EFFECT OF HEAVY METALS CADMIUM SULPHATE AND MERCURIC SULPHATE ON NUCLEIC ACIDS DNA AND RNA IN CRAB SCYLLA SERRATA

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Abstract

In present investigation the estuarine crab Scylla serrata exposed to cadmium sulphate and mercury sulphate (17.2mg/L, 6 mg/L) up to 24, 48, 72, 96, 120 hrs respectively. In present study DNA content was decreased in muscle and hepatopancreas and chelate legs at 24 hrs to 48 hrs and slightly increased at 72 hrs. RNA content was decreased in muscle and hepatopancreas and chelate leg, muscle and gills at 24 hrs, 48 hrs, 96 hrs and 120 hrs and slightly increased at 72 hrs exposure in cadmium exposure crab Scylla serrata. Whereas mercuric sulphate exposure crabs DNA and RNA content was decreased from 24 hrs to 120 hrs in muscle and chelate legs and slightly decreased at 24 hrs and other tissue RNA content decreased. Nucleic acid (RNA and DNA) due to heavy metals toxicity DNA synthesis inhibited ultimately resulted in depletion in RNA level. Any variation in protein affected nucleic acid Synthesis.

Key words: Scylla serrata, RNA, DNA, cadmium sulphate, Mercuric sulphate

Introduction

Nucleic acids are polymer of nucleotides. Nucleotides are consists of combining nitrogenous ring and phosphate group of orthophosphoric acid. The heterocyclic constituent are referred as bases of nucleotides. The structural classes of nucleic acid occur in the cells differing in the nature of the sugar, they contain ribose , a five carbon sugar known as ribonucleic acid (RNA), while other contain the 2-deoxy derivative of ribose known as deoxyribonucleic acid (DNA).

Nucleic acids are the nitrogen containing compounds of higher molecular complex associated with protein in cell. The information in DNA consists of the sequence in which different base pair occur in the centre of stack. The length of molecules in different organisms is roughly proportional to the amount of information it contain. DNA constitute a master file of genetic information carefully reproduced in successive generation of the cells, where as RNA molecules are used as working copies and as a tools for making protein. When cell divide, each daughter cell must have faithful copies of entire genetic instruction in original cell. This accompanied by replication of the DNA in which new chain of complimentary chains are created for each of the original chain in the cell.

The use of the information in DNA to direct the life of a cell involve synthesis of the RNA molecules as a working copies of segments of DNA, Some of the RNA are structural components. Of the apparatus of protein synthesis other act as messengers of DNA in the nucleus carrying instruction for assembly of cellular proteins These instruction are used by the enzymes catalyzing protein synthesis in the cystosol (Leninger).

DNA is a master molecule of life. Heavy metals enter into body of organism through the respiratory organ like gill, Lung etc. and through food and drinking water. It is hazardous to aquatic ecosystem and disturbs the food chain.Nucleic acid contents are considered as an index of capacity of an organism for protein synthesis. Different hormones and stress conditions may exert control over synthesis, activity and break down of nucleic acids. The nucleic acid contents can cause alternation in genetic information and genome functioning.

Material and method

The tissue of crab *Scylla serrata* i.e. hepatopancreas, muscle chelate leg, gill and heart were selected for the DNA estimation in present investigation.

DNA (Diphenylamine Method0

Estimation of DNA content was determined by Diphenylamine Reaction (Barton 1956). After isolating the tissues 1% homogenate were prepared in ice cold distilled water Add 1 ml 10 % trichloroacetic acid, these solution were centrifuged at 2600rpm minutes. Discarded the supernatants and the precipitate were washed three time in 5% TCA each time the supernatants were discarded each times. Again add 2ml of 5% TCA to the precipitate mixed well heated the suspension for 20 minutes at 90^{oc} cooled the tubes centrifuged for 10 minutes at 2500 rpm To o.2 ml of supernatants o. 8ml of 5% TCA and 2ml of diphenylamine reagent were added. The contents were kept in boiling water bath at 70^{oc} for 15 minutes cooled and the colour was read at 600 rpm against blank. The blank consists of 1 ml 5% TCA and 2ml diphenylamine reagent (Barton1956).

RNA (Orcinol)

The tissue hepatopancreas, muscle chelate leg, gill and heart of crab Scylla serrata were selected for the RNA estimation in present investigation.

RNA was determined by Orcinol method (Bial 1962). After isolating the tissues 1% homogenates were prepared in ice cold distilled water. Add 1ml 10 % trichloroacetic acid, these solution were centrifuged at 2600rpm minutes. Discarded the supernatants and the precipitate were washed three time in 5% trichloroacetic acid each time the supernatants were discarded each times. Add 4ml of 0.3N KOH to hydrolyzed RNA incubated 37oc for 1 hour Transferred the tube to ice bath for 5 minutes and then added 10 % TCA leaved the tubes 0^{o}c for 10 minutes. From these 2ml supernatant, 1ml distilled water and 3ml Orcinol reagent were added incubated 37^{o}c for 1 hour. Read colour density against blank. The blank consists of 2ml distilled water, 1ml 5% TCA and 3ml Orcinol reagent.

Result and discussion

In the present study, DNA content in hepatopancreas muscle and chelate leg was decreased at first from 24 hrs to 48 hrs exposures and slightly increased at 72 hrs exposures and then declined in 120 hrs and in gill and heart DNA content decreased from 24 hrs exposures to 120 hrs. In mercuric sulphate exposed crab in muscle and chelate legs DNA was decreased at 24 hrs, 72 hrs, 96 hrs and 120 hrs. (table 1a, 2b). cadmium sulphate exposed crab *Scylla serrata* RNA content in hepatopancreas, muscle, chelate legs gill and heart was decreased at 24 hrs, 72 hrs, 96 hrs and 120 hrs and slightly increased at 48 hrs. In mercuric sulphate exposed crab in muscle and chelate legs RNA was decreased at 24 hrs, 72 hrs, 96 hrs and 120 hrs where as slightly increased at 48 hrs where as in hepatopancreas, gill and heart RNA decreased from 24 hrs .to 120 hrs. (table 2a,2b)

Table1a**: Deoxyribonucleic Acid (DNA) in different tissue of crab Scylla serrata exposed Cadmium Sulphate**

Hours	**Hepatopancreas**	**Muscle**	**Chelate leg**	**Gill**	**Heart**
Control	0.5823 ±0.09	0.568 ±0.12	0.544±0.09	0.343 ±0.11	0.245 ±0.13
24	0.5450 ± 0.04	0.539 ± 0.03	0.532±0.07	0.284±0.02	0.214 ±0.06
48	0.439 ± 0.02	0.525 ± 0.1	0.525 ± 0.12	0.269 ±0.07	0.199 ±0.07
72	0.492 ±0.03	0.410 ± 0.03	0.492 ±0.03	0.235 ±0.22	0.164 ±0.09
96	0.368 ± 0.07	0.373 ± 0.04	0.372 ± 0.04	0.194 ± 0.09	0.088 ±0.02
120	0.313 ± 0.01	0.412 ±0.11	0.286 ± 0.02	0.135 ± 0.04	0.110 ± 0.09

- Values are means ±SD of six individual observation, p>0.05, P< 0.01 p.0.01 significant when student's test was applied between control and experimental groups.

Table 1b**: Deoxyribonucleic Acid (DNA) in different tissue of crab Scylla serrata exposed Mercuric Sulphate**

Hours	**Hepatopancreas**	**Muscle**	**Chelate leg**	**Gill**	**Heart**
Control	0.582 ±0.09	0.568 ±0.12	0.544±0.09	0.343 ±0.11	0.245 ±0.13
24	0.512	0.5	0.501±0.	0.269	0.190 ±0.04

	±0.05	137±0.02	08	±0.03	
48	0.400 ±0.04	0.455±0.04	0.473±0.08	0.258 ±0.03	0.123 ±0.03
72	0.483 ±0.04	0.494±0.07	0.360 ±0.05	0.194 ±0.05	0.115 ±0.05
96	0.345 ±0.07	0.358±0.11	0.276±0.11	0.153 ±0.11	0.088 ±0.02
120	0.3018 ±0.09	0.2720	0.196 ±0.02	0.0850 ±0.09	0.076 ±0.09

		±0.12			

- Values are means ±SD of six individual observation, p>0.05, p < 0.01, p. 0.01 significant when student's test was applied between control and experimental groups.

Table za**: Ribonucleic Acid (RNA) in different tissue of crab Scylla serrata exposed Cadmium Sulphate**

Hours	**Hepatopancreas**	**Muscle**	**Chelate leg**	**Gill**	**Heart**
Control	2.371 ± 0.06	2.161 ± 0.4	2.114 ± 0,04	2.216 ± 0.7	0.961 ± 0.04
24	1.999 ± 0.06	1.657 ±0.1	1.962 ± 0.02	1.809 ±0.06	0.762 ± 0.2
48	2.172 ± 0.03	2.076 ±0.02	1.676 ± 0.02	2.142 ± 0.03	0.837 ± 0.08
72	1.609 ± 0.07	1.523 ±	1.828 ± 0.05	1.562 ± 0.07	0.685 ± 0.03

		0.1			
96	1.581 ± 0.05	1.489 ± 0.09	1.600 ± 0.06	1.457 ± 0.03	0.427 ± 0.03
120	1.514 ± 0.02	1.308 ± 0.10	1.447 ± 0.02	1.278 ± 0.03	0.294 ± 0.02

- Values are means ±SD of six individual observation, p>0.05, p < 0.01, p. 0.01 significant when student's test was applied between control and experimental groups.

Table 2b: **Ribonucleic Acid (RNA) in different tissue of crab Scylla serrata exposed Mercuric Sulphate**

Hours	**Hepatopancreas**	**Muscle**	**Chelate leg**	**Gill**	**Heart**
Control	2.371 ± 0.06	2.161 ± 0.42	2.114 ± 0.06	2.126 ± 0.07	0.961 ± 0.04
4	2.705 ± 0.04	1.666 ± 0.1	1.623 ± 0.08	1.695 0.04	0.705 ± 0.04
8	2.095 ± 0.04	2.018 ± 0.02	1.581 ± 0.04	1.990 ± 0.05	0.742 ± 0.08
72	2.018 ±	2.18	1.39	1.54	0.60

	0.03	1 ± 0.01	0 ± 0.04	7 ± 0.03	9 ± 0.05
96	1.466 ± 0.02	1.085 ± 0.06	1.295 ± 0.08	1.220 ± 0.08	0.399 ± 0.03
120	1.457 ± 0.02	1.028 ± 0.05	1.080 ± 0.1	1.174 ± 0.08	0.232 ± 0.01

- Values are means ±SD of six individual observation, p>0.05, p< 0.01, p. 0.01 significant when student's test was applied between control and experimental groups.

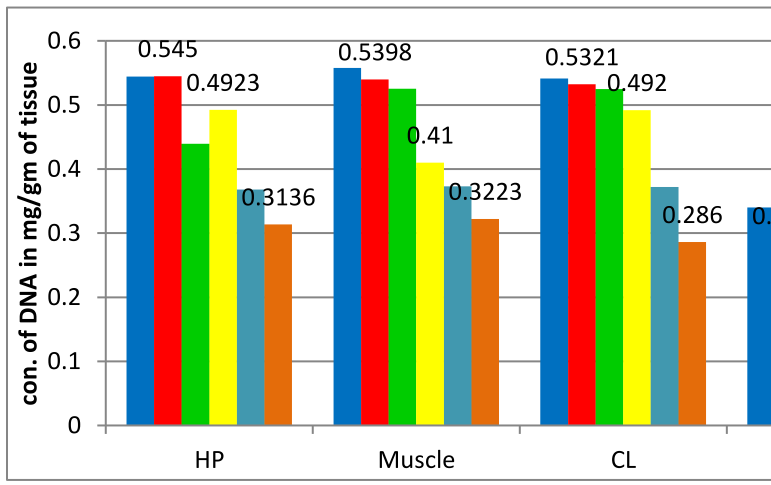

Figure 1a:- DNA content in different tissue of crab Scylla serrata exposed Cadmium Sulphate

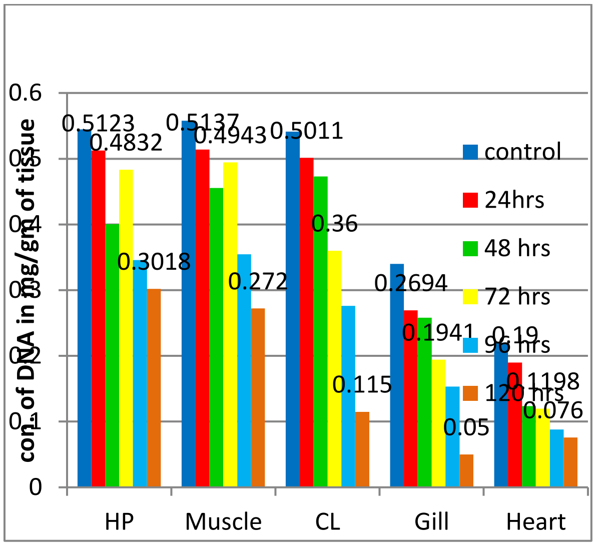

Fig. 1b- DNA content in different tissue of crab Scylla serrata exposed Mercuric Sulphate

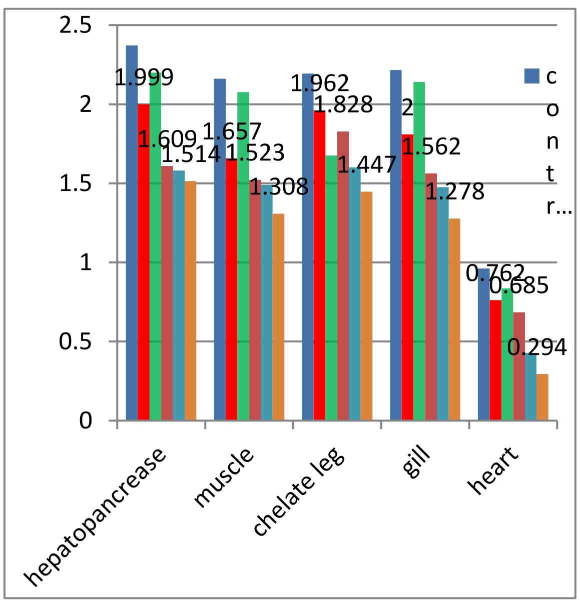

Figure 2a:-RNA content in different tissue of crab Scylla serrata exposed to Cadmium Sulphate

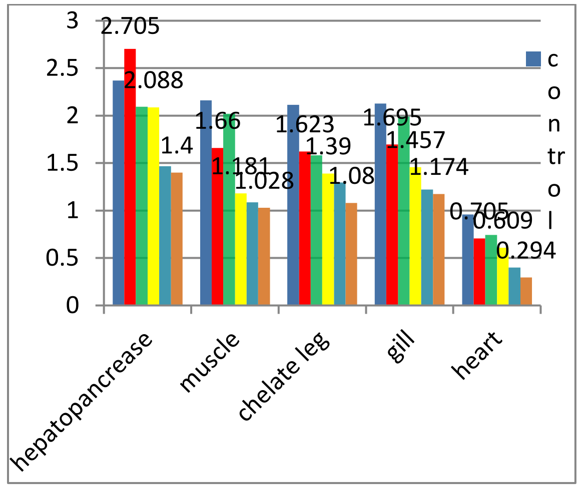

Figure 2b:-RNA content in different tissue of crab Scylla serrata exposed to Mercuric Sulphate

In fish, stress response can be considered an early pollutants induced event that may elicit forms of cellular damage such as different types of DNA damage adduct formation, strand breaks, changes in composition of DNA minor base, increase in the level of DNA repair, oxidative DNA damage and apoptosis, and in particular oxidative DNA damage, which is often used as an indicator of the effects of pollutant since co toxicological studies (Vijayavel and Balasubramanian, 2008; Holden 2000). A wide literature establishes the positive correlation between site contamination and DNA damage, confirming in particular that Comet and Micronuclei assay was useful tools in determining the potential genotoxicity of water pollutants in monitoring programs, both in controlled and natural conditions (Buschini *et al.*, 2004; Matsumoto *et al*., 2006; Nwani *et al.*, 2010; Rocha *et al.*, 2009; Russo *et al.*,2004; Steinert et al., 1998). Furthermore, pollution modulates the expression of some stressrelated proteins, such as metallothionein (MTs) and heat shock proteins (HSPs) (Padmini and Usha Rani, 2008; Wang *et al.*, 2007; Webb and Gagnon, 2009). MTs are cytosolic and or nuclear cysteine-rich proteins, selectively linking their cysteine residues to Cu^{+2} and Zn^{+2} and other toxic metals (Hellou, 2011). Thus, these proteins, involved in the mechanisms of general responses to stress as well as in the tolerance and the detoxification of heavy metals, have been proposed as a sensitive.

Tong Lu *et al.,* (2001) observed that approximately 60 genes (10%) were differently expressed in arsenic exposed human livers compared to control. The differentially exposed genes induced those involved in cell cycle regulation, apoptosis, DNA damage, response and intermediate filaments.

Tripathi and Verma (2000) reported that RNA and protein content was decline by 26-30%. However the decrease in DNA content was only 10% in skeletal muscle if catfish, *Clarius batrachus* exposure to endosulfan. Andhale and zambare (2011) showed that RNA polymate was binds to binding site especially to its DNA template of nucleotide and primer substrates from a new phosphodiester bond and elongates a growing RNA. RNA content was decreased due to acute exposure of mercury and arsenic.

Melathion pesticide disrupt DNA synthesis affected RNA synthesis and consequently protein synthesis (Tripathi and Verma, 2004). Gautam et al., (2002) reported the histo-chemical observations in nucleic acids (RNA and DNA) in the stomach and intestine of *Channa punctatus* (Bloch) after the treatment with endosulfan and diazinon pesticides. Vijayavel and Balasubramanian (2006) observed total protein, RNA and DNA decreased in edible crab *Scylla serrata* exposed to naphthalene.

Rathod and Kshisagar (2010) studied effect of sublethal concentration of two different kinds of pesticides fenvalerate (Synthetic pyrethroid) and monocrotophos (Organophosphate) on fresh water fish *Puctius arenatus*. They observed DNA and protein content in selected tissue like gills, liver, kidney and muscle decreased.. Tilak *et al*., (2005) mentioned reduction in RNA content in fresh water fish *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* exposed to chloropyrifors in different tissues of fishes. Thenmozhi *et al*., (2011) showed significant decrease in nucleic acid content in liver, muscle and gill in fresh water fish Labeo rohita exposed to sublethal concentration of melathion.

Muley *et al.* (2000) found significant alterations in the DNA and RNA contents in gills, liver and brain of the common carp, *Cyprinus carpio* exposed to cadmium chloride and lead acetate, respectively. Both heavy metals decreased DNA content in all tissues. Cd and Pb toxicity decreased RNA content in liver and brain and increased it in gills. Krishnaja *et al*., (1987) examined the toxic effects of mercuric chloride, phenyl mercuric acetate, cadmium chloride, selenium dioxide, arsenic trioxide and lead nitrate on *Scylla serrata*, the common edible Indian marine crab. More *et al.,* (2003) reported depletion of DNA in the bivalve, *Lamellidan marginalis* exposed to heavy metals.

Conclusion

Nucleic acids are polymer of nucleotides. It play important role in biological activities and regulate the protein synthesis any variation in nucleic acid content alter protein synthesis. Nucleic acid (DNA, RNA) content in vital organs like muscle, Hepatopancreas gill and heart the heavy metal inhibit protein synthesis which affect on nucleic ac.id DNA synthesis is inhibited and reduced DNA and RNA content in crab *Scylla serrata* exposed to heavy metals cadmium sulphate and mercuric sulphate

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