorbic acid affected the structure of mid gut epithelial cells, cuticle synthesis and normal formation of nerve ganglion in *L. migratoria* nymphs.



**Treated 1<sup>st</sup> instar**



**Untreated 1<sup>st</sup> instar**



**Treated 5<sup>th</sup> instar**



**Untreated 5<sup>th</sup> Instar**

Figure (2): Longitudinal section in the midgut of the first and fifth instar nymphs. M. = Muscles, F.= Fat body, Mg.= midgut

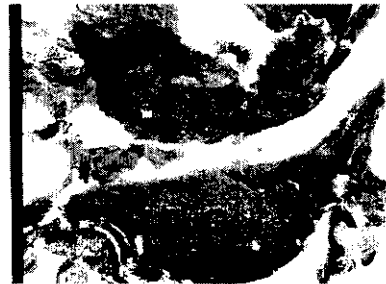


Figure (3): Longitudinal section in the ganglion of the fifth instar nymphs. G. Ganglion

## SUMMARY

The effect of ascorbic acid analogues (D-iso) on the integument, mid gut, fat bodies, muscles and nerve ganglia of *Locusta migratoria* first and fifth instar female nymphs were studied.

The thickness of the integument in the thoracic region was reduced 50% and 28% in the treated first and fifth instar nymphs, respectively. Also, the integument showed curled twisted cuticle, and diminished hypodermal cells with small nuclei. Quantity of fat bodies in the treated insects around the midgut or under the integument was reduced. A reduction in diameter occurred in the midgut of treated insects. The nerve ganglia in treated fifth instar nymphs were malformed.

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