CONCLUSIONS
Though *Shigella flexneri* is known to be the most common causative organism of the disease Shigellosis, but *S. dysenteriae* has been the focus of attention of researchers. The toxin elaborated by the species (*S. dysenteriae*) has been purified and is well characterized. However, there exists a lacuna as far as the toxin of the species *S. flexneri* is concerned. As a result the question whether or not the toxin of the species *S. flexneri* is similar to the shiga toxin of *S. dysenteriae* remained unanswered.

The present study on the isolation, purification and characterization of the toxin from *Shigella flexneri* was therefore carried out.

For the purpose of comparison, all the four species of *Shigella* i.e. *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* were grown in nutrient broth and their lysates prepared using polymxin B sulphate.

The lysates of the four species after estimation of their protein content were run on a 12.5% non denaturing, non-reducing polyacrylamide gel and also on a 10% SDS- polyacrylamide gel. Electrophoresis of the lysates revealed that each species has a unique protein profile.

All the strains were found to have cytotoxic effect.

The species used in the present study was found to be fimbriated. The species *S. flexneri* grew in nutrient agar medium, simple glucose ammonium salt medium with supplementation. It was resistant to ampicillin and amoxillin but was susceptible to gentamycin and chloramphenicol.
The crude extract of the species *S.flexneri* was run on a 10% native gel which revealed the presence of several bands in the range <14.3 to >62 kDa.

The crude extract was run on a column of sephadex G100. Homogeneity of the protein was confirmed on non reducing, non-denaturing PAGE.

Cytotoxicity tests of the lysate and supernatant of *S.flexneri* were performed employing the following:

* Rec Assay
* Rabbit Skin Test
* HeLa Cell Assay

The cytotoxic principle was ineffective on both rec assay and Rabbit skin test. On HeLa cells, however, the fractions (F 11-18) had cytotoxic effect.

The toxicity of the lysate was found to be $8.28 \times 10^4$ /mg protein.

For the supernatant, the value was $9.7 \times 10^3$.

The crude extract of *S.dysenteriae S.boydii* and *S.sonnei* had toxicity values of $0.426 \times 10^4$, $0.832 \times 10^2$ and $0.406 \times 10^2$/mg protein respectively. The toxin from *S. flexneri* was characterized on native polyacrylamide gel electrophoresis which revealed a single band of 66 kDa.

SDS PAGE showed the presence of five bands which could correspond to the A and B subunits along with the dimeric and trimeric forms of the B subunit.

The isoelectric point of the holotoxin was 3.6 which is different from that of the shiga toxin.
The protein was subjected to heat treatments of 50°C and above. It resulted in a loss of cytotoxic activity and at 70°C the cytotoxic effect was completely destroyed.

Proteolytic enzyme treatment of the toxin resulted in a total loss of activity with proteinase K, but not with trypsin. Treatment with reducing agents resulted in a total loss of its cytotoxic activity.

The toxin from S. flexneri is, thus, similar to that of shiga holotoxin in its molecular weight and may have the 1A:5B configuration as does the shiga toxin. Like the Shiga toxin family, it is cytotoxic to HeLa cells but not to prokaryotic cells or rabbit skin. Similarity also exists in their response to reducing agents. However, the other molecular characteristics i.e., isoelectric point, heat stability and behaviour to proteolytic enzymes are somewhat different from that of shiga and shiga-like toxins.

The present study provides an insight into the toxin from Shigella flexneri. It has a molecular weight of 66 kDa. When subjected to SDS PAGE, 5 bands were observed. It has a isoelectric point of 3.6. It cytotoxicity is confined to fraction 11-18.

It may thus be regarded as another different member having an individual place in the broad shiga family. Therefore, the term Shiga-like toxin would be appropriate for this new member.