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Effect of copper sulphate and pod extract of *Acacia sinuata* on biochemical constituent in freshwater snail *Bellamya bengalensis*

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Abstract
Biochemical constituents (glycogen, protein and lipid) from animal body play vital role in its development. The quantity of these constituents is varied in accordance with exogenous and endogenous factors. In the present study, *B. bengalensis* was acutely (Mean LC_{50} concentrations of heavy metal of 0.56 ppm and pod extract 232 ppm) intoxicated with copper sulphate and *A. sinuata.* During exposure, the glycogen, protein and lipid concentration in different digestive organs were declined as per exposure period i.e. 24hrs, 48hrs, 72hrs and 96hrs. The targeted digestive organs of snail such as, salivary gland, oesophagus, intestine, stomach and hepatopancreas were subjected for biochemical determination. The maximum decline biochemical constituents were noted after 96 hours intoxication of copper sulphate and *A. sinuata* in digestive tract. The obtained results of biochemical revealed that, the stored nutrition found to be decreased with increase in the exposure period. Biochemical constituents from digestive tract found directly proportional to the time of exposure period. The decreased concentration and their nutritional values were discussed in relation to the physiology of digestion.

Keywords: Biochemical constituents, Digestive organs, Copper sulphate, *Acacia sinuata,* Freshwater snail *Bellamya bengalensis.*

1. Introduction
In nature heavy metals like iron, cobalt, copper manganese, molybdenum and zinc are beneficial when in stress amount, however in excessive quantity might be cause severe damage to the cellular organization. On the other hand, the heavy metal such as mercury, platanium and lead are more toxic even in least amount. In addition, the successive accumulation of heavy metals in the bodies can cause very serious illness to the living creatures. Comparatively, the effect of heavy metals on all facets of biological organization or system is more prominent in invertebrate group. Among invertebrate, especially molluscan species are adversely affected by toxic heavy metals. Molluscan species (bivalves and `snails) are benthic, sessile and filter feeders inhabiting. In addition molluscan species are pollution indicator, abundantly and easily available. Hence, several investigators have been targeted the molluscan species for conducting the toxicity experiments.
According to literature, the heavy metals directly affected to the tissue and may interact with cell membrane [1]. It is also known that, heavy metal affects the biologically active molecules such as amino acids, co-enzymes and to other binding ions such as sulphur, phosphate, etc. [2]. Higher concentrations of toxicants in aquatic environment cause adverse effect on the aquatic organisms at cellular or molecular level and ultimately disturbed proximate biochemical composition of the organism.
Molluscicide, which plant originated has beneficial valves such as it treated as disinfectant, algal control, regulate molluscan species besides, with its medicinal important in recovering of several diseases. According to literatures, the non-oxidizing molluscicides were originally developed as bacterial disinfectant and also for control of algae from aquatic systems [3]. The plant products often provide better control of adult mussels, due to the inability of mussels to detect chemical constituents as toxicants [4]. While some of plant products were biodegradable, many require detoxification or deactivation to meet state and federal discharge requirements. In recent past, literatures reported that, the plants from a variety of families have shown to possess many classes of products, which has molluscicidal activity from last 20 years [5, 6].
Biochemical constituents like carbohydrates, fats, proteins, vitamins, minerals and water, which provide energy for maintaining tissues architecture in the body. Carbohydrate, protein

and lipid are major important organic elements of different biochemical reactions as well as body metabolism. Proteins plays vital role in animals because, animal cannot survive for long time without energy, so it protein occupies unique position in metabolism because it serves as rich source of energy for the metabolism. Lipid metabolism was involved with Fatty Acid Oxidation to produce energy or in the synthesis of lipids (lipogenesis).

From reviews of literatures, it come to notice that, there is scanty information on comparative effects of heavy metal and molluscicide on major biochemical constituents in freshwater snail *B. bengalensis* particularly in digestive organs. The present work derived to investigate biochemical alterations in digestive tract of *B. bengalensis* after exposed to heavy metals and pod extract

2. Materials and Methods

2.1. Animal collection and maintenance

For present investigation, the freshwater snail *Bellamya bengalensis* (L) was collected from Rajaram tank near Shivaji University, Kolhapur, during period of January to March 2011. The collected population samples were brought to the laboratory, washed and kept for acclimatization. Mature acclimatized healthy snails were exposed to heavy metal (copper sulphate) and molluscicide (pod extract of *Acacia sinuata*) for different exposure periods i.e. 24, 48, 72 and 96 hrs.

2.2. Intoxicants

Two toxicants were selected for this study i.e. one is heavy metal like copper sulphate and other is pod extract of *Acacia sinuata*. For the toxicological analysis, different concentration of pod extract was used against experimental snail *B. bengalensis* using static bioassay method.

2.3. Experimental Design

Experimental animals were exposed to pre-determined mean LC_{50} concentration of heavy metal copper sulphate ($CuSO_4.5H_2O$) at 0.56 ppm and pod extract of *Acacia sinuata* toxicity at 232 ppm to different exposure periods at 24 hrs, 48hrs, 72 hrs and 96 hrs respectively. Treated animals were used for the biochemical analysis. Control group for both was run simultaneously and for biochemical analysis digestive organs such as salivary gland, oesophagus, intestine, stomach and hepatopancreas were selected and processed for as glycogen, lipid and protein estimation by applying following methods: Anthrone method [7], Lowry's method [8] and Folch's method [9].

2.4. Statistical analysis

All results of the biochemical analysis are given as the mean of three readings with ± standard deviation (SD). In the statistical analysis, the one-way analysis of variance (ANOVA) was used to test the any significant difference of biochemical constituents between the experimental and control group after exposure to both heavy metal and molluscicide.

3. Results and Discussion

Digestive system in any animal plays important role to run metabolic processes for complete digestion. Any toxicant, if entered into the cell can disturb the rate of metabolism. In the present study, after intoxication of copper sulphate and pod extract of *A. sinuata*, the biochemical constituents were analyzed at 24, 48, 72 and 96 hours interval.

4. Glycogen

4.1. Glycogen content from control group

In control group, glycogen content was ranged from 3.80 to 7.53 mg/100 mg glycogen in all selected digestive tissues like salivary gland, oesophagus, intestine, stomach and hepatopancreas. The normal glycogen content in selected tissues was plenty which get utilized during the metabolic processes to run normal functions as per requirement.

4.2. Effect of copper sulphate on glycogen content

After exposure to $CuSO_4$, a significant ($p<0.001$) decrease in glycogen content was recorded at 24, 48, 72 and 96 hrs of exposure from selected digestive tissues (salivary gland, oesophagus, intestine, stomach and hepatopancreas). During exposure period, substantially decline in glycogen content have been recorded at 96 hrs of exposure in all tissues. Relatively, maximum decline in glycogen content was recorded in intestine tissue (0.26 mg/100 mg glycogen) followed by oesophagus (0.43 mg/100 mg glycogen), hepatopancreas (1.22 mg/100 mg glycogen), salivary gland (1.29 mg/100 mg glycogen) and stomach (1.43 mg/100 mg glycogen) respectively. (Table 1).

4.3. Effect of pod extract of *A. sinuata* on glycogen content

After intoxication to pod extract of *A. sinuata*, the glycogen content of snail, *B. bengalensis* also decline progressively from 24 to 96 hrs of exposure period. A highest decline in glycogen content was noted at 96 hrs exposure period in all tissues, the intestine tissue showed more loss in glycogen content (0.78 mg/100 mg glycogen) than oesophagus, hepatopancreas, stomach and salivary gland (0.77, 1.73, 1.48 and 1.45 mg/100 mg glycogen) respectively. (Table 2).

Carbohydrate, protein and lipid are vital biochemical constituents in the animal body. It serves as a crucial role in development and survivality of the animals. In the present study, control group of snails showed high amount of glycogen, protein and lipid in their digestive tract. After intoxication of metal and molluscicide, initially metabolism was increased up to 24 hrs; where it was decreased after 48, 72 up to 96 hrs of exposure. Biochemical changes give important indication related to mechanism of action of metals on the cells. Metals may interact with cell membrane [1] and with intracellular organelles and nuclei [10, 11]. Biochemical constituents like glycogen, protein and lipid are considered as sensitive indicators of metabolic activates. Tissue carbohydrate in the form of glucose and glycogen serves as important source of energy for body activities [12]. According to Bayne (1973) [13], irregular catabolism of stored carbohydrate and protein metabolism were specifically related stress syndrome in molluscs. Glycogen, protein and lipid as biochemical content in control animal get utilized to maintain the normal metabolic reactions. Numbers of physiological processes were linked with body maintenance, growth, reproduction, etc. Assessment of metabolic rate during induced toxicity can provide information regarding the biomechanics in the targeted cells [14].

In the present study, the biochemical constituents from selected digestive organs like salivary gland, oesophagus, intestine, stomach and hepatopancreas of snail *B. bengalensis* was significantly altered with proportion to time of intoxication of copper sulphate and *A. sinuata* up to 96 hrs. Intoxication of copper sulphate after 96 hrs, the glycogen level has been reduced as 94% in intestine, 88% in oesophagus, 83% in hepatopancreas, 74% in salivary gland and 73% reduction in stomach (Table 1). On the other hand, after

intoxication of pod extract of *A. sinuata*, intestine showed 82%, oesophagus 80%, hepatopancreas 77%, stomach 73% salivary gland 72% reduction in glycogen level respectively (Table 2). After intoxication of copper sulphate and *A. sinuata* particularly intestine showed major reduction in glycogen level. Similarly, Sarojini *et al.*, (1990a, b) [15, 16] also reported decreased glycogen level in *B. gurerini* when exposed to cadmium chloride and zinc sulphate with respect to exposure interval. Ready *et al*., (1986) [17] studied the effect of mercuric chloride on carbohydrate metabolism of freshwater mussel *Parresysia rugosa* where they reported depletion in glycogen content of selected tissues.

Vutukuru, (2005) [18] reported that, the decreased glycogen concentration of the tissues of animal can be due to its increased utilization as an immediate source to meet energy demands under metallic stress. It could also be due to the prevalence of hypoxic or anoxic conditions, which normally enhanced glycogen utilization [7].

5. Protein

5.1. Protein content from control group

In control group of snail, *B. bengalensis* the protein content was fluctuated from 5.10 to 16.27 mg/100 mg protein in all digestive tissues. Highest protein content was recorded in hepatopancreas tissues than the rest of tissues of control group. (Table 3).

5.2. Effect of Copper sulphate on protein content

Considerably, alterations in protein content of digestive organs of *B. bengalensis* were recorded after intoxication of copper sulphate. During intoxication, the protein content of different selected digestive organs was decreased as increase in the intoxication period. Comparatively, maximum decrease in protein content was observed in salivary gland (0.90 mg/100 mg protein) followed by oesophagus (1.20 mg/100 mg protein), intestine (3.36 mg/100 mg protein), stomach (5.73 mg/100 mg protein) and hepatopancreas (7.13 mg/100 mg protein) at 96 hours of exposure period respectively. (Table 3).

5.3. Effect of pod extract of *A. sinuata* on protein content

The pod extract of *A. sinuata* also induced significant decline in protein content from various selected digestive tissues. Considerably, loss in protein content was recorded in all tissues at 96 hrs of exposure period. A maximum loss in protein content was noticed in oesophagus tissue (1.24 mg/100 mg protein) after 96 hrs intoxication of pod extract. Similarly, salivary gland (1.95 mg/100 mg protein), stomach (8.44 mg/100 mg protein), intestine (5.17 mg/100 mg protein) and hepatopancreas (10.04 mg/100 mg protein) showed highest decline in protein content at 96 hrs of intoxication respectively. (Table 4).

Protein synthesis can be disturbed by a variety of mechanisms either by affecting the nucleic acid metabolism or structure or in the protein forming system itself. In the present study, copper induced snail showed 83% of decreased protein from salivary gland, 76% reduction in oesophagus, while 60% loss of protein content was recorded after 96 hrs of exposure respectively. However, stomach and hepatopancreas showed 59% and 56% decreased in protein concentration of total protein respectively after 96 hrs of exposure to copper sulphate (Table 3). Intoxication of pod of *A. sinuata*, snail showed maximum reduction in protein from oesophagus (76%) followed by salivary gland has (64%). Intestine showed 38% reduction. Hepatopancreas and stomach showed 38% and 41% protein depletion after 96 hrs of exposure (Table 4). Protein content was decreased from selected organs after exposure to metal and pesticide toxicity, Analogous observations were reported in freshwater crab *Uca Marinis* against pesticides Malathion [19]. Whereas, Shanmugam and Venkateshwarlu (2000), [20] also recorded similar results when *B. cunicularis* exposed to endosulphon.

Depletion protein content confers idea about proteolysis and possible utilization of the products for metabolic purpose. They may be mobilised into TCA cycle through amino acid metabolism system to cope up with the excess demand of energy during toxic stress conditions. The fall in protein level during intoxication may be due to increased catabolism and decreased anabolism of protein. The higher depletion of protein in the digestive organs might be due to increased metabolic potency and efficiency of the gland under stressed condition. Mahajan and Zambare (2001) [21] showed the depletion of the protein content in different tissues such as gonad, gill and hepatopancreas of bivalve *Corbicula strialetta*, after exposure to $HgCl_2$ and $CuSO_4$. The acute and chronic exposure to tetracycline and chloramphenicol was recorded decreased protein level in *L. Corrians* with the period of exposure [22].

6. Lipid

6.1. Lipid content from control group

Control group of snail *B. bengalensis* shows marked variation in lipid content from different tissues of digestive tract. In different digestive tissues, the lipid content was ranged from 49.57 to 96.9 mg/100mg lipid. Comparatively, in stomach and intestine acquired maximum lipid value, whereas in salivary gland noted minimum lipid content.

6.2. Effect of Copper sulphate on lipid content

Lipid concentration also decreased significantly from 24 to 96 hrs when exposed to $CuSo_4$ from digestive tract. Relatively, highest decline in lipid content was observed at 96 hrs of intoxication. Salivary gland shows marked decline in lipid content (9.37 mg/100mg lipid) than oesophagus (13.33 mg/100mg lipid), intestine (19.13 mg/100mg lipid), stomach (34.13 mg/100mg lipid) and hepatopancreas (26.70 mg/100mg lipid) at 96 hrs of intoxication respectively. (Table 5).

6.3. Effect of *A. sinuata* on lipid content

After intoxication of pod extract of *A. sinuata*, the maximum loss in lipid content was recorded in salivary gland (15.20 mg/100mg lipid) at 96 hrs followed by rest of digestive tissues such as oesophagus (20.57 mg/100mg lipid), stomach (48.17 mg/100mg lipid), intestine (36.67 mg/100mg lipid) and hepatopancreas (38.97 mg/100mg lipid) correspondingly. (Table 6).

Relatively, at the both intoxication of heavy metal like copper sulphate and molluscicide i.e. pod extract of *A. sinuata*, showed adverse impact on biochemical constituents of digestive organs of snail *B. bengalensis*. Consequently, the quantity of biochemical or organic molecules (glycogen, protein and lipid) of digestive organs was significantly and successively declined from 24 to 96 hours of intoxication period.

Christensen *et al.,* (1977) [23] recorded that the biochemical changes were dependent on the dose response relationship, threshold limit value and reversible or irreversible effects of toxicants. Comparatively, lipid also play vital role in the metabolism, hence after copper sulphate toxicity study, the

lipid content also declined significantly from digestive organs. After 96 hrs of intoxication, the salivary gland showed 81% depletion in lipid content. Oesophagus and intestine showed 76% decreased lipid content. Stomach 68% and hepatopancreas 64% decline was recorded (Table No.5). Due to intoxication of *A. sinuate*, in salivary gland 69% decrease in lipid level was recorded, similarly oesophagus showed 64%, intestine 54%, stomach 55% and hepatopancreas 49% reduction in lipid content of experimental snail after 96 hrs exposures (Table No. 6). Comparable, results were recorded by Patil *et al.,* (2003), [24] found significant decrease in protein, glycogen and cholesterol due to leads toxicity in hepatopancreas and gills of Rock oyster.

In this study, among all digestive organs, the biochemical constituents were prominently declined more or less after intoxication of heavy metal i.e. copper sulphate than pod extract of *A. sinuata*. Among biochemical constituents, the glycogen content was significantly declined from intestine tissue (94% and 82%) when exposed to copper sulphate and molluscicide *A. sinuata*. The protein content was highly reduced in salivary gland (83%) after exposure of copper sulphate, while 76% reduction of protein content has been recorded in oesophagus when exposed to *A. sinuata*. As per as concern the lipid concentration, the maximum decline of lipid was observed in salivary gland (81% and 69%) to both exposures i.e. copper sulphate and *A. sinuata*. (Table 1-6).

Table 1: Alteration in the glycogen content in mg/100mg wet tissue of different digestive tissues of freshwater snail *Bellamya bengalensis* after to copper sulphate induction.

Tissue	Control	Exposure periods			
		24 hrs.	48hrs.	72hrs.	96hrs.
Salivary gland	5.10±0.22	2.95±0.15 ***	2.00±0.50 ***	1.78±0.46 ***	1.29±0.18 *** (74%)
Oesophagus	3.80±0.20	1.57±0.28 ***	1.37±0.15 ***	0.90±0.13 ***	0.43±0.31 *** (88%)
Intestine	4.25±0.02	1.43±0.35 ***	1.28±0.26 ***	0.90±0.10 ***	0.26±0.02 *** (94%)
Stomach	5.40±0.10	3.23±0.86 ***	3.12±0.13 ***	2.25±0.23 ***	1.43±0.11 *** (73%)
Hepatopancreas	7.53±0.15	3.02±0.18 ***	2.42±0.52 ***	1.72±0.10 ***	1.22±0.02 *** (83%)

All the values are mean of one observations ± Standard deviation, P<0.001=***. Value in parenthesis is indicates the total percentage of decline glycogen content.

Table 2: Alteration in the glycogen content in mg/100mg wet tissue of different digestive tissues of freshwater snail *Bellamya bengalensis* after pod extract of *Acacia sinuata* induction.

Tissue	Control	Exposure periods			
		24 hrs.	48hrs.	72hrs.	96hrs.
Salivary gland	5.10±0.22	5.06±0.05 NS	4.80±0.20 NS	1.84±0.29 ***	1.45±0.30 *** (72%)
Oesophagus	3.80±0.20	2.93±0.16 *	1.77±0.45 ***	0.90±0.18 ***	0.77±0.25 *** (80%)
Intestine	4.25±0.02	3.25±0.22 ***	2.13±0.13 ***	1.06±0.05 ***	0.78±0.55 *** (82%)
Stomach	5.40±0.10	4.38±0.10 **	3.13±0.03 ***	2.80±0.48 ***	1.48±0.24 *** (73%)
Hepatopancreas	7.53±0.15	3.24±0.02 ***	2.75±0.05 ***	2.21±0.04 ***	1.73±0.44 *** (77%)

All the values are mean of one observations ± Standard deviation, P<0.001=***. Value in parenthesis is indicates the total percentage of decline glycogen content.

Table 3: Alteration in the protein content in mg/100mg wet tissue of different digestive tissues of freshwater snail *Bellamya bengalensis* after Copper sulphate induction.

Tissue	Control	Exposure periods			
		24 hrs.	48hrs.	72hrs.	96hrs.
Salivary gland	5.37±0.15	3.91±0.08 ***	3.00±0.10 ***	2.37±0.23 ***	0.90±0.10 *** (83%)
Oesophagus	5.10±0.56	4.23±0.25 NS	3.23±0.25 **	2.40±0.05 ***	1.20±0.30 *** (76%)
Intestine	8.37±0.15	7.23±0.31 ***	6.73±0.15 ***	5.03±0.06 ***	3.36±0.30 *** (60%)
Stomach	14.20±0.20	13.80±0.26 NS	10.30±0.20 ***	8.33±0.28 ***	5.73±0.20 *** (59%)
Hepatopancreas	16.27±0.25	13.89±0.50 NS	11.46±0.45 ***	10.70±2.02 ***	7.13±0.15 *** (56%)

All the values are mean of three observations ± Standard deviation, P<0.001=***, P<0.01=**, P>0.05= NS. Value in parenthesis is indicates the total percentage of decline protein content.

Table 4: Alteration in the protein content in mg/100mg wet tissue of different digestive tissues of freshwater snail *Bellamya bengalensis* after pod extract of *Acacia sinuata* induction.

Tissue	Control	Exposure periods			
		24 hrs.	48hrs.	72hrs.	96hrs.
Salivary gland	5.37±0.15	3.95±0.70 *	3.63±0.15 **	2.63±0.55 ***	1.95±0.75 *** (64%)
Oesophagus	5.10±0.56	4.80±0.20 NS	3.68±0.36 **	2.53±0.55 ***	1.24±0.22 *** (76%)
Intestine	8.37±0.15	8.30±0.36 NS	7.41±0.08 **	6.12±0.10 ***	5.17±0.29 *** (38%)
Stomach	14.20±0.20	14.08±0.13 NS	11.35±0.31 ***	10.08±0.07 ***	8.44±0.70 *** (41%)
Hepatopancreas	16.27±0.25	15.66±0.57 NS	13.22±0.26 ***	12.33±0.15 ***	10.04±0.06 *** (38%)

All the values are mean of three observations ± Standard deviation, P<0.001=***, P<0.01=**, P<0.05=*, P>0.05= NS. Value in parenthesis is indicates the total percentage of decline protein content.

Table 5: Alteration in the lipid content in mg/100mg wet tissue of different digestive tissues of freshwater snail *Bellamya bengalensis* after Copper sulphate induction.

Tissue	Control	Exposure periods			
		24 hrs.	48hrs.	72hrs.	96hrs.
Salivary gland	49.57±0.38	32.50±0.26 ***	25.90±0.82 ***	20.83±0.06 ***	9.37±0.15 *** (81%)
Oesophagus	56.73±0.54	49.57±0.21 ***	37.87±0.71 ***	24.52±0.20 ***	13.33±0.45 *** (76%)
Intestine	80.63±0.55	40.23±0.67 ***	30.27±0.23 ***	26.43±0.21 ***	19.13±0.32 *** (76%)
Stomach	96.9±0.35	56.17±0.76 ***	45.67±0.12 ***	44.03±3.44 ***	34.13±0.06 *** (68%)
Hepatopancreas	76.00±1.00	53.33±1.53 ***	43.57±0.32 ***	38.23±0.21 ***	26.70±0.26 *** (64%)

All the values are mean of three observations ± Standard deviation, P<0.001=***. Value in parenthesis is indicates the total percentage of decline lipid content.

Table 6: Alteration in the lipid content in mg/100mg wet tissue of different digestive tissues of freshwater snail *Bellamya bengalensis* after pod extract of *Acacia sinuata* induction.

Tissue	Control	Exposure periods			
		24 hrs.	48hrs.	72hrs.	96hrs.
Salivary gland	49.57±0.38	42.87±0.69 ***	28.80±0.22 ***	24.43±0.31 ***	15.20±0.16 *** (69%)
Oesophagus	56.73±0.54	54.47±0.46 **	42.67±0.62 ***	34.00±0.16 ***	20.57±0.40 *** (64%)
Intestine	80.63±0.55	75.80±2.69 *	62.00±0.41 ***	46.73±0.61 ***	36.67±1.25 *** (54%)
Stomach	96.9±0.35	94.60±0.43 ***	84.80±0.62 ***	65.53±0.68 ***	48.17±0.12 *** (55%)
Hepatopancreas	76.00±1.00	72.40±0.43 ***	68.53±0.34 ***	51.43±0.42 ***	38.97±0.78 *** (49%)

All the values are mean of three observations ± Standard deviation, P<0.001=***, P<0.01=**, P<0.05=*. Value in parenthesis is indicates the total percentage of decline lipid content.

In this study, biochemical contents were significantly decreased in selected digestive organs of freshwater snail *Bellamya bengalensis* after intoxication of copper sulphate and *A. sinuata*. Similarly, Rao and Jayashree, (1990) [25] reported the depletion in level of total glycogen, lipid and protein from selected foot, mantle and digestive gland of the adult *Bellamya dissimills* when intoxicated against LC_{50} concentrations of copper sulphate and zinc sulphate after 96 hrs of exposure.

Magare and Kulkarni (1994) [26] studied the effect of pH on biochemical content like glycogen, protein and lipids in snail *Cerastus moussonianus* and noticed that during acidic pH condition, various biochemical contents were utilized, while in alkaline pH, they were stored. Rao and Ramamurthi (1980) [27] have studied the effect of sub lethal concentration of sumithion on some biochemical constituents of the snail, *Pila globosa*. They have shown that, the sub-lethal concentration of sumithion, leads to decrease the concentration of glycogen and protein in the tissue. Kamble, (2007) [28], noted that, carbohydrates, protein and lipids play important role in metabolic activities, in such context this organic constituents were provide the energy. Awati, (2004) [29] reported decreased concentration of glycogen, protein and lipid in the gills and gonads of *Viviparous bengalensis* due to synthetic and natural molluscicide intoxication.

Varadraj *et al*., (1994) [30] noticed that glycogen, protein and lipids were reduced in liver, gills and mantle of snail *Pila globosa* after treatment of tannery effluents. They found decreased free amino acids, total proteins, total glycogen and lipids in all the tissue of *Pila globosa*. Increase in acidic mucosubstances in all the vital organs showed decrease in glycogen, protein and lipids in order to overcome the chemical stress caused after heavy metals induction.

In the present investigation, toxicity effect of metal copper sulphate and pod extract of *Acacia sinuata* was assessed for biochemical alterations on the basic organic constituents as glycogen, protein and lipid. The results obtained were comparatively interpreted in which maximum glycogen reduction found in intestine due to exposure of both intoxicants (copper sulphate and *A. sinuata*). Similarly, major lipid depletion was in salivary gland of experimental snail. Comparatively copper sulphate induced salivary gland showed maximum reduction in the protein molecules from salivary gland, whereas at *A. sinuata* exposure maximum protein depletion found in oesophagus. In the experimental snail the lipid content was significantly decreased up to the 96 hrs of exposure, especially at copper sulphate induction than the induction of pod extract of *A. sinuata*. Results proved that copper sulphate is more toxic as that of *A. sinuata* against freshwater snail *B. bengalensis.*

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