Preface
Cancer is a major public health problem in India and many other parts of the world. According to Cancer statistics of India published by cancer support society, every year about 8,50,000 new cancer cases are diagnosed in India resulting in about 5,80,000 cancer related death every year. In males, oral, lungs and stomach cancers are the three most common causes of cancer incidence and death. It is suspected that in 2008 about 565,650 Americans were died by cancer, corresponding to over 1,500 deaths per day (Jemal et al, 2008). Since 1985, the cancer registries in India, which are under the National Cancer Registry Program, have reported a 12% increase in cancer cases, which is much higher than the rate of increase in the US (Pal and Mittal, 2004).

Cancer is not just one disease, but a large group of almost 100 diseases. Its two main characteristics are uncontrolled growth of the cells in the human body and the ability of these cells to migrate from the original site and spread to distant sites. If the spread is not controlled, cancer can result in death. The term cancer derives from the observation by Hippocrates in 400 BC, that the veins radiating from a breast cancer resembled the legs of a crab, hence karkinoma in Greek and cancer in Latin. He later added the suffix -oma, Greek for swelling, giving the name carcinoma.

Cancers are classified by the type of cell that resembles the tumor and, therefore, the tissue presumed to be the origin of the tumor. The following general categories are usually accepted:

- Carcinoma: malignant tumors derived from epithelial cells. This group represent the most common cancers, including the common forms of breast, prostate, lung and colon cancer.
- Lymphoma and Leukemia: malignant tumors derived from blood and bone marrow cells
- Sarcoma: malignant tumors derived from connective tissue, or mesenchymal cells
- Mesothelioma: tumors derived from the mesothelial cells lining the peritoneum and the pleura.
- Glioma: tumors derived from glia, the most common type of brain cell
• germ cell tumors: tumors derived from germ cells, normally found in the testicle and ovary
• Choriocarcinoma: malignant tumors derived from the placenta

Cancer cells can break away from a primary tumor, penetrate into lymphatic and blood vessels, circulate through the bloodstream, and grow in a distant focus (metastasize) in normal tissues elsewhere in the body, which is known as metastasis. The process of metastasis and secondary site tumor formations keeps repeating and allows the cancer to spread through the organism (Lodish et al, 2002). Even if cancer is forced into remission by today’s best treatment methods, the threat of metastasis remains. Experiments on model organisms, whose many metabolic processes parallel our own, help us to understand the underlying factors that are involved in causing cancer and other diseases (Griffiths et al, 2002). Studies in the mouse and the fly have revealed numerous molecules and genes that are involved in cancer formation and metastasis.

Matrix metalloproteinases (MMPs) belong to a family of structurally related calcium and zinc-dependent endopeptidases that have the ability to digest a broad range of extra cellular matrix molecules. These enzymes, which include the collagenases, gelatinases, stromelysins and membrane type (MT)-MMPs, have been implicated in the turn over of the extra cellular matrix during tumor development and progression (Bellon, et al, 2004; Polette et al, 2004; Sood et al, 2004). MMP activity can be controlled at various levels: transcription, proteolytic activation of the zymogen form, and inhibition of the active enzyme (Stamenkovic, 2003). MMPs are overexpressed in multiple tumor types when compared to normal tissues (Mannello et al, 2005; Egeblad and Werb, 2002). However, in cases in which increased MMP levels have been shown to be strong indicators of a negative prognosis, it is more likely that targeting those enzymes will impact tumor progression; several studies pointed to the possible use of MMPs in the future to augment treatment strategies in specific cancers (Agnantis et al, 2004).
Angiogenesis is the process of forming new blood vessels from existing ones and requires degradation of the vascular basement membrane and remodeling of the ECM in order to allow endothelial cells to migrate and invade into the surrounding tissue. Various MMPs are also necessary for releasing proangiogenic factors like Vascular endothelial growth factor (VEGF) (Joyce and Rundhaug, 2005). VEGF has been well described as a cytokine most important for endothelial cell proliferation and the process of angiogenesis which is essential for the tumor development (Tammela et al, 2005). VEGF acts on endothelial cells to induce cell migration and proliferation, as well as acting as a vascular permeability factor, which allows leakage of plasma proteins such as fibrinogen/fibrin that create a temporary support structure for migrating epithelial cells, leukocytes, and endothelial cells (Li et al, 2003; Conway et al, 2001).

Nitric Oxide (NO) is a gaseous signaling molecule that regulates various physiological and pathophysiological responses in the human body, including circulation and blood pressure, platelet function, host defense and neurotransmission in central nervous system and in peripheral nerves. Since its discovery in 1987 (Palmer et al, 1987; Ignarro et al, 1987) NO has been a target of intensive research and drug development. There are several studies which have pointed to the critical role of nitric oxide (NO) in VEGF-induced vascular permeability, as well as angiogenesis (Parenti et al, 1998). Cellular responses to oxidative and nitrosative stress are often regulated at the level of transcription (Marshall et al, 2000). Both prokaryotic and eukaryotic cells have transcription factors that are regulated by NO, for example NF-κB, AP-1 and Jak-STAT pathways.

Apoptosis, a physiological process for killing cells, is critical for the normal development and function of multicellular organisms. Abnormalities in the control of cell death can contribute a variety of diseases, including cancer. Apoptosis is a programmed cell death that can be induced by a variety of different stimuli such as growth factor deprivation (Cornelius et al, 2005), ionizing radiation (Konemann et al, 2005), chemotherapeutic drugs (Sordet et al, 2003), ultraviolet (UV) radiation (Kulms and Schwarz, 2002) and activation of cell death receptors (Wajant et al, 2005). The events of apoptosis involve membrane blebbing, cytosolic condensation, breakdown of
nuclear DNA with subsequent nucleosomal fragmentation and finally formation of well-enclosed apoptotic bodies. This enables phagocytic cells to remove apoptotic cells, minimizing tissue inflammation, avoiding damages to neighboring cells and efficiently degrading host DNA (Fiers et al, 1999). Signaling for apoptosis occurs through multiple independent pathways that are initiated either from triggering cells with in the cell or from outside the cell, for instance the ligation of death receptors. Caspases can be thought as central executioners of the apoptotic pathway, because they induce most of the visible changes that characterize apoptotic cell death. In the caspase family caspase-3 is a distal executioner, which can transform proenzyme to active form by apoptotic-triggers induced caspase-cascade activation and then mediates apoptotic cascade (Ramp et al, 2003; Jimbo et al, 2003). p53 functions as a transcription factor regulating downstream genes which are important in cell cycle arrest, DNA repair, and apoptosis. The critical role that p53 plays is evident by the large number of tumors that bear a mutation in this gene. After DNA damage, p53 holds the cell at a checkpoint until the damage is repaired. If the damage is irreversible, apoptosis is triggered. A central player in that genetic program, and the link between apoptosis and cancer, emerged when Bcl 2 (B-cell lymphoma 2), the gene that is linked to an immunoglobulin locus by chromosome translocation in follicular lymphoma, was found to inhibit cell death, rather than promote proliferation (Vaux et al, 1988). This unexpected discovery gave birth to the concept, now widely embraced (Cory et al, 1999; Hanahan and Weinberg, 2000; Green and Evan, 2002; Johnstone et al, 2002) that impaired apoptosis is a crucial step in tumorigenesis.

Several inflammatory cytokines have been linked with tumorigenesis, which suggests that inflammation is associated with cancer development (Lazar-Moinar et al, 2000). The studies indicated the elevation of constitutive production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and granulocyte monocyte-colony stimulating factor (GM-CSF) in tumor cells. Regulation of the expression of these pro-inflammatory cytokines involves the transcription factor, Nuclear Factor (NF)-κB, which can be activated by cytokines such as TNF-α. The host environment promotes the constitutive activation of NF-κB and pro-inflammatory cytokine expression during metastatic tumor progression of
murine squamous cell carcinoma (Dong et al, 1999). These transcription factors play an important role in cellular transformation either by providing continued positive growth stimuli such as mediated by cytokines or by inhibiting apoptotic pathways (Seibentlist et al, 1994; Karin et al, 2000; Silverman and Maniatis, 2001). NF-κB transcription factors have emerged as major regulators of programmed cell death and necrosis. NF-κB acts in an intrinsic fashion to confer resistance to cell death by activating the expression of antiapototic genes.

Throughout history, natural products have afforded a rich source of compounds that have found many applications in the fields of medicine, pharmacy and biology. Within the sphere of cancer, a number of important new commercialized drugs have been obtained from natural sources, by structural modification of natural compounds, or by the synthesis of new compounds, designed following a natural compound as model. The search for improved cytotoxic agents continues to be an important line in the discovery of modern anticancer drugs. The huge structural diversity of natural compounds and their bioactivity potential have meant that several products isolated from plants can serve as “lead” compounds for improvement of their therapeutic potential by molecular modification. In the present study the effect of natural products such as Punarnavine, an alkaloid from *Boerhaavia diffusa* and naturally occurring terpenoids such as Glycyrrhizic acid, Ursolic acid, and Limonene on the activation of NF-κB and iNOS gene expression during metastastic tumor progression is assessed.