INTRODUCTION
In recent decades, advances in biotechnology have increased the potential usage of many enzymes and the emergence of new and promising research activities in molecular biology and genetic engineering is continuously expanding as the number of enzymes that need to be purified and characterized (Verma N, 2007). Wide varieties of enzymes are used as pharmaceuticals and in addition, are also found in industrial and domestic applications. Enzymes are an important class of conjugated proteins and are ubiquitous in nature. Enzymes are widely used in industry, continuing and extending many processes which have been used since the dawn of history.

The remarkable properties of enzyme include their high catalytic power, high specificity which transform the substrates into the products, their ability to catalyze the reactions under normal temperature and pressure and also to get regulated by a variety of metabolites and environmental conditions.

The prospects of enzyme application are due to:

1. Higher yields and selectivity obtained by genetic manipulation.
2. Mild fermentation conditions which results in low costs of nutrients, optimal utilization of components in nutrient solution and amenability to fed-batch fermentation conditions.

Most notably the enzymes are the ideal catalysts for a given substrate, much more efficient and specific in their reaction characteristics than any man-made catalyst. The commercial usage of enzymes started when fungal cell extracts were first added to brewing vats to facilitate the breakdown of starch into sugars. Today, enzymes form a major subject for research and finds innumerable applications in process industries.

Enzymes produced by living cells are usually obtained by growing microorganisms in a pure culture or directly obtained from plant and animals and are absolutely essential in all biochemical reactions. Despite the importance of enzymes from plant and animal sources, their large scale extraction poses technical, economical and possibly ethical problems. Hence, the trend has been towards the utilization of microorganisms as a source of enzymes. Large scale extraction of commercial enzymes is mainly from microbial sources because a wide range of enzymes are available in microorganisms further, the cells producing the required enzyme can be easily cultivated and obtained, as most of the enzymes of commercial importance are extracellular (Michel Duval et al., 2002).

Enzymes have been used in various industrial processes like baking and brewing for centuries. Apart from these, enzyme applications have revolutionized many fields, for example many enzymes are essential components of recombinant DNA technology which has lead to major advances in medicine and
pharmaceutical industries. The manufacture of an enzyme for use as a drug is an important facet of today's pharmaceutical industry (Oettgen, 1971). Traditionally, the source of enzymes would have been extracted animal tissue, serum or other natural materials. However, with the advent of recombinant DNA techniques, sources now include cultured microorganisms (both prokaryotic and eukaryotic expression systems) and mammalian cells.

Enzymes have been used for therapy of variety of diseases for a long time, their pharmacology, however, is being recognized only in the course of last decades owing to their importance when used in cancer therapy. Biotechnological advancements have enabled for enhanced potency and specificity among enzymes with a production at a lower cost (Pajdak, 1977). The term 'therapeutic enzyme' has been known for at least 40 years. Therapeutic enzymes have a broad variety of specific uses as oncolytic, anticoagulants or thrombolytic, and as replacements for metabolic deficiencies. The favored kinetic properties of these enzymes are low Km and high Vmax in order to get maximal efficiency even at very low enzyme and substrate concentrations. It is of great importance to fully understand the enzyme properties and catalytic activity, in order to optimize its use and limit potential side effects (Kumar, 2009).

Cancer is the most mystifying disease is defined as a disturbance of growth characterized primarily by excess proliferation of cells without apparent relation to the physiological demands of the organism involved. The disease still remains unconquered in spite of the fact that a very large number of studies are being carried out all over the world. Within the area of cancer treatment one
exploits the knowledge of neither differences between normal and malignant cells, i.e. malignant cells lack of certain functions (Hyakuna et al., 2004).

Cancer drug therapy is undergoing a major transition from the pre-genomic era to the post genomic era. New technologies particularly high throughput screening, combinational chemotherapy, gene expression micro array and computer aided drug designing are increasing the speed and efficiency of drug development (Michel Vellard, 2003). Improved systemic drug therapy is particularly important for the treatment of leukaemia, where surgery and radiation can no longer be curative.

The development of microbial enzymes for cancer therapy, has added to the choice of anti-leukaemic drugs which promises prospects for better treatment. The use of microbial enzymes in leukemia therapy makes it important that the enzyme should be produced in large quantities and are usually required to be in their pure forms; therefore, their production costs are very high. If such a difficulty is circumvented, the potential use of enzymes in clinical medicine, in general and cancer chemotherapy in particular, would be extended (Huisman and Gray, 2002).

Leukaemia a disease of the reticuloendothelial system is characterized by the uncontrolled proliferation of leucocytes. It is a result of excessive proliferation of the blood forming tissues. In this disease there is a considerable increase in number of white blood cells sometimes their number in blood increase 4, 50,000 per cubic mm compare to the normal level of 8,500 – 10,000. Different forms of leukemia derive their name from the type of cell that has become malignant.
1. Chronic lymphatic leukaemia
2. Chronic myeloid leukaemia
3. Acute lymphatic leukaemia
4. Acute myeloid leukaemia, etc.

The new insights into the underlying molecular biology of leukemia have changed our understanding of the disease (Hill et al., 1967). Not only there are prospects of better treatment and the introduction of new biologically based therapies, but, as the causes of disease are also being unraveled, the possibility of prevention may not just be wishful thinking.

A pre-requisite for making an effective medication for the treatment of cancer is that some fundamental difference between normal cells and cancer cells must be defined. The chemotherapeutic agent must exploit this cellular difference in such a way that normal cells are spared and only cancer cells are injured. L-Asparaginase exploits the unusually high requirement tumor cells have, for the amino acid asparagine (Guo Qing Long et al., 2002). The enzyme L-Asparaginase has been a clinically acceptable anti tumor agent for the effective treatment of acute lymphoblastic leukemia and lymphosarcoma. Acute leukemia is an uncommon malignant disorder resulting from the clonal proliferation of hematopoietic precursors of the myeloid or lymphoid lineages (Haley et al., 1961).

Of the two major subgroups, acute lymphoblastic leukemia is more common in children, while acute myelogenous leukemia predominates in adults. This is predominantly a disease of childhood and is the most common childhood cancer, accounting for 85% of childhood leukaemias (Crowther, 1971). The
incidence of this disease is highest in the three to four year old age group, falling off by ten years. Generally the risk of any child developing acute leukaemia is about 1 in 2000. With modern chemotherapy 60%-70% of all children with acute lymphoblastic leukemia can be long-term survivors and are potentially cured. The advancements in treatment of acute leukaemias have improved the chance of cure, depending upon the patient's age and the type of leukaemia (Aytemiez Gurgey et al., 2004). The purpose of the therapy is to reduce the number of neoplastic cells, to improve the conditions and if possible to prolong survival while maintaining an adequate quality of life (Michel Duval, 2002). The chemotherapeutic potential of L-Asparaginase in treating acute lymphoblastic leukemia and lymphosarcoma has been one of the most eminent discoveries of modern times. Children with, Acute lymphoblastic leukemia were found to be improved with more intensive combination chemotherapy from 4% in the early 1960s to more than 80% in the 1990s. The continuously improving treatment results through the years are an example of medical development and the handling of therapeutic schedules according to often international treatment protocols (Chen et al., 2004).

L-Asparaginase appears to be highly effective especially in children with newly diagnosed Acute lymphoblastic leukemia (Broom, 1968). Prolonged L-Asparaginase intensification improved the outcome significantly. Nowadays L-Asparaginase is an essential drug that is used to treat children with Acute lymphoblastic leukemia all over the world. Its potential is now well established, as it has remarkably induced remission in most of the patients suffering with this disease. This therapy has brought a major breakthrough in modern oncology
and with the development of its new functions, a great demand for L-Asparaginase is expected in the coming years (Aytemiz Gurgey et al., 2004).

L-Asparaginase (L-Asparagine amidohydrolase; EC 3.5.1.1) catalyzes the conversion of L-Asparagine to L-aspartate and ammonia and to a lesser extent the formation of L-glutamate from L-glutamine. L-Asparagine is a major requirement by the cells for the production of protein. It can be produced within the cell by an enzyme called asparagine synthetase or can be absorbed from the outside (consumed in the diet, absorbed into the body and made available to the body's cells). Tumor cells, more specifically lymphatic tumor cells, require huge amounts of asparagine to keep up with their rapid malignant growth. Thus, the asparagines from the diet as well as what can be made by themselves (which is limited) is utilized by them to satisfy their large asparagine demand. L-Asparagine is an essential amino acid for the growth of tumor cells, whereas the growth of normal cells is not dependent on its requirement as it can be synthesized in amounts sufficient for their metabolic needs with their own enzyme L-Asparagine synthetase (Adamson and Fabro, 1968). The presence of L-Asparaginase deprives tumor cells of an important growth factor and they fail to survive. The antineoplastic activity results from depletion of the circulating pools of L-Asparagine by L-Asparaginase. Unlike normal cells, malignant cells can only synthesize L-Asparagine slowly and are dependent on an exogenous supply. In contrast, normal cells are protected from asparagine starvation by their ability to produce this amino acid. Unlike conventional cancer therapy, L-Asparaginase therapy is highly selective. For this reason the commonest therapeutic practice is to inject
intravenously free enzyme in order to decrease the blood concentration of L-
Asparagine affecting selectively the neoplastic cells (Broom, 1968).

This extraordinary behavior of these neoplastic cells was broken by the
scientific community 50 years ago, with the observation that guinea-pig serum
treated lymphoma-bearing mice underwent rapid and often complete regression
(Kidd, 1953). In the 1960s, and continuing research in this field, Broome reported
that Asparaginase activity in guinea-pig serum was responsible for the anti-
lymphoma effects. The final proof that Asparaginase was the tumor-inhibitory
agent of guinea pig serum was furnished by other investigators who isolated the
enzyme to homogeneity as judged by immunoelectrophoresis and demonstrated
that it was strongly inhibitory to lymphoma tumors. Thus the development of this
enzyme as a potent anti-tumor or antileukaemic drug has gained more
importance.

The giant step in the development of L- Asparaginase as an effective
antitumor agent took place only after Mashburn demonstrated that L-
Asparaginase produced by the microorganism *Escherichia coli* had the same
antitumor activity as that gained from guinea pig serum and commercial
production of L-Asparaginase appeared desirable only after these findings.

Later it was reported that L-Asparaginase could be extracted from two
bacterial sources: *Escherichia coli* and *Erwinia chrysanthemi*. Thus these
bacterial sources made it possible to produce and utilize larger quantities of the
enzyme and a series of preclinical and clinical studies was initiated (Aguayo et al.,
1999). A poly ethylene glycol modified version of the enzyme (PEG-Asparaginase) was developed in the 1970s and 1980s and was first used in clinical trials in the 1980s. A large number of sources were screened for the production of L-Asparaginase to find a better source of this enzyme. They include plant leaves, developing seeds, Yeast, Fungi, Mycobacterium, Nocardia and a large number of gram positive and gram negative bacteria. It is interesting however that only the enzyme derived from gram negative bacteria showed anti tumor activity with few exceptions.

Among all the microbes, producing L-Asparaginase the bacterial L-Asparaginases have been studied in great detail till date as, enzymes obtained from yeast and fungi did not show appreciable enzyme activity for clinical trials. Many genus of bacteria, such as, *Erwinia carotovora*, *Thermus thermophilus* (Pritsa et al., 2001), *Thermusaquaticus* (Curran et al., 1985), *Vibrio succinogenes* *Citrobacter freundii*, *Streptomyces griseus* (Dejong, 1972), *Escherichia coli* (Howard and James, 1968), *Erwinia aroideae* (Peterson and Ciegler, 1969), *Proteus vulgaris* (Tosa et al., 1971), *Enterobacter aerogenes*, *Zymomonas mobilis*, *Bacillus licheniformis*, *Pseudomonas aeruginosa* (Abdel-Fattah et al., 2002) were found to be potent producers of L-Asparaginase. But, L-Asparaginase from bacterial origin can cause hypersensitivity in the long-term use, leading to allergic reactions and anaphylaxis, apart from these, they pose problems of protein purity and limited pharmacokinetic (PK) distribution in a mammalian system, immunogenicity to the host etc.
Therefore these bacterial proteins must be purified extensively to eliminate toxic reactions and to minimize immune reactions (Amardeep Khushoo et al., 2004). The main restrictions to the use of L-Asparaginase as a therapeutic agent include its premature inactivation and rapid clearance, thus necessitating frequent injections to maintain therapeutic levels, and several types of side effects ranging from mild allergies and development of immune responses to anaphylactic shock are often observed during clinical therapy, which may be life-threatening. Therefore the availability of two or more different L-Asparaginases would be advantageous in clinical trials (Grundmann and Oettgen, 1970).

Despite these problems, native *Escherichia coli* and *Erwinia* Asparaginases have made major contributions in the treatment outcome of Acute Lymphoblastic Leukemia patients. Further L-Asparaginase was the focus of intensive investigations during 1970s that made large scale industrial manufacture of this enzyme practical from wild type bacterial strains. However, no sufficient reports are available concerning the production of this important anti-cancer drug using recombinant DNA techniques. Currently, most of the commercial L-Asparaginase is produced by wild-type fermentation. Unfortunately, production of large quantities of L-Asparaginase through this process is restricted by the extremely low efficiency of the technique. Consequently, L-Asparaginase for most clinical applications is largely depended on imports from Japan and USA. The extremely high price of this drug poses the severe restriction on the clinical application of this potent drug to patients suffering from Acute Lymphoblastic Leukaemia. Therefore, it is remarkably attractive to search for systems that display high expression levels of L-Asparaginase and construct
suitable systems for this enzyme through DNA recombinant techniques (Gilbert et al., 1986). Therefore L-Asparaginase has been a major research subject for many researchers worldwide.

Although, much has been unraveled, it appears that there is still a long way to go in exploring this amazing enzyme. The most important requirements for an L-Asparaginase to be of therapeutic use is that it should be easy to isolate, stable at physiological pH and temperature, less immunogenic to the host, and finally the production cost should be low for the extensive use of this drug worldwide for the treatment of Acute leukaemias (Rizzari et al., 2000). If such difficulties are curbed L-Asparaginase would become a wonder drug for the future generations to come.