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COPPER SULPHATE INDUCED NEURONAL HISTOLOGICAL CHANGES IN THE FRESHWATER SNAIL *BELLAMYA BENGALENSIS* (L)

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ABSTRACT
Present study was carried to investigate the nuropathological effect of induced copper sulphate in cerebral ganglion of the freshwater snail *Bellamya bengalensis*. Histopathological and ultrastructural changes found hypertrophy, hypotrophy, shrinkage in the neuronal cells with include degeneration in neurons leading to changes in axonal processes. Ganglionic histopathology were compared and discussed with normal neuronal cells of snail Bellayma bengalensis.

INTRODUCTION

Heavy metals are recognized as a strong biotoxicants, because of their persistent nature and cumulative action to the aquatic flora and fauna (Sharma and Agrawal, 2005). In the aquatic invertebrate, Beaby and Eaves (1983), observed that molluscs can accumulate higher concentration of metal ions than other groups of invertebrates. Among the heavy metals copper is important metal which is mostly used in the industries, paints and ceramics. The fertilizers are the main sources of copper, zinc and mercury which cause the pollution to the different media (Simkiss, 1984; Crop and Morgan, 1991). Intoxication of copper reduces growth, survival and rate of reproduction in the aquatic invertebrates.

The molluscan nervous system has been extensively studied by electrophysiologists, comparative neurologists, and recently cytologists are very much interested in the structure, formation, and secretion of neurosecretory substance in different animals (Bonga, 1970). The molluscan nervous system play important role in controlling growth and metamorphosis. The heavy metal accumulation at the cellular level is capable of interacting with many biological legends and interfere with different mechanisms (Gurd and Wilcox, 1956), some of which may affect to the nerve function (Scheinberg and Sternlieb, 1976). In the molluscan fauna, it is worthy to mention that in the freshwater snails nervous system has been proved to be sensitive to many toxic materials and cytotoxicants that may induce injurious consequences. (Hernadi and Vehovszky, 1992; Boer *et al.,* 1995; Wiemann *et al.,* 1995).

The paper deals with histopathological alteration in the cerebral ganglia of freshwater snail *Bellamya bengalensis* due to induced copper sulphate toxicity.

MATERIALS AND METHODS

The freshwater snail *Bellamya bengalensis* was collected from 'Rajaram tank', near to the Shivaji University, Kolhapur. To study the induced effect of copper sulphate, healthy, adult snails were divided into five troughs, out of five one trough was considered as control group of snail. The remaining four were used as experimental groups. Snails in each group were intoxicated by predetermined (mean LC_{50} 0.40 ppm) of water miscible copper sulphate (Cu SO_4. $5H_2O$). Experimental snails in each trough were exposed for 24, 48, 72 and 96 hr. After completion of exposure period, snails from each trough were dissected out for nervous system specifically for ganglionic mass. The cerebral ganglia was selected for histological investigation, the morphological changes were detected by staining the slides by Haematoxylene-Eosin technique by following normal microtechnique procedure.

RESULTS

The central nervous system of freshwater snail *Bellamya bengalensis*, consist of ring of ganglia around the anterior part of the digestive tract (buccal mass). The dorsal nerve mass is composed of two cerebral ganglia connected by a short commissure. The ventral nerve mass is composed of two pedal ganglia and visceral complex. This complex consists of two pleural, two parietal, and one visceral ganglia. All these ganglia are very close together, joined by very short commissure. Physiologically, the cerebral ganglion contains neurosecretory cells and neurons. They have been act as neurohormones, neromodulators or neurotransmitters in the body.

Histological organization of the unexposed cerebral ganglia in snail *B. bengalensis*

The paired cerebral ganglia that are connected by a short commissure (Fig. 1), histological the neurons have oval well defined nucleus. The neurophil (fibrous mass) was found centrally. Gilal cells were found between the nerve neuronal cells. The cells were distinguished by their dense longish small nuclei (Fig. 2). Also few gilal cells and large number of nerve axons that was found in different directions in the section. Some viable sized neurons were also found at the border layer of the cerebral ganglia. The nerve cells mostly unipolar with one axonal process (arrow) which were often found divided into collaterals (Fig. 3).

Histological alteration in the cerebral ganglia due to induced copper sulphate

The neuronal alterations in the experimental snails were found in different manner, it was depending on the induced time and also to the concentration of metal. As per the Concentration and exposure period histopathological changes are as follows.

24 hr exposure: After 24 hr obvious alterations were not found in the cerebral ganglia of *Bellamya bengalensis,* the gaint neurons are slightly shrunken. The neurophile (fiber mass) did not show marked changes (Fig. 4).

48 hr exposure: Cerebral ganglia after this period showed shrinkage in giant neurons with multinucleated cellular architecture in it. Also gaint and medium sized neurons showed little vacuolation with degeneration of neurons in the ganglionic mass (Fig. 5).

72 hr exposure: After 72 hr exposure the gaint neurons were darkly stained and showed ruptured plasma membrane. The dissolved neurons have formed diffused structure. The axonal processes in the ganglia was found disturbed (Fig. 6).

96 hr exposure: After 96 hr of exposure the gaint neurons showed heavy shrinkage with poor nuclear membrane integrity. The nuclei of the neuronal cells appeared with irregular structure. The large and medium sized neurons displayed shrinkage of there cell bodies. The surrounding extra space found enlarged and disturbed. The gaint neuron was greatly distorted and showed dark staining with completely disturbed axonal processes (Fig. 7).

DISCUSSION

The mode of action of copper sulphate in the nervous system of gastropods has predominantly have been studied at a neurophysiological, or behavioral level. The current study has been performed to show the histopathological and ultrastructural alteration in the cerebral ganglia of snail *Bellamya bengalensis* associated with copper sulphate

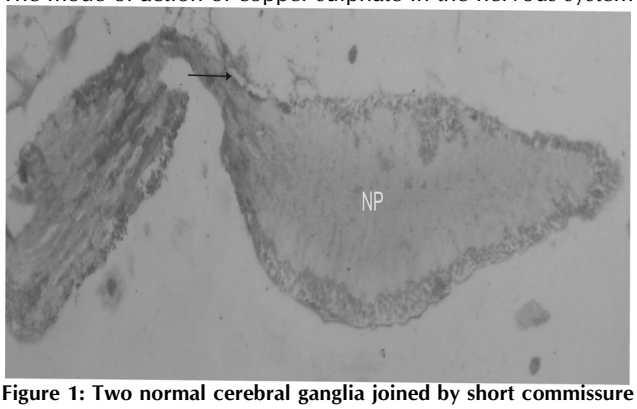

Figure 1: Two normal cerebral ganglia joined by short commissure stained with HE X 100 (arrow); NP- Neurophile

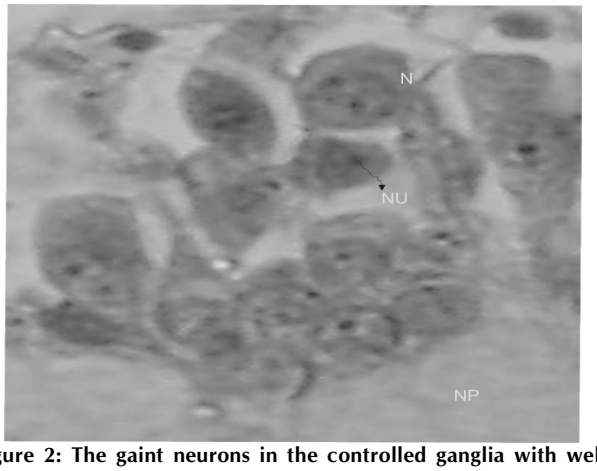

Figure 2: The gaint neurons in the controlled ganglia with well defined nucleus HE X500; N- Neurons NU- nucleus NP- Neurophiles

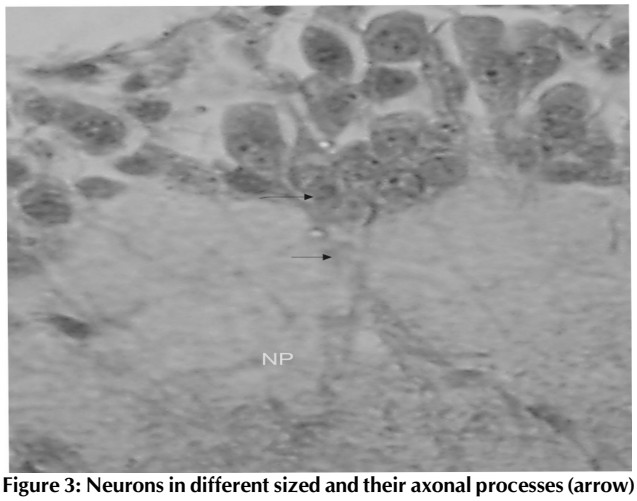

Figure 3: Neurons in different sized and their axonal processes (arrow) into the neurophile of control ganglia. HE X 400; NP- neurophile

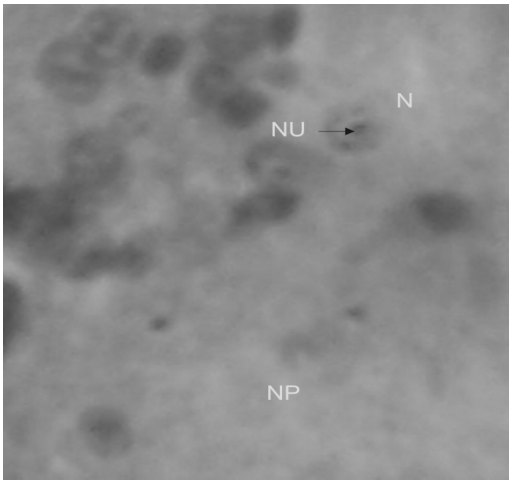

Figure 4: Copper sulphate induced cerebral ganglia after 24 hr Stained with HE X400; N- Neurons; NU-Nucleus; NP- Neurophiles

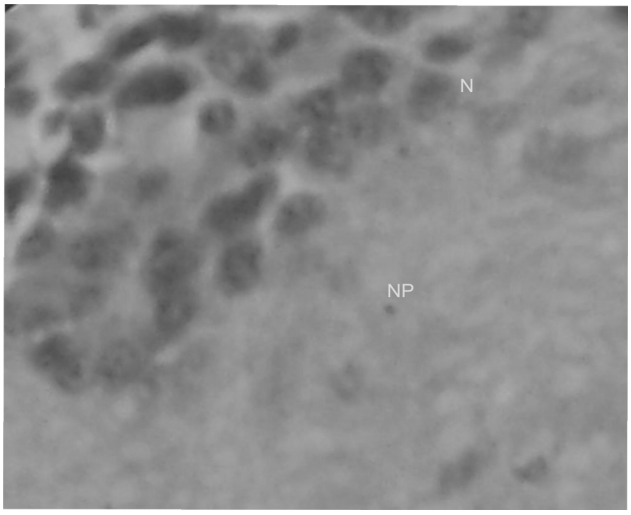

Figure 5: Copper sulphate induced cerebral ganglia after 48 hr Stained with HE X400; N- Neurons; NP- Neurophiles

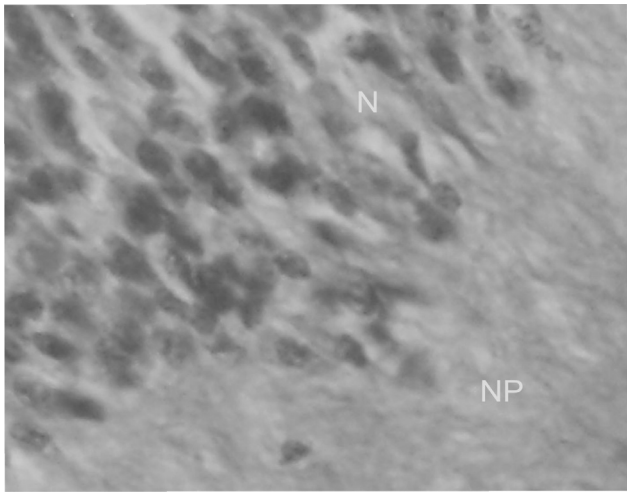

Figure 6: Copper sulphate induced cerebral ganglia after72 hr Stained with HE X400; N- Neurons; NP- Neurophiles

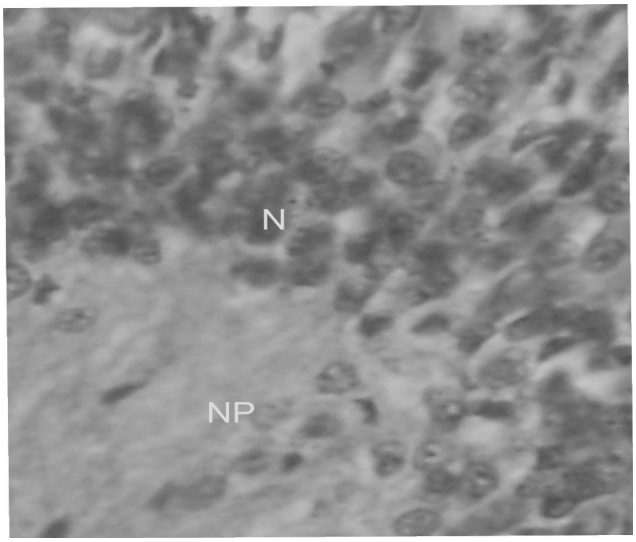

Figure 7: Copper sulphate induced cerebral ganglia after 96 hr Stained with HE X400; N- Neurons; NP- Neurophiles

induction. Observations revealed that, molluscan cerebral ganglion contains several neurons with variable sizes *i.* e. gaint, large, medium, and small neuronal cells. In previous studies similar results have been noted in the neuronal cells and fibers with slight differences in the neurons of various pulmonate species (Steffens, 1980; Kruatrachue *et al.,* 1993; Essawy, 2001).

Light microscopic investigations of cerebral ganglion showed that the alteration in neurons was due to induced copper sulphate in which neurons of all size were greatly affected. They showed shrinkage of cell, cytoplasm *i.* e. disturbed, multinucleated, as well as completely degenerated neurons. The Sterman (1982), Jones and Cavanagh (1984), have observed pathological changes in the cell cytoplasm of all sized neurons and the ganglia due to Acrylamide intoxication, in which they found dilation of intracellular space between the neurons in the cerebral ganglia of treated snail *Bellamya bengalensis*.

Cotran *et al.,* (1999), found that these dilations probably reflected by dysfunction of iron and osmotic balance of cells. Mcllwain and Hoke (2005), observed that shrinkage of cell bodies, eccentric nuclei could be attributed to the effect of the two tested carbomate molluscicides on the affected neurons. Singh and Singh, (1984) reported that insecticides treatment induced enhanced transmitter release and mitochondrial damages resulting in the neurophile and nerve cell bodies in the metathorasic ganglion of *Periplantea americana*. The neurotoxic action of carbamate was due to the inhibition of AChE activity and the accumulation of ACh at synaptic junction were result in the alteration of locomotion (Wedgwood and Bailey, 1988) and feeding behavior (Wright and Williams, 1980, Wedgwood and Bailey, 1986).

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