1. Introduction

1.1 *Bellamya bengalensis*
   1.1.1 Systematic position of *B. bengalensis*
   1.1.2 Habitat, characteristic features and life history in brief

1.2 Detergent-Washing soda
   1.2.1 Chemistry
   1.2.2 Washing soda as aquatic contaminant

1.3 Immunological characteristics of molluscs in general

1.4 Hemolymph of *B. bengalensis*

1.5 Hemocytes of *B. bengalensis*
   1.5.1 Washing soda induced morphological damage in the different subpopulation of hemocytes

1.6 Functional attribute of hemocytes under the exposure of washing soda
   1.6.1 Non self adhesion
   1.6.2 Cell-cell aggregation

1.7 Phagocytic response of hemocytes under the exposure of washing soda

1.8 Cytotoxic response of hemocytes under the exposure of washing soda
   1.8.1 Superoxide anion generation and respiratory burst activity
   1.8.2 Nitric oxide generation
   1.8.3 Estimation of iNOS activity

1.9 Activity of phenoloxidase in hemocyte, gill, digestive gland and mantle under the exposure of washing soda

1.10 Biochemical adaptive response of hemocytes, gill, digestive gland and mantle under the exposure of washing soda
   1.10.1 Activity of antioxidant of *B. bengalensis* under the exposure of washing soda
   • Catalase
   • Superoxide dismutase
   1.10.2 Activity of detoxification of *B. bengalensis* under the exposure of washing soda
   • Glutathion S transferase

1.11 Activity of phosphatases in hemocytes under the exposure of washing soda
1.12 Activity of transaminase in the hemocyte, gill, digestive gland and mantle under the exposure of washing soda

1.13 Screening of hemocyte lysosomal membrane stability under the exposure of washing soda

1.14 Hemocyte apoptosis and necrosis under the exposure of washing soda

1.15 Histopathological analyses of *B. bengalensis* exposed to washing soda
   1.15.1 Gill
   1.15.2 Digestive gland
   1.15.3 Mantle

1.16 Study of selected behaviours of *B. bengalensis* under the washing soda exposure

2. Scope of the study

3. Review of literature

4. Materials and methods
   4.1 Collection, transportation, acclimation and laboratory maintenance of *B. bengalensis*

4.2 Detergent (Washing soda, sodium carbonate, anhydrous)
   4.2.1 Determination of LC$_{50}$
   4.2.2 Rationale of selecting the experimental concentration
   4.2.3 Experimental concentration
   4.2.4 Treatment methodology

4.3 Collection of hemolymph and isolation of hemocytes

4.4 Isolation of hemocytes

4.5 Screening of viability of hemocytes following the principle of vital dye exclusion in control and washing soda treated *B. bengalensis*

4.6 Preparation of hemocyte and tissue lysate

4.7 Morphological analyses of hemocyte under the washing soda exposure
   4.7.1 Phase contrast microscopy
   4.7.2 Bright field microscopy
   4.7.3 Scanning electron microscopy
4.8 Enumeration of hemocyte under the washing soda exposure
   4.8.1 Total hemocyte count
   4.8.2 Differential hemocyte count

4.9 Flow cytometry of hemocytes employing fluorescence activated cell sorter (FACS) under the washing soda exposure
   4.9.1 Determination of relative size (FSC) and complexity (SSC) and gate wise sorting of cells
   4.9.2 Determination of cell morphological analysis from sorted fractions of hemocytes of control and treated B. bengalensis

4.10 Estimation of no observed effect level (NOEL)(Parameter: Total hemocyte count)

4.11 Functional attributes of hemocytes under the washing soda exposure
   4.11.1 Estimation of nonself surface adhesion
   4.11.2 Estimation of cell cell aggregation

4.12 Estimation of phagocytic response under the washing soda exposure

4.13 Cytotoxic response of hemocytes under the washing soda exposure
   4.13.1 Estimation of generation of superoxide anion
   4.13.2 Estimation of nitric oxide generation
   4.13.3 Detection of iNOS activity

4.14 Activity of phenoloxidase in hemocytes, gill, digestive gland and mantle

4.15 Recovery of toxicity of washing soda

4.16 Biochemical adaptive response of hemocyte, gill, digestive gland and mantle under the washing soda exposure
   4.16.1 Antioxidant activity
      Estimation of activity of catalase
      Estimation of activity of superoxide dismutase
   4.16.2 Detoxification response
      Estimation of activity of glutathione-S-transferase

4.17 Metabolic interaction of hemocytes and washing soda
   4.17.1 Estimation of activity of acid phosphatase
   4.17.2 Estimation of activity of alkaline phosphatase
4.18 The activity of the transaminase in hemocyte, gill, digestive gland and mantle under the washing soda exposure

4.18.1 Estimation of activity of glutamate oxaloacetate transaminase
4.18.2 Estimation of activity of glutamate pyruvate transaminase

4.19 Screening of lysosomal membrane stability by neutral red retention assay under the washing soda exposure

4.20 Hemocyte apoptosis-necrosis assay under the washing soda exposure

4.20.1 Detection of apoptosis and necrosis employing FACS
4.20.2 Detection of hemocyte apoptosis and necrosis employing fluorescence microscopy

4.21 Histopathological analyses of gill, digestive gland and mantle of B. bengalensis

4.22 Estimation of total protein

4.23 Study of selected behaviours of B. bengalensis under the washing soda exposure

4.23.1 Relative mobility
4.23.2 Foot protrusion response
4.23.3 Clustering behavior

4.24 Statistical analyses

5. Result

5.1 LC$_{50}$ of washing soda

5.2 Screening of viability of hemocytes of B. bengalensis exposed to the experimental concentration of washing soda along with the control

5.3 Morphological analyses of hemocyte under the washing soda exposure

5.3.1 Phase contrast analysis
5.3.2 Bright field microscopic analysis
5.3.3 Ultrastructural analysis

5.4 Enumeration of hemocyte under the detergent exposure

5.4.1 Total hemocyte count
5.4.2 Differential hemocyte count

5.5 Screening of relative size (FSC) and granularity (SSC) of hemocytes and cell sorting employing FACS

5.5.1 Determination of relative size (FSC) and complexity (SSC)
5.5.2 Sorting of principal morphotypes of hemocytes and morphological identification
5.6 Estimation of no observed effect level (NOEL) with reference to total hemocyte count

5.7 Functional attributes of hemocytes under washing soda exposure
   5.7.1 Assay of nonself surface adhesion
   5.7.2 Assay of cell cell aggregation

5.8 Estimation of phagocytic response of hemocyte under the washing soda exposure

5.9 Cytotoxic response of hemocytes under the washing soda exposure
   5.9.1 Generation of superoxide anion
   5.9.2 Generation of nitric oxide
   5.9.3 Generation of iNOS

5.10 Activity of phenoloxidase in hemocytes gill, digestive gland and mantle
   5.10.1 Hemocytes
   5.10.2 Gill
   5.10.3 Digestive gland
   5.10.4 Mantle

5.11 Recovery response of toxicity of washing soda
   5.11.1 Total hemocyte count
   5.11.2 Differential hemocyte count
   5.11.3 Non self surface adhesion
   5.11.4 Cell cell aggregation
   5.11.5 Phagocytosis assay
   5.11.6 Estimation of nitric oxide generation
   5.11.7 Estimation of superoxide anion generation
   5.11.8 Estimation of phenoloxidase activity

5.12 Biochemical adaptive response in hemocyte, gill, digestive gland and mantle of *B. bengalensis* under the washing soda exposure
   5.12.1 Antioxidant activity
      Estimation of activity of catalase
      Hemocyte
      Gill
      Digestive gland
      Mantle
5.12.2 Detoxification response
Estimation of activity of glutathione-s-transferase
Hemocyte
Gill
Digestive gland
Mantle

5.13 Metabolic interaction of hemocytes and washing soda
5.13.1 Estimation of activity of acid phosphatase
5.13.2 Estimation of activity of alkaline phosphatase

5.14 Transaminase activity in hemocyte, gill, digestive gland and mantle of *B. bengalensis* under the washing soda exposure
5.14.1 Estimation of activity of glutamate oxaloacetate transaminase
5.14.2 Estimation of activity of glutamate pyruvate transaminase

5.15 Screening of hemocyte lysosomal membrane stability by neutral red retention assay under the exposure of washing soda

5.16 Hemocyte apoptosis-necrosis assay under the washing soda exposure
5.16.1 Detection of apoptosis and necrosis employing FACS
5.16.2 Detection of hemocyte apoptosis and necrosis employing fluorescence microscopy

5.17 Histopathological analyses of gill, digestive gland and mantle of *B. bengalensis* under the washing soda exposure

5.18 Study of selected behaviours of *B. bengalensis* under the washing soda exposure
5.18.1 Relative mobility
5.18.2 Foot protrusion response
5.18.3 Clustering behavior

6. Discussion
7. Conclusion
8. Bibliography
9. Publications