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Research Article

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Effect of N-Nitroso-N-Methylurea on Midgut in *Periplaneta americana*

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ABSTRACT

N-nitroso-N-methylurea, a chemical carcinogen was injected in the abdomen of Periplaneta americana at an effective dose of 100 µg/g body weight on alternate days. The insects were vivisected after 4, 8, 12, 16 and 20 days of treatment. Histological changes were observed in midgut. The columnar cells were more or less normal in shape but their nuclei were clumped. The regenerative nidi were also showing degenerated chromatin material. The gut epithelium was distorted in shape and their nuclei were comparatively very small and pycnotic. The cytoplasm was degenerated and weak in construction and the circular muscle layer was thickened. Most of the cells of the gut epithelium had lost their normal architecture and some epithelial cells were showing additional growth of cells towards the gut lumen due to the proliferation of the cells. Longitudinal muscles were distorted in shape, vacuolated and obliterated.

Keywords: *Periplaneta americana, Midgut, N-nitroso-N-methylurea.*

INTRODUCTION

N-nitroso-N-methylurea is a potent direct acting carcinogen that has been shown to induce cancer of various organs, mainly of the forestomach, brain and the nervous system, in a wide variety of animal species[11,16]. NMU belongs to a class of compounds generally known as N-nitrosamides, which are unstable in aqueous solution, especially at pH > 5.

Several authors have studied the effects of insecticides on midgut epithelium of different insects[2,15,22] studied the effect of organo chlorines and aromatic amine on the midgut of the *P. pictus*. and reported that "brown coloured bodies" were evident. Singh[21] studied the effect of benzidine and 1-nitroso-2-napthol on the midgut of *Periplaneta americana* and reported that additional epithelial layer was noticed towards lumen. Some out growths were also formed due to mitotic activity in the nidi.

In the present investigation is aimed to evaluate the carcinogenic effects of N-nitroso-N-methylurea on the midgut of *Periplaneta americana*.

MATERIALS AND METHODS

Periplaneta americana were collected from hotel, garbage, dark wet places and were kept in insect cages in the laboratory. They were regularly fed with balanced diet and water.

Adult insects of more or less similar weight in each set were considered for these experiments. N-nitroso-N-methylurea (technical grade) was supplied by Sigma. NMU was dissolved in 0.9% sodium chloride/3mM sodium citrate, pH 6.0 and prepared freshly. N-nitroso-N-methylurea was injected in the abdomen at an effective dose of 100 µg/g body weight on alternate days to *Periplaneta americana* and the insects were vivisected after 4, 8, 12, 16 and 20 days. Control insect received similar quantity of saline. The midgut was dissected out from the adult female in Ringer´s saline. The midgut was removed, fixed overnight in aqueous Bouin's fluid and processed for histological studies. The paraffin blocks were prepared in the usual way, sectioned at 6µ and were stained with Delafield's haematoxylin and eosin[7].

RESULTS

(a) Normal histology of the midgut

Internally the gut is lined by the stratum of enteric epithelium, the outer ends those cells rest upon a basement membrane, the latter is followed by an inner layer circular muscles and an outer layer of longitudinal muscles. The outer most coat of the mid gut is a thin peritoneal muscles. In the structure enteric epithelium two main types of cells are distinguished these are the columnar cell, their boundaries as usually well defined they almost invariably possess a striated border and these cells have well defined nucleus. The other type of cells are the regenerative nidi, their function is to renew other epithelial cells when they are destroyed (Fig. 1).

(b) Histopathological observation in the treated series from 4 to 20 days

4 to 12 days:

During 4 days, columnar cells of gut epithelium are more or less normal in shape. The chromatin material of the nuclei of some cells of the epithelial layer is clumped. The regenerative nidi are also showing degenerated chromatin material. The striated border is visible in some cells. The circular and longitudinal muscle layers are also distorted in shape (Fig. 2a and b). During 8 days, the gut epithelium appears to be accumulated together, vacuolated at places and weak in construction (Fig. 3a and b). The circular muscle layer is thickened and the longitudinal muscles are distorted in shape. Striated border is also distorted at few places. During 12 days, most of the cells of the gut epithelium have lost their columnar shape and some epithelial cells are showing additional growth of cells towards the gut lumen (Fig. 4a). Longitudinal muscles are vacuolated and degenerated. The cytoplasm of the epithelium is degenerated and weak in construction (Fig. 4b).

16 to 20 days:

During 16 days, the columnar cells of the gut epithelium have lost their normal architecture. An outgrowth is formed due to the proliferation of the cells (Fig.5a). The regenerative nidi are with degenerated chromatin material. Longitudinal muscles are distorted in shape (Fig. 5b). During 20 days, the columnar cells of the gut epithelium have lost their normal architecture. Columnar cells are distorted and degenerated in shape. The nuclei of gut epithelium are comparatively small. The cytoplasm is severely weak and vacuolated (Fig. 6a and b). The peritrophic membrane is completely obliterated. Striated border is also distorted. Longitudinal muscles are obliterated.

Fig. 1: Section of the midgut of normal adult *P. americana* showing columnar cells with prominent nuclei (arrow 1), well developed regenerative nidi (arrow 2), well developed circular as well as longitudinal muscles (arrow 3-4) (X 100) Haemotoxylin / Eosin.

Fig. 2a: Showing more or less distorted shape of columnar cells (arrow) after 4 days treatment with n-nitroso-n-methylurea (X 100) Haemotoxylin / Eosin.

Fig. 2b: Same showing enlarged view of degenerated chromatin material nuclei (arrow) after 4 days treatment with n-nitroso-n-methylurea (X 400) Haemotoxylin / Eosin.

Fig. 3a: Showing distorted shape of columnar cells (arrow) after 8 days treatment with n-nitroso-n-methylurea (X 100) Haemotoxylin / Eosin.

Fig. 3b: Showing thickened circular muscles (arrow) after 8 days treatment with n-nitroso-n-methylurea (X 100) Haemotoxylin / Eosin

Fig. 4a: Showing the newly formed gut epithelium (arrow1),vacuolated longitudinal muscles (arrow2) 12 days treatment with n-nitroso-n-methylurea (X 100) Haemotoxylin / Eosin.

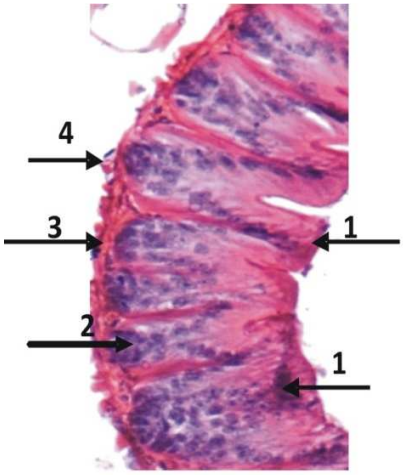

Fig. 1

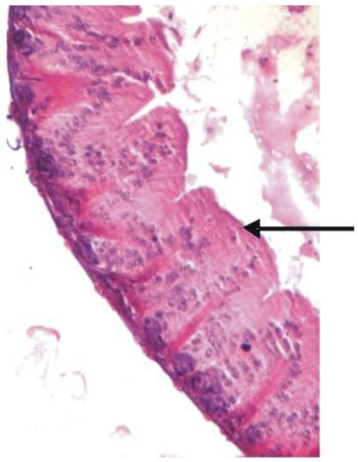

Fig. 2a

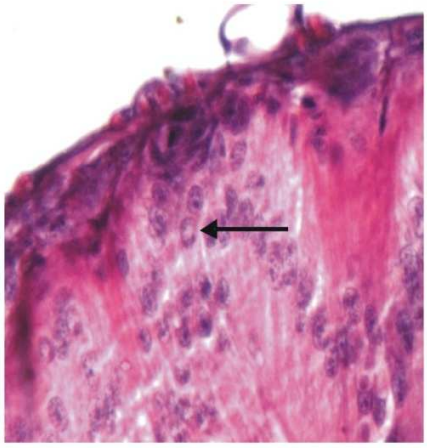

Fig. 2b

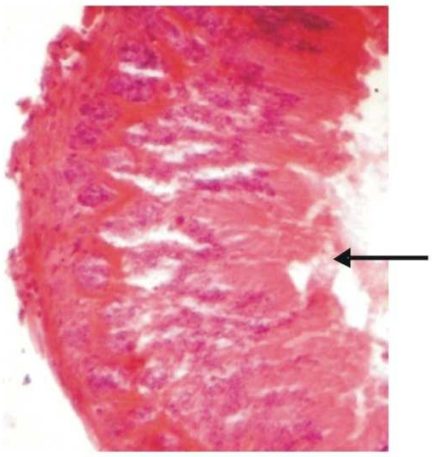

Fig. 3a

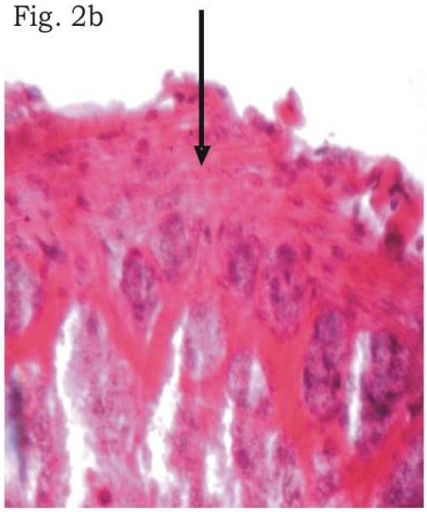

Fig. 3b

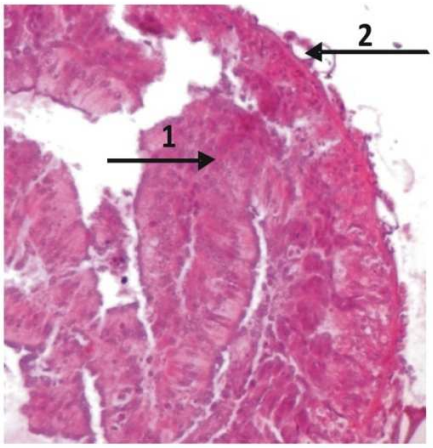

Fig. 4a

Fig. 4b: Showing distorted shape of columnar cells after 12 days treatment with n-nitroso-n-methylurea (X 100) Haemotoxylin / Eosin.

Fig. 5a: Showing elongated columnar cells after 16 days treatment with n-nitroso-n-methylurea (X 100) Haemotoxylin / Eosin.

Fig. 5b: Showing a growth of cells on the outside of the gut after 16 days treatment with n-nitroso-n-methylurea (X 100) Haemotoxylin / Eosin.

Fig. 6a: Showing distorted and thickened circular muscles (arrow 1) and weak and vacuolated cytoplasm (arrow 2) after 20 days treatment with n-nitroso-n-methylurea (X 100) Haemotoxylin / Eosin.

Fig. 6b: Showing shrunken and necrotic nuclei (arrow) after 20 days treatment with n-nitroso-n-methylurea (X 100) Haemotoxylin / Eosin.

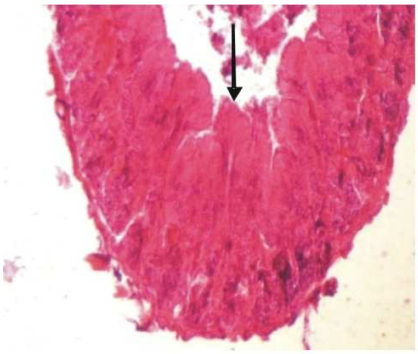

Fig. 4b

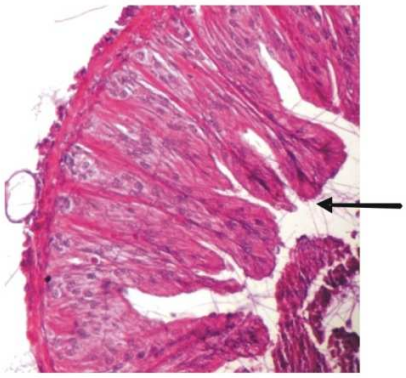

Fig. 5a

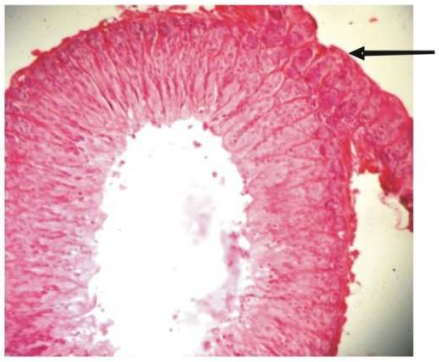

Fig. 5b

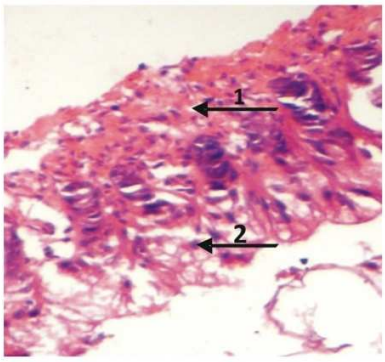

Fig. 6a

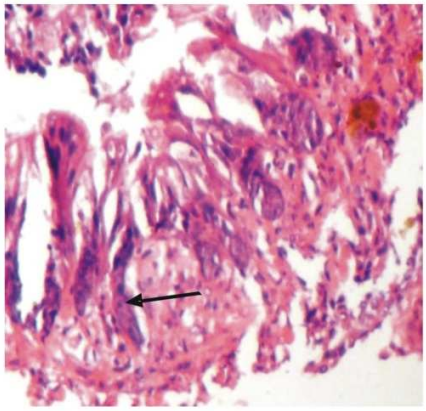

Fig. 6b

DISSCUSION

In the present study, it has been observed that the treatment with sub-lethal concentration of N-nitroso-N-methylurea for a peroid of 20 days has caused notable changes such as: disruption of peritrophic membrane, striated border, regenerative cells and longitudinal muscles. The remarkable histopathological changes observed in the midgut of *Culex pipiens* larvae are in conformation with the present findings, Ahmed[3] also 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. Ngoh *et al.*[14] studied the insecticidal activity and repellent properties of nine volatile constituents of essential oils against *P. americana*.

Here in *Periplaneta americana* similar observation have been made as mentioned by Cantwell *et al.*[6], Sutherland[23], Bearwald and Boush[5] and Ahi[2]. The gut epithelium showed hyperactivity and additional layers of epithelium was seen with degenerated cytoplasm. The ciruclar muscles were swollen and distorted. The longitudinal muscles were degenerated.

Pilate[15] reported proliferation of eptihelial cells in the lumen of the midgut, as mitotically active epithelial cells were produced as a result of some regenerative process. Sharma (1966) studied nuvan poisoning on *Poekilocerus pictus* F. and reported that the epithelium and their nuclei were badly shrunken and their cytoplasm became granular and cell structure and cell boundary was completly lost. In conformation with the above findings, shrinkage and disintegration of epithelial cells, disappearance of cell boundaries and vacuolization of the epithelial cells of midgut were observed in *Periplaneta americana*. Similar histopathological changes were reported in *P. americana* treated with lindane, Sharma and Chattoraji[19], *Spodoptera litura* treated with endosulfan, dizinon and dichlorvos, Lal *et al.*[10] and in *Plebiogryllus guttiventris* treated with fenitrothion, Balakrishannan[4].

Alterations in the alimentary canal of insects in response to insecticidal stress have been recorded by Mukherji and Haridas[13], Lal *et al.*[10], Mishra[12] and Singh[20]. In extreme cases, such as treatment with a-BHC, malathion, phosphamidon, parathion, carbaryl and endrin, severe necrosis of the epithilial lining cells of the alimentary canal has been observed in *Hieroglyphus nigrorepletus*, Mishra[12] and *Chrotogonus trachypterus*, Singh[20]. Breakdown of the epithelial layer in the alimentary canal in insects appears to be a generalized stress response resulting not only from exposure to wide range of toxicants but also from physiological stress such as starvation, Rosaish and Mukherji[17].

Against *S.littoralis*, Salam and Ahmed (1997) found that, the *Melia azedarach* extract caused destruction of epithelial cells. Also, Younes *et al.*[25] observed the degeneration of the epithelial cells and decay of its boundaries when *S. littoralis* larvae were treated with the extracts of both *Clerodendro inerme* and *Conyza dioscoridis* caused slight and severe disintegration of the epithelium, fading of the boundaries of epithelial cells and detachment of epithelial cell, Emara and Assar (2001). In contrast to these observations, Schluter and Schulz[18] found no effect of azadirachtin on the midgut epithelium of *Epilachna varivestis* larvae.

Hussein *et al.*[9] mentioned that the effect of plant extract on midgut of *Erias insulana* may due to digestion and absorption of plant oil. On the other hand Ahmed[3] 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. Abdel- Ghaffar[1] reported that effect of Margosan-o combined with sesame oil against the berseem hopper *Euprepocnemis plorans* and Wanderley–Teixeir *et al.*[24] described the midgut and pyloric valve alterations of the Orthopteran, *Tropidacris collaris*. The longitudinal muscles were distorted and ruptured the epithelial cells increased in number and become bigger in size. Some additonal growths were formed due to the peoliferation of cells with NMU treatment.

In conclusion, results obtained from this investigation showed that N-nitroso-N-methylurea caused cellular deformation in the midgut. It is important to mention that cellular defense mechanism of insects probably plays a strong defensive role in inhibiting the formation of tumors in insects as this potent carcinogen, NMU did not cause tumors in the midgut.

However, in the midgut an additional layer of epithelium were seen both outside and inside the normal epithelium, which was probably due to increased mitotic activity of regenerative nidi.

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