In the present investigation, the effect of N-nitroso-N-methylurea was studied on different tissues of Periplaneta americana. This investigation includes histopathological study of midgut, fatbody and ovary and biochemical estimation of the following parameters: protein, carbohydrate, lipid, amino acid, DNA, RNA and alkaline phosphatase.

The concentrations of N-nitroso-N-methylurea were determined according to the highest rate of mortality with a high dose and doses below it, till safe concentration was reached which served as experimental concentration. The experimental concentration (LC₀) for present investigation was 100 µg/g body weight.

Adult insects of more or less similar weight in each set were considered for these experiments. NMU was dissolved in 0.9% sodium chloride / 3mM sodium citrate, pH 6.0 and prepared freshly. N-nitroso-N-methylurea (100 µg/g body weight) was injected in the abdomen on alternate days to Periplaneta americana and the insects were vivisected after 4, 8, 12, 16 and 20 days of treatment. Control insects received similar quantity of saline. The midgut, fatbody and ovary were 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 (Davenport, 1960).

The tissues selected for the biochemical estimation were haemolymph and the rest of the tissues were the same as those for
histophothological studies. Method of treatment was similar to that of the histopathological studies but insects were vivisected after 5, 10, 15 and 20 days. Control insect received similar quantity of saline.

The parameters used for biochemical estimation were:

1. Protein – Bradford reagent
2. Carbohydrate – Anthrone reagent
3. Lipid – Sulpho-phosphovanillin reagent
4. Amino acid- Ninhydrin reagent
5. DNA- Diphenylamine reagent
6. RNA- Orcinol reagent
7. Alkaline phosphatase- Disodium phenyl phosphate

Histopathological study

The changes observed in the midgut were: 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. 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. The regenerative nidi were with degenerated chromatin material. The columnar cells of the gut epithelium had lost their normal architecture. The nuclei of gut epithelium were comparatively very small and pycnotic. The cytoplasm was weak and vacuolated. The peritrophic membrane was also completely obliterated.
As regards the fatbody, the cell boundaries of most of the fat cells had lost their identity and their cytoplasm was vacuolated. The peripheral and central globules had lost their architecture. Nuclei were small and pycnotic. Some of the nuclei were aggregated and were degenerated. The entire adipose tissue was seen to be degenerated and obliterated.

As regards the female gonads the mature oocytes were distorted in shape and were with ill developed, degenerated and contracted ooplasm. The ooplasm was severely vacuolated. The follicular epithelial cells were pycnotic, degenerated and thin. In mature oocytes the follicular epithelium was thickened with many additional nuclei. The follicular epithelial cells were degenerated, obliterated and distorted in shape. They were seen migrating in the ooplasm and had lost contact among themselves.

**Biochemical studies:**

Proteins contents of midgut decreased significantly on 15\textsuperscript{th} and 20\textsuperscript{th} day from that of their corresponding controls. However, the decrease of protein levels on 5\textsuperscript{th} and 10\textsuperscript{th} day was non-significant.

Proteins contents of fatbody and ovary decreased significantly on 5\textsuperscript{th}, 10\textsuperscript{th}, 15\textsuperscript{th} and 20\textsuperscript{th} day from that of their corresponding controls.

Proteins contents of haemolymph decreased significantly on 15\textsuperscript{th} and 20\textsuperscript{th} day than that of their corresponding controls. However, the decrease of protein levels on 5\textsuperscript{th} and 10\textsuperscript{th} day was non-significant.
Summary

Amino acid

Amino acid contents of midgut increased significantly on 15th and 20th day from that of their corresponding controls. However, the increase of amino acid levels on 5th and 10th day was non-significant.

Amino acid contents of fatbody, ovary and haemolymph increased significantly on 10th, 15th and 20th day than that of their corresponding controls. However the increase of amino acid levels on 5th day was non-significant.

Carbohydrate

Carbohydrate contents of midgut decreased significantly on 5th, 10th, 15th and 20th day from that of their corresponding controls.

Carbohydrate contents of fatbody and ovary decreased significantly on 10th, 15th and 20th day than that of their corresponding controls. However, the decrease of carbohydrate levels on 5th day was non-significant.

Carbohydrate contents of haemolymph decreased significantly on 15th and 20th day than that of their corresponding controls. However, the decrease of carbohydrate levels on 5th and 10th day was non-significant.

Lipid

Total lipid contents of midgut, fatbody, ovary and haemolymph decreased significantly on 5th, 10th, 15th and 20th day than that of their corresponding controls.

DNA

DNA contents of midgut, fatbody and ovary decreased significantly on 10th, 15th and 20th day than that of their
corresponding controls. However, the decrease of DNA levels on 5\textsuperscript{th} day showed non-significant decrease.

**RNA**

RNA contents of midgut, fatbody and ovary decreased significantly on 10\textsuperscript{th}, 15\textsuperscript{th} and 20\textsuperscript{th} day than that of their corresponding controls. However, the decrease of RNA levels on 5\textsuperscript{th} day showed non-significant decrease.

**Alkaline phosphatase**

Alkaline phosphatase contents of midgut decreased significantly on 15\textsuperscript{th} and 20\textsuperscript{th} day than that of their corresponding controls. However, the decrease of protein levels on 5\textsuperscript{th} and 10\textsuperscript{th} day was non-significant. Alkaline phosphatase contents of fatbody and ovary decreased significantly on 5\textsuperscript{th}, 10\textsuperscript{th}, 15\textsuperscript{th} and 20\textsuperscript{th} day than that of their corresponding controls.

Alkaline phosphatase contents of haemolymph decreased significantly on 10\textsuperscript{th}, 15\textsuperscript{th} and 20\textsuperscript{th} day than that of their corresponding controls. However the decrease of protein levels on 5\textsuperscript{th} day was non-significant.

The results obtained from this investigation showed that N-nitroso-N-methylurea caused cellular deformations in the fatbody, midgut and ovary.

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, fat body and ovary. 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.

Several vacuoles observed in the treated fatbody cells are probably an indication for an immediate utilization of these food reserves as an immediate energy source, when the insect is under stress.

In the ovary, it has been observed that yolk deposition was arrested and the yolk platelets present prior to the treatment became disintegrated and destroyed.

The protein content of midgut, fatbody, ovary and haemolymph decreased in NMU treated insects. In general, the breakdown of protein dominates over their synthesis due to enhanced proteolytic activity and during chronic period of stress they are also a source of energy.

The carbohydrate content of midgut, fatbody, ovary and haemolymph also decreased in NMU treated insects. The decrease in carbohydrate levels in the midgut, fatbody, ovary and haemolymph could be due to their utilization as energy sources under stress condition.

The lipid content of midgut, fatbody, ovary and haemolymph decreased in NMU treated insects. The decrease in lipid levels in the midgut, fatbody, ovary and haemolymph could be due to their utilization as energy sources under stress condition and physiological stress caused by toxicants or environmental contamination.

The amino acid content of midgut, fatbody, ovary and haemolymph increased in NMU treated insects. The increase in free amino acid levels in the hemolymph and fatbody could be due to decreased protein synthesis, resulting from the insecticidal stress.
The DNA and RNA content of midgut, fatbody and ovary decreased in NMU treated insects. In the present study the reason for decrease in nucleic acid level may be due to disturbed cell division.

The alkaline phosphatase content of midgut, fatbody, ovary and haemolymph decreased in NMU treated insects. ALP is a set of hydrolytic enzymes which hydrolyze phosphomonoesters under alkaline conditions and reflects the absorption, digestion and positive transportation of nutrients in the midgut. Different stress and disease cause considerable decrease in the activity of ALP.

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