INTRODUCTION
Shigellosis or bloody diarrhea is an enteric disease accounting for 140 million episodes worldwide (WHO, 1993). It is known to be responsible for the death of 3 million children annually in developing countries, where the problem is most frequent (WHO 1997). Shigellosis has been reported to affect 50% of the cases of clinically severe bloody diarrhea (WHO, 1995). Among five year old children, about 10% of all diarrhea episodes are bloody in nature which account for 15% of diarrhea associated deaths in this age group. The incidence of bloody diarrhea has also been known to be associated with more complications, which are likely to affect the child’s growth leading to a higher fatality rate (WHO, 1995).

The disease is known to be endemic throughout the world. Its major epidemics have been reported from developing countries. Eleven African countries were hit by epidemics of the disease in 1994. It was the leading cause of death among refugees in Rwandan camps, Tanzania and Zaire (WHO, 1997). Cases of epidemic and endemic dysentery have also been recorded in Asia. In India, 10.4% of the population of the Andaman and Nicobar Islands was affected by the disease annually (Ghosh and Sehgal, 1996). The incidence was, however, higher (22.9 %) in pre- and post-monsoon season with maximal episodes occurring in the month of June.

The etiological agent of the disease is the microorganism *Shigella*. *Shigella* are Gram-negative rods belonging to the family
Enterobacteriaceae. These are non-motile and non-capsulated. *Shigella* have been divided into four groups or species based upon their biochemical reactions and antigenic structure. The groups A, B, C and D correspond to the species *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* respectively (Sleigh, 1989).

The strains of *S. flexneri* and to a lesser extent, *S. dysenteriae* and *S. boydii* are known to be primarily responsible for the dysentery (WHO, 1993), though the disease caused by *S. dysenteriae* is the severest and that by *S. sonnei* is the mildest.

Shigellosis is a disease known exclusively to affect human beings and primates. The onset time is known to vary between 12-50 hours and the infective dose has been reported to be as low as 10 cells (Todar, 1993). The major cause of concern for the disease is the reported increase in the incidence of drug resistance, which makes it difficult to bring epidemics under control.

The organism responsible for the disease is transmitted by fecal contamination of food, hands or water. After ingestion the bacteria passes innocuously through the stomach and the small intestine to invade the colon where they multiply in the epithelial cells. The bacteria rarely penetrate beyond the intestinal mucosa and have never been reported to invade the blood (WHO, 1993).

The known symptoms of the disease are fever, diarrhea, with blood and mucous, abdominal cramps and tenesmus. Megacolon, rectal
prolapse and occasionally perforation of the colon have also been reported to occur. Among the systemic complications, seizures have often been in children during early stages of illness.

Rapid weight loss and deterioration of the nutritional status due to anorexia, increased catabolism and loss of protein from the damaged intestine are known to occur if the disease persists unchecked. Severe and rapid hemolysis together with anuria (hemolytic uremic syndrome) and septicemia are often known to be associated with fatal outcome.

In malnourished children, the disease often causes death particularly when complicated with other infections (WHO, 1993). The malady is also known in AIDS patients. Though rare, the organism is known to cause reactive arthritis (Twam-Danso et al., 1993) and osteomyelitis (Wilson, 1996).

The toxin producing strains of *Shigella dysenteriae* and *E. coli* 0157 have also been linked with hemolytic uremic syndrome (HUS) particularly in children aged less than five years. This disease is typified by the development of hemolytic anemia, thrombocytopenia and acute renal failure. It is not yet known which factors predispose individuals to the development of this disease, however, there is evidence of the involvement of toxins in causing vascular endothelial damage and inflammatory response (WHO, 1993).

Bonventre (1970) defined microbial protein toxins as a special class of poison which differs from other poisons like cyanide and
mercury by virtue of their microbial origin, protein nature, high molecular weight and antigenicity (Stephan and Piętrowski, 1986).

Enterotoxins are a subclass of toxins. These are extra cellular bacterial proteins, that exert their action on the gastrointestinal tract. Bacterial toxins are to be the most powerful human poisons, which retain their activity even at high dilutions. These protein toxins resemble enzymes in being proteinaceous in nature with high biological activity and specificity. Many of these toxins are known to consist of two components – subunit A which is responsible for their enzymatic activity and subunit B which is not only concerned with the binding to a specific receptor on the host cell membrane, but also transfer subunit A across the membrane. The enzymatic component becomes active intracellularly once it gets released from the native toxin (Todar, 1997).

The strain S. dysenteriae is known to produce the Shiga toxin. The Shiga family includes Shiga toxin from Shigella dysenteriae type I and Shiga-like toxins produced by enteropathogenic E. coli, Salmonella typhimurium and Vibrio cholerae. The family belongs to the A-B class of bacterial toxins.

Shiga toxin, the prototype toxin, was originally described 96 years ago (Conradi, 1903). It possesses the following biological properties:

i. Lethal to rabbits and mice.

ii. Cytotoxic to several cell lines.
iii. Entertoxic, i.e. it is known to cause fluid accumulation in rabbit ileal loops.

iv. Able to inhibit cell-free protein synthesis.

The biochemical mode of action of this toxin is known to be identical to that of plant toxin, ricin from *Ricinus communis*.

Shiga-like toxins or verotoxins can be divided into two groups - Shiga-like toxin 1 and 2 or SLT-I (Stx 1) and SLT-II (Stx 2) respectively. Further the terms SLT I and SLT II are completely interchangeable with the terms verocytotoxins I and II (Bitzan *et al.*, 1994).

The knowledge about toxins and their mode of action is very important for understanding of diseases at the molecular level. Toxins also find use as drugs to prepare vaccines. Protein toxins have long been used to construct immunotoxins and other toxin conjugates in attempts to find efficient drugs in the treatment of cancer and other diseases (Sandvig and vanDeurs, 1994).

Although it is well known that the *Shigella* species are the etiological agents of the disease Shigellosis, yet only the toxin produced by *S.dysenteriae* has been purified and is well characterised. Hence, the present study was undertaken with the following aims and objectives:

- To compare the protein pattern and toxicity of the different species of *Shigella* i.e., *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*.
- To isolate and purify the toxin from *S. flexneri*.
- To assay the cytotoxic activity of the purified proteins from *S. flexneri*:
• To characterize the purified toxin with respect to the following properties:
  • Molecular weight.
  • Isoelectric point.
  • Effect of heat treatment on its toxicity.
  • Effect of proteolytic enzymes on its toxicity.
  • Effect of reducing agents on its toxicity.