The most prominent characteristic of the living system is the interface between different components of the body, their adaptation and assembly into an autonomous self engineered system, outlining the dynamic nature of the human body which is always eternally involved in the process of transformation (Wolkenhauer and Muir, 2009). A dynamic balance between the cell restitution and death is critical for the normal functioning of the cell. A number of signaling pathways command the fate of normal cells as to whether they should proliferate and differentiate or undergo senescence. Certain catastrophic sequential events causing disruption in the functioning of the cell at the molecular, biochemical and cellular levels underscore the genesis of cancer which involves the transition of a normal cell into a malignant one (Hejmadi, 2010). Series of somatic alterations in DNA, including mutations in DNA, may result in the unrestrained cellular proliferation and consequently results into cancer.

CANCER is thus, an intricate disease interlinked with multiple signaling pathways and is an aftermath of multiple deregulated molecular and cellular events (Pietras and Ostman, 2010). Carcinogenesis is an underlying etiologic channel that leads to cancer. According to GLOBOCAN 2012 reports about 14.1 million new cancer cases and 8.2 million cancer related deaths worldwide have been recorded in 2012. The prevalence estimates clearly exhibited that there is rising burden of cancer in the developing countries which are going through rapid societal and economic revolution along with a transition towards lifestyles typical of the industrialized nations. 8% more deaths were documented in 2012 than 2008. It is expected that if this menace of cancer remains unrestrained, the number of cancer cases may rise to 19.3 million by 2025 (Ferlay et al., 2013, Bray et al., 2013). Cancer has struck deep roots in India also, which reportedly claimed 5.5 million lives in 2010 (Dikshit et al., 2012).

Remodelled lifestyle, improper dietary regimen and exposure to both polluted environment and toxins are amongst the crucial factors that have greatest contribution in establishing the “disease of civilization”- Cancer (Theodoratou et al., 2014; Anand et al., 2008). Intoxication of environment with several hazardous xenobiotics has contributed significantly in the rising trend of cancer incidence (Jain et al., 2013). Exposure of humans to chemical carcinogens
consequently results in several genetic and biochemical modulations in the cell that further aggravate the onset of carcinogenesis. Colorectal cancer has been revealed to account for the third most commonly diagnosed cancer in men and second in women worldwide, according to GLOBOCAN 2012 estimates. The statistics depict that 52% deaths resulting from colorectal cancer occurred in the lesser developed regions of the world which reflects a poorer survival in these nations. In India, the incidence of colorectal cancer cases in 2012 was observed to be 64,000 which are expected to rise to 80,000 by the year 2020 (Ferlay et al., 2013, Bray et al., 2013).

Most of the colorectal cancer cases have been found to be sporadic i.e. with no apparent evidence of having inherited the disorder. Only a small fraction of the patients have a family history of colorectal cancer which is suggestive of hereditary contribution, common exposures among family members, or a combination of both. Genetic mutations have been recognized as the cause of inherited cancer risk in some colon cancer-prone families and accounts for only 5%-6% of CRC (colorectal cancer) cases overall in conjunction with non-genetic risk factors (Burt, 2000). The etiology of CRC involves a multifarious interaction between low penetrance polymorphisms in carcinogen-metabolizing enzymes and environmental risk factors (Gertig and Hunter, 1998). Sedentary lifestyle, smoking, obesity, ingestion of heterocyclic amines and aromatic hydrocarbons, low consumption of folate and methionine and high alcohol intake are amongst the most common risk factors associated with CRC (Kushi and Giovannucci, 2002). About 70% of the colorectal cancer cases are known to be a consequence of the adherence to inappropriate and unhealthy dietary regimen (Anand et al., 2008). It has been suggested by the researchers that diets high in total fats increase the risk of CRC. Inappropriate and unrestricted inclusion of dietary fats in our meals abundantly increases the colonic primary bile acids and their further transformation to cytotoxic, mutagenic and anti-apoptotic secondary bile acids (deoxycholic acid and lithocolic acid) that act as cancer promoters (Jia et al., 2013; Casimiro, 2002; Potter, 1995). The chromosomal instability pathway (CIN) and microsatellite instability pathway (MSI) have been recognized to be the two major pathways of carcinogenesis in CRC (Armaghany et al., 2012). DNA methylation in alliance with structural changes and mutations in chromatin and tend to dysregulate the conserved signaling networks that exert effects on essential cell phenotypes, which includes the regulation of cellular metabolism, proliferation, differentiation and survival (Fearon, 2011).
Exposure of normal cells to carcinogens leads to the induction of chemical carcinogenesis, during which they acquire distinct and complementary capabilities that enable growth of tumors and metastasis. The induction of chemical carcinogenesis in various experimental models has made it feasible for the researchers to gain better understanding of the cause of cancer, its development, treatment and prevention. Two methylating agents, 1, 2-dimethylhydrazine (DMH) and its metabolite azoxymethane (AOM), are amongst the list of most potent carcinogens being predominantly used to induce colon tumors in the experimental rat models. DMH induced colon carcinogenesis in a rat model is widely used for the assessment of environmental, dietary and chemopreventive agents, morphological and molecular mechanisms involved in the process of colon carcinogenesis and also for elucidating new targets for chemoprevention (Rosenberg et al., 2009; Chen and Huang, 2009). The mechanism underlying the carcinogenic action of DMH involves a series of oxidative events where metabolic activation of DMH in the liver through intermediates azoxymethane (AOM) and methylazoxymethanol (MAM) ultimately lead to its conversion into highly reactive methyldiazonium ion (Fiala et al., 1977). This carcinogenic metabolite (an electrophile) can either be detoxified by reacting with a free electron pair or can react with the negative centre on DNA which leads to the formation of adducts (N7-methylguanine, O6-methylguanine and O4-methylthymidine) on specific bases in DNA. The methylation of the DNA bases results in the loss of colonic cells, increased proliferation and an apparent elevation in the mutations of colonic epithelial cells (Netto et al., 1992; Chang, 1984).

Chemoprevention of cancer by dietary agents has substantially been scrutinized in the past and provides a strong decree for adopting this measure as a preventive strategy against cancer in humans. Chemopreventive strategies include the utilization of natural or synthetic chemical compounds to reverse, restrain or prevent the cellular events associated with multistage and multifactorial process of carcinogenesis and are known to have strong impact on the functioning of macromolecules, release of carcinogens, formation of DNA adducts and repair events linked to carcinogenesis. Among the known natural compounds, dietary polyphenols have specifically gained remarkable attention among the researchers worldwide due to their incredible antioxidant properties, their preventive role in combating various diseases associated with oxidative stress and their enormous abundance in our diet.

Curcumin is one such polyphenol which has extensively been explored by researchers for chemopreventive action against several diseases and still holds the distinction to be under lens for several other investigations. Curcumin, popularly known as Golden Spice, is the most
active component of turmeric (*Curcuma longa*). This brilliant yellow-orange crystalline powder is obtained from the roots of *Curcuma longa* and makes up 2-5% of the spice. It has long been exploited as a traditional medicine in India and China. Curcumin, [(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] diferulolylmethane, has a molecular weight of 368.37 g/mol, melting point of 183°C and molecular formula of $C_{21}H_{20}O_6$ (Aggarwal *et al.*, 2003). Relative importance of phenolic hydrogens with regard to the antioxidant potential of curcumin has well been illustrated and phenolic hydrogens are considered to be labile for abstraction than $\text{CH}_2$ hydrogens in curcumin (Priyadarshini *et al.*, 2003). Extensive scientific research on curcumin in the past has unravelled its wide spectrum of therapeutic applications such as anti-carcinogenic, anti-inflammatory, anti-mutagenic, antioxidant, anti-bacterial, anti-angiogenic (Saha *et al.*, 2012; Garg *et al.*, 2005; Shishodia *et al.*, 2005; Joe *et al.*, 2004; Aggarwal *et al.*, 2003). Curcumin has also been found to augment the cytotoxic effects generated by the chemotherapeutic drugs such as doxorubicin, tamoxifen, cisplatin, camptothecin, vincristine (Bharti *et al.*, 2003; Harbottle *et al.*, 2001; Verma *et al.*, 1997).

Curcumin, a well known chemopreventive phytomedicine, curtails the process of carcinogenesis at all the three stages – initiation, promotion and progression. It averts the activation of carcinogen at the initiation stages of cancer and also inhibits the proliferation of malignant cells during promotion and progression of tumorigenesis (Duvoix *et al.*, 2005). Numerous biochemical cascades of events including various transcription factors, growth factors, cytokines, protein kinases and several enzymes are known to regulate this chemopreventive action of curcumin which include several transcription factors, growth factors, cytokines, protein kinases and various other enzymes (Lin, 2007). Curcumin is known to block the induction of nitric oxide synthase (iNos) in the macrophages which are activated during tumor initiation and scavenges free radicals or reactive oxygen species generated due to oxidative stress (Sreejayan and Rao, 1997). It is also known to check phosphorylation and degradation of the inhibitor IκBα, thereby inhibiting NF-κB activation which is known to have implications in several cancers (Pan *et al.*, 2000). Curcumin has been shown to exert apoptotic action by activating caspases and suppressing the levels of various cell survival and cell proliferative genes such as Bcl-2, cyclin D1, IL-6, cyclooxygenase-2 (COX-2), and MMP-9 (Aggarwal *et al.*, 2004; Malhotra *et al.*, 2014). Curcumin has been shown to inhibit IL-6 induced STAT phosphorylation and consequent STAT-3 nuclear translocation which is essential for arresting the tumor (Bharti *et al.*, 2004).
Our ecosystem has been found to be enriched with numerous metals and their compounds which are critical for the emancipation of modern world from hunger, ailments and discomfort. Majority of these metals penetrate our environment through their widespread use in various biocides, preservatives and paints or through inhalation, ingestion of food and contaminated water (Januszko et al., 2012; Craig et al., 2003). Zinc, a bluish white shiny metal, has been recognized as one of the quintessential micronutrients required in the body for its proper functioning (Adamo et al., 2010; Popescu et al., 2009). Zinc expresses its role as a perfect metal cofactor by acting as a redox stable ion which is a prerequisite for a large number of reactions for their activity (McCall et al., 2000; Butler, 1998).

Zinc has been extensively explored and is reported to play a unique and well defined role in various biological processes. More than 300 proteins are known to have dire requisite of zinc for their functions in microorganisms, plants and animals. It has also been stated to be vital for enzymes of all the classes as well as transcription and replication factors (Morgan et al., 2011; Ibs and Rink, 2003). Approximately 50% of the proteins required for regulation of transcription are zinc binding proteins (Nyborg and Peersen, 2004). With regard to its role in enzymes, zinc is known to perform three major functions- catalytic, coactive (or cocatalytic) and maintenance of structural integrity. It is also known to be involved in the regulation of cell division and differentiation (Vallee & Falchuk 1993). It also has implications in various essential processes such as synthesis of protein, nucleic acids, metabolism of carbohydrates and lipids, development of brain, induction of programmed cell death, and inhibiting cellular proliferation and oxidative stress (Gower-Winter and Levenson, 2012; Oteiza and Mackenzie, 2005; Barceloux, 1999). Zinc-importer family (having 14 proteins that transports zinc into the cytosol), and the zinc transporter family (with 10 proteins transporting zinc out of the cytosol) are also known to regulate the intracellular distribution of zinc (Zheng et al., 2008; Eide, 2006). Zinc has been suggested to exhibit its anti-oxidant potential via the action of metallothioneins, which represents an intriguing link between cellular levels of zinc and the redox state of the cell (Santos et al., 2012; Maret, 2011; Joshi et al., 2004; Sidhu et al., 2004).

Zinc is one of the most important components of DNA-binding proteins (with zinc fingers), Cu/Zn SOD and various proteins involved in DNA repair. Perturbation in the zinc status can lead to single and double strand DNA breaks which can consequently result into oxidative damage to DNA that may further augment the risk for cancer development (Kayatekin et al., 2008; Tapiero and Tew, 2003; Mocchegiani and Muzzioloi, 2000). It has been suggested in literature that malignant cells develop inherent mechanism to contain zinc efflux, thereby,
maintaining intracellular zinc levels under zinc deficient conditions. This underpins the vitality of intracellular zinc in rapidly dividing cells (Dutta et al., 2010).

Serine Phosphorylation (addition of a phosphate (PO4) group to a protein molecule or a small molecule) of p53 is an area of prime interest for researchers investigating the process of carcinogenesis. Both curcumin and zinc have been shown to exhibit an imperative role in intensifying the expression of p53 under cancerous conditions (Malhotra et al., 2014; Li et al., 2005; Cho et al., 1994). The strong chemopreventive action exhibited by curcumin and zinc makes it excellent contenders to be investigated further for their combined action against colon carcinogenesis.

In addition to the chemoprevention, the early detection of cancerous growths is of matter of great magnitude that also needs the attention of the health professionals and researchers throughout the world. The early diagnosis of cancer helps in assessing the overall prognosis and survival of the patients. The screening tests available for the diagnosis of cancer are indecisive and uncertain. Tumors notoriously represent heterogeneous nature and the temporal alterations in the expression of gene after tissue abstraction from tumor can depreciate the value of gene array data in human specimens. The procedure of biopsy is invasive which bear its own inherent risks. Moreover, it can be painful and technically non-diagnostic. It may further encroach upon the tumor microenvironment and may escape the most essential parts of the tumor. Also, technical factors and tumor heterogeneity limit the acquisition of a representative sample from the similar region of tumor (Kurdziel et al., 2008). Modalities such as Computed Tomography (CT) and magnetic Resonance Imaging (MRI) furnish only limited insight into the tumor pathophysiology as the anatomic images lack molecular information. Further, it becomes arduous to image tumor with CT in case where tumor and its surrounding tissue share same physical properties (e.g. the same density) (Seaman et al., 2010; Evans, 2008). Recent innovations in the fields of molecular biology and chemistry coupled with improved understanding of the mechanisms of disease through proteomics, genomics and animal models has provided impetus for the development of molecular imaging. Molecular imaging can be better stated as the visualization, characterization and measurement of biological processes at the molecular and cellular levels in humans and other living systems (Mankoff, 2007). It provides minute details about the cellular and molecular events ensuing inside the body and has a great prognostic value, allowing precise staging and stratification of patients for appropriate treatment planning which is further relies on staging and the pathways that are atypical for each patient.
Moreover, the procedures involved are non-invasive, painless and safe (Seaman et al., 2010). Molecular imaging has evidently paved way for more sensitive approaches that allows early detection of cancer along with the exceptional insights into the molecular pathogenesis of evolving cancers. The temporal and dynamic details dispensed by non-invasive molecular imaging are more valuable in the investigations involving different experimental animal models (Naik et al., 2008).

Nuclear medicine is that branch of molecular imaging that has asserted its role as a dynamic medical speciality exploiting the nuclear properties of radioactive and stable nuclides for diagnostic and therapeutic interventions. The procedures involved in this approach are non-invasive and dispenses a broad array of tools for probing normal and diseased states at the molecular and cellular levels and also defines response to the different treatment strategies. Investigation and analysis of the uptake of radiotracer and its specific turnover in target organ help in predicting the health status of an individual. The functional processes that underscores the basis of nuclear medicine include interactions between proteins, blood flow and metabolism of the tissue, expression of cell receptors in normal and diseased state, intracellular interactions, cell trafficking, tissue invasion, and apoptosis (Advancing Nuclear Medicine Through Innovation, 2007). SPECT and PET imaging in combination with CT are two such nuclear medicine imaging modules that are currently being used in the medical centres worldwide for the detection and therapeutic applications. Different SPECT and PET radiotracers (e.g. $^{99m}$Tc, $^{201}$Tl, $^{18}$F-FDG, $^{131}$I etc) are presently being used and still many are under investigation for their potential application in the field of nuclear medicine. Although several reports document the importance of $^{18}$F-FDG PET in detecting and staging many different tumors such as lung, breast, and colon cancers (Strauss and Conti, 1991; Conti et al., 1996), yet several disadvantages posed by $^{18}$F-FDG necessitates the development of new radiopharmaceuticals for the detection of cancer. It is not a tumor-specific probe and can also be found to be accumulated in the non-neoplastic pathologic conditions such as infections (Alavi et al., 2002; Sumpe et al., 2000; Bakheet et al., 1998), autoimmune disease such as Grave’s disease, (Yun et al., 2001; Boerner et al., 1998) tuberculosis and sarcoidosis (Chen and Chen 2003; Bakheet et al., 1998; Yasuda S et al., 1996). Further, the uptake of $^{18}$F-FDG can be augmented by inflammatory induced modifications like postoperative healing scars and postradiation therapy. The limitation posed by PET with regard to its intrinsic lower limit of spatial resolution is not observed in SPECT imaging. Moreover, the readily available $^{99m}$Tc
radiopharmaceuticals, conjoined with the favourable nuclear properties of this radionuclide, make it a radiotracer of choice that can be best utilized for the development of new probes.

The favourable characteristics possessed by curcumin and zinc make them potential candidates to be investigated against several ailments. These phytochemicals have the added advantage of being safe, non-toxic, easily accessible and their availability at low cost. The spectrum of benefits acquired by these phytomedicines has been unfolded by several researchers in the past. It has also been proven that the potency of a single chemopreventive agent can be augmented by simultaneous or sequential supplementation of multiple agents. It has been proven that curcumin when given in conjunction with chemotherapeutic drugs augments their therapeutic effect. Therefore, it seemed probable to us to further investigate the chemopreventive excellence of curcumin and zinc in synergism against 1,2 dimethylhydrazine (DMH) induced colon carcinogenesis in rat models. Both the agents under investigation share some similar features (such as inhibition of proliferation, inflammation and induction of apoptosis) in terms of their molecular mechanisms which intrigued us to investigate their role in combination for synergistic chemopreventive effects against colon carcinogenesis.

Also, the excellent properties of curcumin impelled us to further investigate its role as a diagnostic agent in detecting cancer. So far, there are just a very few reports that asserts the radiolabeling and characterization of radiolabeled curcumin. Pignedoli et al (2010) reported radiolabeling of Re(I) and $^{99m}$Tc(I) complexes of curcumin, its un-substituted derivative and asymmetrical Curcumin conjugated to glucose but have not investigated its efficacy in targeting malignant lesions in experimental animals including its biokinetics.

Therefore, the present study was conceived with an aim to label curcumin with $^{99m}$Tc so as to develop curcumin not only as a diagnostic tool but also to assess its chemopreventive efficacy when used in combination with zinc against colon carcinogenesis.

The specific objectives of the present study are following:

- To label Curcumin with $^{99m}$Tc.
- To characterize $^{99m}$Tc labeled Curcumin and perform quality control parameters.
- To perform in vitro protein binding test and lipophilicity test of the prepared radiopharmaceutical.
To study the distribution pattern of the radiopharmaceutical in different organs in normal and tumor bearing rats as a function of time after oral administration.

To analyze the bio-distribution pattern of the prepared radiopharmaceutical in different organs as a function of time after its intravenous administration in both normal and tumor bearing rats.

To perform scintigraphic studies for analyzing the bio-distribution of $^{99m}$Tc labeled Curcumin albumin complex in experimental rat models.

To induce colon carcinogenesis in the rat models with the help of 1, 2 dimethylhydrazine (DMH) and to further classify it histologically at 8 weeks and 16 weeks duration using Hematoxylin and Eosin (H&E) staining.

To demonstrate statistically the chemopreventive effect of curcumin and zinc against DMH induced colon carcinogenesis by assessing tumor incidence, multiplicity, tumor volume and ACF’s.

To assess the total sialic acid levels in the serum or normal and treated rats.

To demonstrate the effect of curcumin and zinc in modulating lipid peroxidation levels during DMH induced colon carcinogenesis.

To determine the membrane stabilizing effect of curcumin and zinc separately and in combination on normal and tumor bearing rats.

To determine the modulatory effect of curcumin and zinc on the expression of proteins like caspases 3, caspases 9, p53, TNF- \(\alpha\), COX-2 and bcl-2 using western transfer analysis in the colon tissues of normal and tumor bearing rats.

To demonstrate apoptosis using DNA fragmentation and TUNEL assay in the normal and treated rats.

To investigate the effect of curcumin and zinc separately and in combination on $^{14}$C-Glucose uptake and turnover and $^{3}$H-Thymidine uptake.

To analyze $^{65}$Zn Biokinetics and bio-distribution in the normal as well as treated rats.