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Reactivity of digestive mucins in freshwater snail *Bellamya bengalensis* (l) against copper sulphate induction

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Abstract

Freshwater snail *Bellamya bengalensis* was used for the toxicological study against copper sulphate in pre-determined LC_{50} concentrations (0.56ppm) up to 96 hrs. Histopathology was investigated by using standard H-E (Hematoxylene-Eosine) staining technique. Cellular damage was found in midgut (stomach cells) mucus cells, epithelial lining and typhosolar cells. Depleted mucin was found in PAS and Alcian blue (AB) staining technique. Highly reactive proteioglycans was found in mucus secretary cells. Histopathology and chemical changes were comparatively discussed in relation to digestive mechanism of experimental animals.

INTRODUCTION

High concentration of metals in the environment orders a new ecological factor, to which organism must adapt to survive in it. For living organisms, number of metals are vital elements, since they play important roles in the control of gene transcription, redox reactions and oxygen transport. The concentration and length of exposure is related to the severity of toxicity to any animal. Thus in bioassay living organisms becomes an agent for the evaluation of potency for toxic substances [1] . Metal accumulation in water and sediment alters the physiological mechanisms in organism as per the environmental factors [2]. However, essential and non essential metal may also be prejudicial, causing mutagenic [3-4], carcinogenic effects [5-6]. Copper sulphate found to be an essential element for organisms and has been reported to often occur in freshwater habitats also, at concentrations of approximately 2 to 3 ppb [7]. At high concentrations in the metabolism, copper regulatory processes were found to be impaired, which may be because of its cytotoxic and genotoxic properties [8].

Mucin in the digestive tract may have several functions such as the protection of the vital epithelium from proteolytic damage, mechanical injuries and bacterial infection, lubrication and also to increase digestive efficiency [9-10]. Functions are related to their different structure in the various parts of the digestive tract [11-12]. Epithelial mucins have been classified into neutral and acidic types, further divisible into three subgroups, namely, sulphated mucins, sulphated sialomucine and carboxylated biochemical components [13]. Opisthobranchs have been dominated by mucus secreting cells in the stomach epithelium however, the secretary material of digestive cells was found enriched in *Aplysia depilans* [14].

The gastric shield protects the stomach wall from the abrasive effect of the rotating crystalline style, although it may also assist in the trituration of stomach contents [15]. The carnivorous Cephalaspidean, *Melanochlamys cylindrica* and the herbivore, *Bulla quoyi* seem to be devoid of secretary cells in the stomach [16-17]. The stomach epithelium of *Haminoea hydatis* found more complex, containing additional types of secretary cells [18,16]. Major alterations included are increase in the mucus vesicles, mucus production and dilation of cells in the midgut (Stomach). By taking literature review, we have selected the above problem to discuss changes in histochemical components of midgut through toxicity of copper sulphate in the freshwater snail *Bellamya bengalensis.*

MATERIALS AND METHOD

Animal Collection:

Healthy, adult freshwater snails *Bellamya bengalensis* were collected from Rajaram tank, near Shivaji University, Kolhapur. After acclimatization, selected snails were divided into five groups, first as control and remaining four as experimental groups. Experimental animals were exposed to water miscible metal Copper sulphate ($CuSO_4.5H_2O$) toxicity, at 0.56 ppm (pre-determined LC_{50} concentration), for the different exposure periods as, 24hrs, 48hrs, 72hrs and 96hrs. After completion of exposure period, live snails from each group was immediately dissected out for midgut (stomach) and were processed for histological and histochemical investigations.

Histology and Histochemistry:

Standard Haematoxylene-Eosin technique was used for the histological investigations as, it differentiates the different cell structure of the stomach. Tissue was dissected out and fixed in the Calcium-Acetate-Formalin (CAF) for 24 hrs., followed by washing, dehydration, clearing in xylene and finally blocks were prepared by paraffin embedment. Trimmed tissue blocks were sectioned at 4-5µm and were used for histopathological staining procedure.

Histochemical detection of midgut (stomach cells) was performed by using standard Periodic Acid -Schiff (PAS), Alcian blue (AB) pH 2.5 and AB pH 1 staining technique. PAS was applied to investigate the alterations in glycoproteins, polysaccharides [19-20]. Section were treated with AB pH- 2.5 [21], for detection of mucosubstanses. Treatment of AB pH-1 [22] to the cells was for the detection of sulfomucins after intoxication of copper sulphate as per the time of exposure.

RESULTS

Histopathology of Midgut / Stomach:

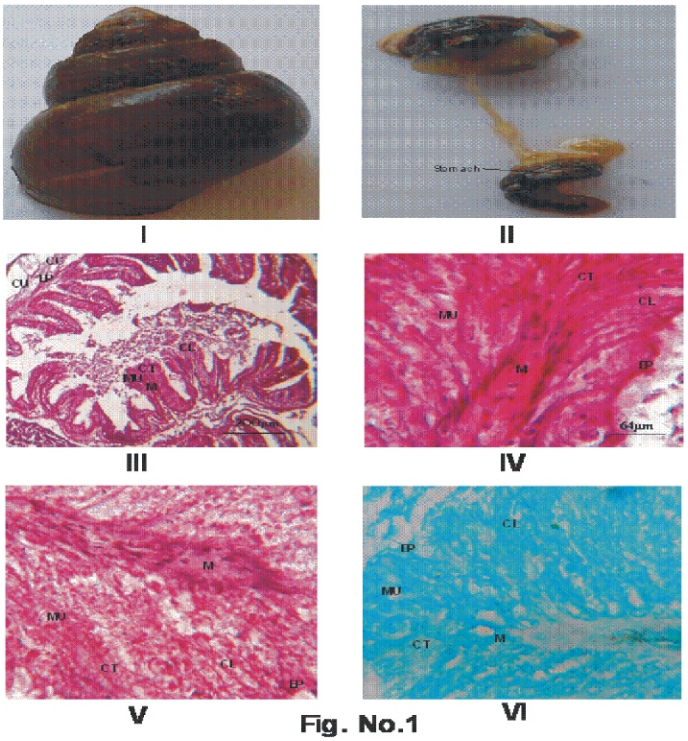

Fig. 1 : Freshwater Snail *Bellamya Bengalensis*, **II**- Digestive Tract of *Bellamya Bengalensis* - Stomach, **III**-T. S. of stomach, **IV**- Normal HE Stomach section, **V**- Normal PAS stomach section, **VI**- Normal Alcian Blue (AB) stomach section, **Abbreviations:** - M-Muscle, Cl-Cilia, MU-Mucous cell, EP-Epithelial cell, CC- Columnar cell, CT- Connective tissue, CU-Cuboidal cells.

In the digestive tract, stomach was found at the narrow tip of the digestive gland, connecting to the digestive gland and intestine. Midgut is a part of digestive system, it plays an important role in digestion process. It digests the food by secreting essential enzymes. In the normal histology, stomach showed different type of cells as mucus secreting cells, goblet cells which are enriched by protein material along with modified, epithelial cells, columnar cells, cuboidal cells, supporting cells and glandular cells providing conformed cellular frame work to stomach. The secretary layer followed by connective tissue and muscular layer composed of circular muscle and longitudinal muscle fibers. Single nucleated flat epithelial cells were arranged at the outer lining to form conform covering for to protect internal parts. In controlled animal midgut showed many folds, each of one was measured and found 64µm in diameter.

Differentiation of cellular architecture was found varied as per the exposure period from 24 hrs. to 96 hrs. After induction of copper sulphate stomach cells were found damaged, degenerated and displaced in the tissue. Muscular layer was found squized with vesicles in it. The mucosal lining was found ruptured. Cellular debris was found in most of the section. Then number of mucus secreting cells was quantitatively increased due to metal toxicity. The supporting cells, cuboidal cells were showed cellular damages. Cellular hypertrophy was observed in the section. In section epithelial cells and connective tissue were found ruptured. The connecting tissue was found displaced. Vacuolization, cellular degeneration, rupturing of cells was found in the different layer in toxicity as per the exposure period.

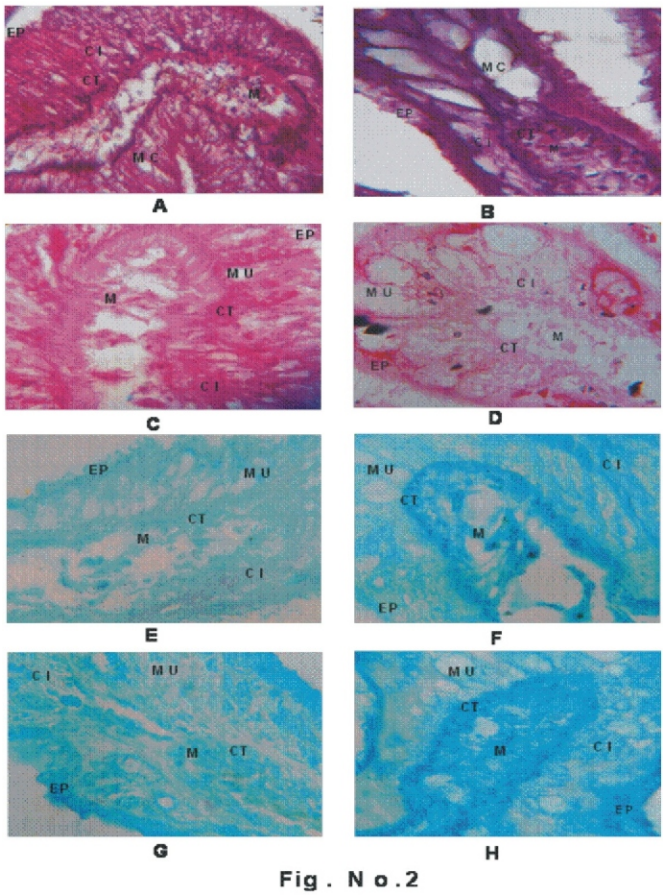

Fig. 2 : **A-** After induction of Copper sulphate changes in stomach cells 24 hrs. HE, **B-** 96 hrs. HE, Histochemical alteration of staining intensity in different cells of Stomach, **C-** 24 hrs. PAS, **D-** 96 hrs. PAS, **E-** 24 hrs. AB pH-2.5, **F -** 96 hrs. ABpH-2.5, **G-** 24 hrs. AB pH-1, **H -** 96 hrs. AB pH-1.
Abbreviations: - M-Muscle, Cl-Cilia, MU-Mucous cell, EP-Epithelial cell, CT- Connective tissue,

Normal Mucins in Stomach:-

Mucus secretary cells play important role in protection from injury by its mechanism of lubrication, balancing the enzyme activity in mid gut of snail *Bellamya bengalensis*. The mucopolysaccahride are neutral or acidic. A neutral mucopolysaccahride contains carbohydrate made up of hexose units usually acetylated in the form of glucosamine. The acid mucopolysaccahride contain carboxylated glucose units (glucornic acid). Hyluronic acid is a simple acid i.e. mucopolysaccahride composed of chain of glucosamine and glucuronic acid. Chondroitin sulphuric acid was found widely distributed in connective tissues and formed complex of acid mucopolysaccahride made up of sulphated galactosamine and glucuronic acid. The mucoproteins, usually considered to be contain predominantly more than four percent of a hexosamine with polysaccharide.

Histochemical methods revealed that, protein secreting cell among lining midgut were PAS positive and showed the presence of glycoproteins in controlled midgut section. Stomach cells showed both neutral and acidic mucosubstances in the form of glycoconjugates. The outer epithelial lining showed presence of glycogen material and large amount of hyluronic acid. In the

Table 1. Histochemical staining reaction of midgut in control and copper sulphate intoxicated snail *Bellamya bengalensis*.

Sr.No.	Histochemical techniques	Cell	Control group	Animal groups Experimental group			
				24hrs.	48hrs.	72hrs.	96hrs.
1)	PAS	EP	++++P	+++P	+++P	++P	++P
		Cl	++++P	+++P	+++P	++P	++P
		MC	++++P	+++P	+++P	++P	+P
		M	++++P	+++P	+++P	++P	+P
		CT	++++P	+++P	+++P	++P	+P
2)	AB pH-2.5	EP	++B	++B	++B	+++B	++++B
		Cl	++B	++B	++B	+++B	++++B
		MC	++B	++B	++B	+++B	++++B
		M	++B	++B	++B	+++B	++++B
		CT	++B	++B	++B	+++B	++++B
3)	AB pH-1	EP	++B	++B	++B	+++B	++++B
		Cl	++B	++B	++B	+++B	++++B
		MC	++B	++B	++B	+++B	++++B
		M	++B	++B	++B	+++B	++++B
		CT	++B	++B	++B	+++B	++++B

+ - moderate reaction, ++ -strong reaction, +++ -very strong reaction

staining, it showed intensely blue and pink coloured (Fig.1 V, VI).

Mucin after induction of copper sulphate:

Generally, mucus cells showed decreased glycoproteins with respect to increased content of hyluronic acid, sulphomucin and other acidic mucopolysaccahride. Initially in the experiment, protein and carbohydrate concentration was found minimized and acidic mucosubstance sulfomucines were slightly increased. The connective tissue showed reduced content of neutral mucosubstances at the initial toxicity period. Glycoprotein was found lowered with respect to increased carboxylated (COOH) ions. In AB pH-2.5 reaction, the carboxyl group containing hylaluronic acid and sialomucins were found increased in mucoid cells. PAS reactive chemical moiety in the toxicated cells showed that, hexose containing glycogen was decreased; similar chemical components were found in muscle, epithelial cells and mucus cells (Fig. No. 2-C, D)

Mucus secretary cells showed negative reaction to the neutral mucosubstances. The columnar cells showed presence of strongly acidic mucosubstances, sulfated mucins in the lumen. The connective tissue showed less concentration of neutral mucosubstances where as, the hyaluronic acid and sialomucins was found greatly increased. Similar results were found in typhosolar cells of stomach. The result as per the staining intensity is mentioned in table no.1.

DISSCUSION

Toxicity of heavy metal copper sulphate damages the structure of stomach which can be clearly seen in histological and histochemical techniques. Histopathological alteration in organs may cause the metabolic disorder, cellular damages or some time death of animal. Histochemically seen that, due to metal stress in the cells, it effect the neutral and acidic mucosubstances. Therefore, several scientists have observed histopathological and histochemical alteration in various organisms. Tyagi and Panwar (1990) [23], studied the effect of copper sulphate and mercuric chloride on the freshwater snail *Viviparus bengalensis*. They found histological changes in mucocytes of buccal cavity notated, ruptured cells and hyper secretion of white mucus after 72 hrs. of exposure. Vaint *et. al.,* (2002) [24] exposed *Halotis rufescens* to the heavy metal copper and observed vacuolization and cellular degeneration, rupturing of cells in the foot and digestive gland. Hernadi (1981) [25], observed glycoprotein rich sulphahydral groups and aromatic amino acids in various cells and glands of the snail *Helix pomatia*. Many investigator have used histochemical technique to evaluate the stress effect caused by heavy metal on the animals [26,-28]. With respect to this they, found decreased concentration of neutral mucosubstances due to metals toxicity in the cells and the glands of many organs [29-32]. The response of the liver to high copper concentrations was far more marked than in the case of low concentrations, where, in most cases, the copper content was less than that of the control of snail *Lymnaea peregra* [33].

Mucus-secreting cells are allegedly responsible for the lubrication of the luminal surface of the digestive tube and were also found in the stomach of many other gastropods [34-37]. The sulphated acid mucin in the gastric secretion may provide a resistance against bacteria in the stomach. Because of the heavy sulphation rate it has been noted that, it had restricted the degradation of mucin by bacterial mucin through degrading glycosidase [38]. Abd Allah and Moustafa (2002) [39] reported the accumulation of lead and cadmium and were noted histopathological effects in the marine prosobranch *Nerita saxtilis*. Triebskorn and Kunast (1990) [40], have reported, the histopathological alterations of the digestive tract after exposure to molluscicides in snails such as *Deroceras reticulatum*.

By considering histopathology and altered histochemistry, it was found that, copper sulphate is highly toxic to the snail *Bellamya bengalensis* and is having direct effect over the digestive system by interference to number of biochemical constituents in the cell. As a result, during experiment snail was found sluggish or immotile, inactive in the metabolism leading to behavioral changes and facing mortality problems.

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REFERENCES

1. James R, Sampath K, Ponamani KP (1992) Effect of metal mixture on activity of two respiratory enzymes and their recovery in *Oreochromis mossambicus. Indian* J. Exp. Biol. 30**:** 496-499.

2. Regoli F, Principato G (1995) Glutathione glutathioned dependent and antioxicant enzymes in mussel, *Mytilus galloprovincialis*, exposed to metals under, field and laboratory conditions Toxicol., 31: 143-164.

3. Filipic M, Hei TM (2004) Mutagenicity of cadmium in mammalian cells: implication of oxidative DNA damage. Mutat Res- Rev Mutant Res 546: 81-91.

4. Hei TM, Filipic M, (2004) Role of oxidative damage in the genotoxicity of arsenic. Free Radic Biol Med 37 (5): 574-581.

5. Waalkes MP, (2003) Cadmium carcinogenesis. Mutat Res Rev Mutat Res 533: 107-120.

6. Waisberg M, Joseph P, Hale B, Beyersmann D, (2003) Molecular and cellular mechanism of cadmium carcinogenesis. Toxicology 192: 95-117.

7. Wedepohi KH. (1990) *Handbook of Geochemistry*. Springer-Ver-lag, Berlin, Germany.

8. Vijayram K, Geraldine P (1996): Are the heavy metals cadmium and zinc regulated in freshwater Prawns? Ecotoxicol Environ Saf 34: 180-183.

9. Gupta BL (1989) The relationship of mucoid substances and ion and water transport, with new data on intestinal goblet cells and a model for gastric secretion. Symposia of the Society for Experimental Biology, 43: 81-110.

10. Domeneghini C, Arrigihi S, Radaelli G, Bosi G, Veggettti A (2005) Histochemical analysis of glycoconjugate secretion in the alimentary canal *Anguilla anguilla* L. *Acta Histochemica*, 106: 477-487.

11. Loo SK, Wong WC, (1975) Histochemical observations on the mucins of the gastrointestinal tract in the toad (*Bufomelanostictus*). Acta Anatomica, 91: 97-103.

12. Ferri D, Liquori GE, Natale L, Santarelli G, Scillitani G (2001) Mucin histochemistry of the digestive tract of the red-legged frog *Rana aurora aurora*. *Acta Histochemica*, 103: 225-237.

13. Bancroft JD, Cook HC (1984) Manual of Histological Techniques, Churchill Livingstone, New York, USA.

14. Lobo-da-Cunha A, Batista- Pinto C, (2003) Stomach cells of *Aplysia depilans* (Mollusca, Opisthobranchia): J. Submicrosc. Cytol. Pathol.34: 217-225.

15. Morton B, Prezant RS, Wilson B, (1973) In P. L. Beesley, G.J.B. Ross and A. Wells, eds., Mollusca: The Southern Synthesis Fauna of Australia Vol. 5. CSIRO Publishing, Melbourne, Australia. Class Bivalvia, pp. 195-234.

16. Rudman WB, (1971) Structure and functioning of the gut in the Bullomorpha (Ophithobranchia). Part 1. Herbivores. Journal of Natural History, 5:647-675.

17. Rudman WB (1972c) Structure and functioning of the gut in the Bullomorpha (Opisthobranchia). Part 4. Aglajidae. Journal of Natural History, 6: 547-560.

18. Fretter V (1939) The structure and function of the alimentary canal of some tectibranch molluscs, with a note on excretion. Transaction of the Royal Society of Edinburgh, 59: 599-646.

19. Mc Manus JFA (1946) Histological and histochemical use of periodic acid. Stain. *Technol*.23:99.

20. Hotchkiss RO, (1948) A micro chemical (reaction resulting in the staining of polysaccharide structure in fixed tissue preparations. *Arch. Biochem*. 16: 131.

21. Mowry, RW, Winkler CH, (1956) The coloration of acidic carbohydrates of bacteria and fungi in tissue sections with special reference to capsules of *Cryptococcus neoformans, Pneumococcus and Staphylococcus*. Amer. J. Path. 36: 628.

22. Lev R, Spicer SS (1964) Specific Staining of sulfate groups with Alcian blue at low pH. *J. Histochem. Cytochem*. 12:309.

23. Tyagi, R K, Seth RN, Panwar R S, (1990) Toxicity of 2-methoxy ethyl mercuric chloride, copper sulphate and mercuric chloride to freshwater snail *Viviparus bengalensis* (Swainson). J. Environ. Biol. 11(3): 263-267.

24. Viant MR, Walton JH, Tjeeroema RS, (2002) Sublethal action of copper in abalone (Haliotis rufescens) as characterized in Vivo 31P NMR Aquatic Toxicology 57: 139-151.

25. Heranaoi, L, (1981) Acta, Biol. Acad. Sci. Hung. 32: 19-32.

26. Jackim, E, Hamlin M, Souis S (1970) Effect of metal poisoning on live liver enzymes in killifish Fundulus heteroditus. J. Fish. Res. Biol Canada. 27: 383-390.

27. Sastry KV, Sharma P, (1980) Effect of mercuric chloride on the brain enzyme in a freshwater teleost, Ophiocephalus Punctabis. Arch. Environ. Contam. Toxico. 9: 425-430.

28. Thurberg FP, Calabrese A, Tucker RK (1977) Response of the lobster Homarus americanus to sublethal concentration of Cd and Hg. In "Physiological responses of marine biota to pollutants", Ed. F.J. Vernberg. Acad. Perss. New York, 185-197.

29. Verma S R, Chand R (1986) Toxicity effects of mercuric chloride on freshwater enzymes of carbohydrate metabolism of Notopterus notopterus. Indian J. Environ. Heath, 28:1-7.

30. Lomte, VS, Patil PN, (1989) Effect of Pesticide on the activity of invertase in the armyworm Mythimma Separata. Proc. Nat. Acad. Sci., 55-59.

31. Sontakke, YS (1992) Some physiological variation associated with pollutant treatment in the snail, Thiava tuberculata. Ph.D. Thesis, Marathwada University, Aurangabad (M.S.).

32. Valarmathi S (2000) Environmental quality of coastal zone of Madras and impact of pollutants on Sesarma quadrtum (Fabricus) Ph.D. Thesis University of Madras, Tamilnadu, India.

33. Fabisiak J P, Tyurin VA, Tyurina YY, Borisenko G G, Korotaeva A, Pitti BR, Lazo JS, Kagan VE (1999) Redox regulation of copper metallothioneins.

34. Bolognani Fanti AM, Bolognani L, Ottaviani E, Franchini A, (1982) The digestive apparatus of *Murex brandaris* (L.) and *Trunculariopsis trunculus* (L.). Z. mikrosk. Anat. Forsch. 96: 561-582.

35. Roldan C, Garcia-Corrales P, (1988) Anatomy and histology of the alimentary tract of the snail *Theba pisana* (Gastropoda: Pulmonata). Malacologia 28: 119-130.

36. Soto M, Gil JM, Marigomez JA Angulo E, (1990) Histochemistry and elemental composition of the stomach cells in Littorina littorea (L.) Folia Histochemica et Cytobiologica, 28: 239-248.

37. Leal-Zanchet AM (1998) Comparative studies on the anatomy histology of the alimentary canal of the Limacoidea and Milacidae (Pulmonata: Stylommatophora). Malacologia, 39: 39-57.

38. Robertson AM, Wright DP (1997) Bacterial glycosulphatases and sulphomucin degradation *Canadian Journal of Gastroenterology*, 11: 361-366.

39. Abd Allah AT, Moustafa MA (2002) Accumulation os lead and cadmium in the marine prosobranch Nerita saxtilis, Chemical analysis, light and electron microscopy. *Eviron pollut* 116: 185-191

40. Triebskorn R, Kunasi C, (1990) Ultrastructural change in the digestive system of Deroceras reticulatum (Mollusca; Gastropoda) induced by lethal and sublethal concentration of the carbamate molluscicide cloethcarb. Macologia 32 (1): 89-106.