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Histopathological effects of *Datura alba* leaf extract on the midgut of *Periplaneta americana*

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Abstract

In the present work, the effect of crude leaf extract of *Datura alba*, which is a medicinal plant was studied on the American cockroach, *Periplaneta americana*. The efficacy of leaf extract of *D. alba* in disturbing the normal metabolic and physiological processes was determined in the cockroach midgut. Histological studies revealed disruption of the cellular structures of the cockroach midgut, fed on different doses of leaf extract of *D. alba* (10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml & 100µg/ml). The most characteristic effects were disruption of peritrophic membrane, striated border, secretory cells, regenerative cells and longitudinal muscles. Nuclei of the epithelial cells disintegrated as compared to the control. The columnar cells were moderately disturbed. Overall tissue destruction leaving gaps and lacunae at various layers was observed. Thus, as a biological, plant-derived preparation, extract of *D. alba* could be used as an effective botanical insecticide to be included in the Integrated Pest Management Programme for *P. americana* and other insect pests as well.

Keywords: *Datura alba*; Cockroach; Midgut; Histology.

Introduction

Periplaneta americana, also known as the American cockroach, is an urban cockroach found in places such as homes and shops (Lee and Lee, 2000). They also serve as potential carriers of the causes of bacterial diarrhea and nosocomial infections in hospitals (Agbodaze and Owusu, 1989; Fotedar *et al.*, 1991). There is ample evidence that substances produced by cockroaches are involved in producing allergic symptoms (Kongpanichkul *et al.*, 1997; Pumhirun *et al.*, 1997). Besides that, cockroaches also secrete a mixture of xanturenic acid, kiturenic acid and 8-hydroxycuinalic acid, which are tryptophan derivatives that have mutagenic and carcinogenic properties (Mullins and Cochran, 1973). Cockroaches can be controlled primarily by the use of synthetic insecticides. However, repeated application of insecticides has resulted in the development of resistance to chlorinated hydrocarbons, organophosphate, carbamates and pyrethroids insecticides (Prabhakaran *et al.*, 1996). Serious problems of resistance by insect species, pest resurgence, residual toxicity, phototoxicity, vertebrate toxicity, widespread environmental hazards and increasing costs of application of the presently used synthetic pesticides have directed the need for effective, biodegradable pesticides (Zettler and Cuperus, 1990; Glenn *et al.*, 1994; Ewete *et al.*, 1996; Guedes *et al.*, 1997; Talukder and Howse, 2000; Elhag, 2000). This awareness has created

worldwide interest in the development of alternative strategies, including the re-examination of using plant derivatives against agriculturally important insect pests. Plant-derived materials are more readily biodegradable. Some are less toxic to mammals, may be more selective in action and may retard the development of resistance. Their main advantage is that they may be easily and cheaply produced by farmers and small-scale industries as crude, or partially purified extracts. In the last two decades, considerable efforts have been directed at screening plants in order to develop new botanical insecticides as alternatives to the existing insecticides. Research using plant extracts for controlling cockroaches is limited. Recently, the essential oil of catnip (*Nepeta cataria* L.) was reported to have repellency against adult male *B. germanica* (L.) (Peterson *et al.*, 2002). Thavara *et al.* (2007) studied seven commercial essential oils for repellency against cockroaches and found *Citrus hystix* exhibited complete repellency against *P. americana* and *B. germanica*. The histopathological changes induced by different plant extracts on different insects were documented by many authors (El-Ghar *et al.*, 1994; El-Din, 1999; Abdel-Ghaffar, 2004; Wanderley-Teixeir *et al.*, 2006; Mohammad, 2009).

Almost all the research work has been concentrated on agricultural pests and stored product pests and limited attempts have been

made on the use of biological pesticides to control household pests. The present study is aimed to evaluate the efficacy of crude leaf extract of *Datura alba*, on the cockroach midgut in disrupting the normal structure at midgut level.

Materials and Methods

Laboratory rearing of *P. americana*: Cockroaches were collected from dark, damp places like drains and the required species i.e. *P. americana* was separated. They were then reared in captivity on diet bread crumbs and some cellulose containing materials like rough papers. After continued rearing for 1-2 weeks, the adult cockroaches were separated out and used for the present experiment.

Preparation of leaf extract of *D. alba*: The leaves of *D. alba* were collected from Botanical Garden, A.M.U., Aligarh, dried in shady and dark place and were powdered using electrical grinder. 50 gm of the powdered plant material were soaked in 100 ml of methanol and then filtered. The filtered material (crude extract) were concentrated in water-bath at 60°C and the residue obtained called as crude extract was stored at 4°C to make stock solution from which desired concentrations were prepared.

Application of different concentrations of leaf extract of *D. alba*: 1 ml of each concentrations of leaf extract of *D. alba* (10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml & 100µg/ml) was sprayed on the bread crumbs with the help of pipette and individually fed to starved cockroaches. The control was also run beside these samples.

Histopathology: The cockroaches fed with different doses (10, 20, 40, 80, 100 µg/ml) were sacrificed after 24h and dissected to expose the alimentary canal and the midgut were separated out. It was then placed in Bouin's solution for 6 hrs that functions as fixative for the specimen. Later the sample was washed several times with water until yellow colour of bouin's solution disappeared. Dehydration was carried out using ascending alcohol graded from 30% to 100% for an hour each. Later, the specimen was placed in xylene and wax (1:1) and then pure wax for about an hr. The specimen was embedded in wax. Ribbon of wax was then cut at 7 µm with the help of Rotary Microtome. The ribbon was placed on to the egg albumin coated clean glass slide with the help of hair brush and the slides were subjected to heating plate to straightened

the creases of the ribbon. Later, dewaxing was done using xylene. The sample was then hydrated using descending grades of alcohol and was stained with haemotoxylin and counter stained in 70% eosin. Further, dehydration was completed and specimen was mounted in DPX. Then the slide was subjected to microscopic observation and photomicrographs were taken at 10X.

Results

Effect of various doses of crude leaf extract of *D. alba* on midgut of *P. americana*: The midgut section of the control specimen shows no abrupt changes about the structure. The peritrophic membrane of the control section is clearly visible with no changes (Fig 1A). At the highest dose i.e. 100 µg/ml various tissue layers lost their identity in the sections, only secretory cells are present at some places (Fig 1B). The peritrophic membrane is damaged at great extent and the striated border was very much affected almost lost their identity. The circular muscles and longitudinal muscles were apparently disrupted. There was overall tissue destruction leaving gaps and lacunae at various layers. At 40 µg/ml *D. alba* caused progressive destructive changes in the mid gut as shown in Fig.1C. Whereas, in the treated cockroaches fed on 10 µg/ml *D.alba* shows slight change in the histology of the midgut. The peritrophic membrane disrupted slightly. The striated border was affected to some extent. There was not much effect on secretory cells and regenerative cells and circular muscles remain intact as shown in Fig.1D.

Discussion

Plant extracts have been used worldwide as an alternative method to control pests. *D. alba* is a commonly used medicinal plant for inflammatory diseases such as asthma, rheumatism, muscle spasm and whooping cough. It is clearly proved that crude or partially purified plant extracts are less expensive and highly effective for the control of insect pests rather than the purified compounds or extracts (Jang *et al.*, 2002; Cavalcanti *et al.*, 2004). The present study demonstrated that there is marked disruption of peritrophic membrane, striated border, secretory cells, regenerative cells and longitudinal muscles of the treated midgut sections of *P. americana* with different doses of the leaf extract of *D. alba* as compared to that of control.

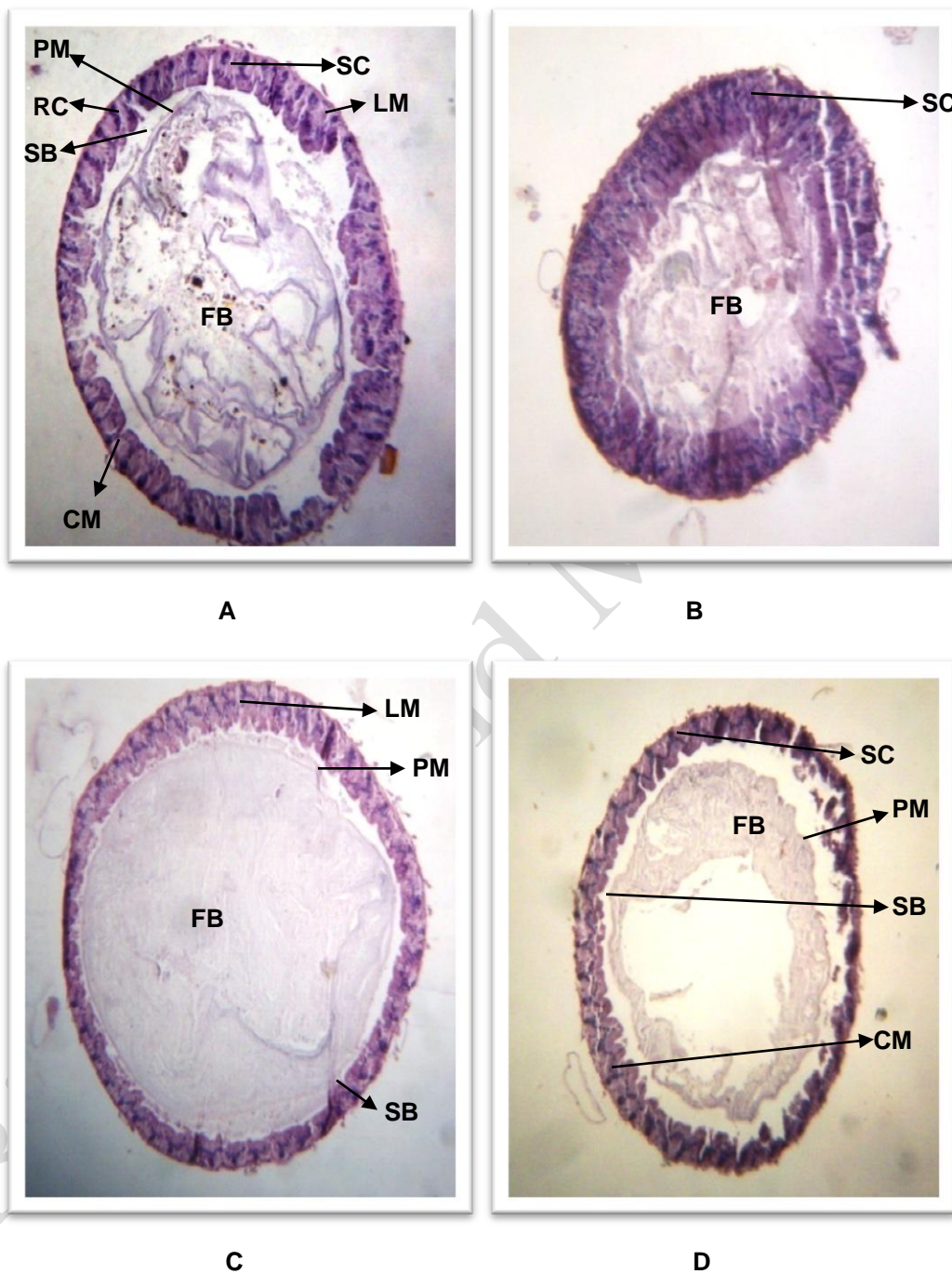


Fig 1. T.S. of mid gut of *P. americana*: (A)Control (10X), (B)treated with 100µg/ml *D. alba* via feeding application (10X), (C) treated with 40µg/ml *D. alba* via feeding application (10X) & (D) treated with 10µg/ml *D. alba* via feeding application (10X). Abbreviations: CM-Circular muscles, FB-Food bolus, LM-Longitudinal muscles, PM-Peritrophic membrane, RC-Regenerative cells, SB-Striated border, SC-Secretory cells.

Ahmed (1995) reported that histopathological effect of oil extract of chamomile plant produced enlargement of epithelial cells, appearance of vacuoles at the apical part of the cell and destruction of the peritrophic membrane of *Culex pipiens* larvae. Jung *et al.* (2007) evaluated in Korea the hexane fraction of extracts from seeds of *Myristica fragrans* against *B. germanica* (L.) and found that the (1S)-(-)- β -pinene (0.06mg/ cm²) was the most toxic insecticide and comparable to permethin (0.05 mg/cm²). Ngoh *et al.* (1999) studied the insecticidal activity and repellent properties of nine volatile constituents of essential oils against *P. americana*.

Thus, it is concluded that as a biological, plant-derived preparation, extract of *D. alba* could be used as an effective botanical insecticide to be included in the Integrated Pest Management Programme for *P. americana* and other insect pests as well.

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