CHAPTER I

INTRODUCTION

INTRODUCTION

Cancer is a complex disease, resulting in the production of tumors (metastasis). There have been some attempts to understand the role played by trace elements in either initiating or promoting or inhibiting the growth of cancer (Maenhaut et al., 1987; Tanaka et al., 1987; Valkovic et al., 1993; Yin et al., 1996; Kubala – Kukas et al., 1999; Ashok et al., 2001; Reddy et al., 2002). Metals must act intracellularly, if any, interaction or equilibriation with the nucleic acids or enzymes is to take place. By themselves metals are unable to enter cells, they may be transported, however, as a part of a chelate system (Frust, 1963). One of the widely studied groups of complexes in cancer chemotherapy is that of noble metals like platinum. Platinum coordination complexes in cancer chemotherapy and the possible mechanism of their anti-tumor activity have been widely reviewed (Rosenberg. 1985). Comparison between the anti-tumor action of rhodium (II) carboxylated with enzymes (Howard et al., 1976) and between the rhodium and ruthenium complexes with platinum complexes (Giraldi, et al., 1977), the anti-leukaemic properties of 46 organoplatinum complexes (Meischen, et al., 1976) and several others have widely been studied. Cis-dichloro-diamine Platinum (II)(Cisplatin) has been effectively used against variety of cancers including head and neck cancer (Loehrer, et al., 1984). Also, a novel platinum complex with binucleating napthazarinate ligand (diamine –dichloro) - (napthazarinato) – diplatinum (II) has been proved to be potent anti-neoplastic activity (Papageorgiou and Christianopoulou,

1986) comparable with that of the cisplatin with much lower toxicity. The primary mechanism of the anti-tumor activity of cisplatin probably resides in its ability to inhibit DNA synthesis (Ciccarelli et al., 1985). However, the drug is also toxic to several tissues like kidney, gastro-intestinal tract and bone marrow (Von Hoff et al., 1979) and the severity of cisplatin nephrotoxicity is related to platinum concentration in the kidney (Barnard, 1989).

Though the anti-cancer activity of different platinum metal complexes appears to be promising, yet platinum being unnatural to the living body from their biological view point, the system does not develop any effective mechanism for their rejection like those metals viz. copper, iron, etc. Hence, it can rationally be argued that, if any, complex having anti-cancer activity of these biologically involved metal ions are available, its toxicity would be lessened reasonably to a great extent.

Numerous compounds of copper are known to contain organic ligands exhibiting antiproliferative activity in them. The ligands of the schiff base type have been found to possess antiproliferative activity against experimental animal tumors was revealed for the natural complexes trans – bis (salicylaldeoximato) copper II (Elo & Lumme, 1985) as well as for the copper (II) complex salt 15 of the macro-cyclic ligand tetrabenzo [b, f, j, n] – 1, 5, 9, 13 – tetra-azacyclo-hexadecene (Sadler et al., 1984). Another complex, Dipthalate complex of copper (II) have been found to be active against leukemia and many of the copper complexes (Petering & Petering,

1975; Agarwal & Sartorelli, 1975; Pickart and Thalar, 1980; Saryan et al., 1981).

Administration of copper (II) dimethyl-glyoxime chelate Cu (dmg $H)_2$ has been found to inhibit the tumor growth of the animal with Ehrlich Ascites Tumor and Sarcoma 180. Different bis (thiosemicarbazone) complexes of copper (II) such as copper pyruvaldehyde, bis – (thiosemicarbazone), copper – 2 – keto-3-ethoxybutyraldehyde bis- (thiosemicarbazone) have been found to be active against varieties of tumors (Das, 1989). They are known as CuKTS and are remarkably effective against different types of tumor, such as Walker 256, nitrogen mustard resistant carcinosarcomas, Sarcoma 180, spontaneous mammary tumor of DBA_2 mice; but it is not effective against leukemias. Administration of CuKTS also shows anti-tumor activity but it is toxic to the host (Das, 1989). Certain ligands such as 1-formyl isoquinoline thiosemicarbazone and 5-hydroxy-2-formyl-pyridine thiosemicarbazone have an excellent anti-tumor activity in some animals.

Lumme et al. (1984) established that salicylaldoxime and 2,4-dihydroxy benzaldoxime copper (II) complex are found to be quite successful against L-1210 and Ehrlich ascites carcinoma. Patil et al. (1989) had synthesized and characterized Cu (II) complex coded as BRH_2 (5-phenylazo-3-methoxy salicylidene thiosemicarbazone) and used in the growth inhibition of P388 lymphocytic leukaemia cell sensitive and resistant to adriamycin, however, their toxic effects are yet to be investigated.

The role of elements either in macro or micro quantities in the biological system has been established. The research of the relation between cancer and trace element contents is especially a hot point (Schrauzer et al., 1996). There have been some attempts to understand the role played by trace elements either in initiation and promotion or inhibition of the growth of cancer (Valkovic et al., 1993; Yin et al., 1996; Kubala-Kukas et al., 1999; Ashok et al., 2001; Reddy et al., 2002). Their role in the process of development of the progression or inhibition has been studied as mentioned elsewhere. Of the several elements copper has been found as one of the active metals. Among the trace elements copper, zinc and iron are present in significant quantities in blood. Copper and zinc may have some role in promoting tumor cell growth (Schwartz, 1975). The serum copper level and serum zinc level were reported to correlate with various cancers (Martin – Lagos et al., 1997; Poo et al., 1997; Magalova et al., 1999; Ferrigno et al., 1999). Magalova et al. (1999) reported that serum copper level in subjects with gastric and colorectal cancer were increased. Serum copper level did not correlate with breast cancer and serum zinc level was slightly decreased in breast cancer (Magalova et al., 1999).

Increased serum copper and ceruloplasmin have been reported in various types of malignant tumors in man (Schwartz, 1975; Chakravarty, et al., 1984). Huang et al. (1999) reported that the mean serum copper level in patients with breast cancer was significantly higher than the control group and the mean serum selenium level were significantly lower than the

control, while the concentrations of zinc and iron remain unaltered. It was further established that in cancer patients' serum copper levels are of considerable importance in assessing disease activity in prognosis (Hrgoveic et al., 1973; Roguljic, et al., 1980). Elevated serum copper and decreased serum zinc levels have been detected in patients with Sarcoma (Breiter et al., 1978; Huhti et al., 1980; Inutsuka & Arki, 1978). Some researchers suggested that the copper content in serum can be used as a marker or screening test for diagnosing ovarian or hepatic cancers (Miatto et al., 1985; Margalioth et al., 1986). Ferrigno et al. (1999) concluded that serum copper level, serum zinc level and Cu / Zn ratio have some prognostic significance in lung cancer. It has been demonstrated as well that a Zn – metalloprotein implicated in tumor invasion and metastases, is highly expressed in advanced stage Neuroblastoma (Ara et al., 2000).

Bishayee and Chatterjee (1995) have established Vanadium as a significant biological regulator in assessing the physiological and biochemical state of the animals in a dose related manner. Relatively, high values for Rb, Zn and Sc in the glioblastomas and medulloblastoma, Rb and Se in neurinomas, Zn in ependynomas and Rb in spongioblastomas have been worked out (Schicha et al., 1972). In relation to normal cerebral cortex, brain tumors showed increased concentration of Rb, Zn and Se with increasing malignancy. Thus it has been proved that several trace elements have appeared as marker in the process of carcinogenesis and they could well be

used in the prognostic process. Se, Mg, Zn, Fe, Mn and also Cu could be used as biomarkers in the assessment of neoplastic diseases.

Of the enzyme system associated with carbohydrate metabolism, LDH needs special call, since the LDH has been considered as the biochemical tumor marker in a variety of malignancy (Singh et al., 1993; Burgess et al., 1963; Kawakatsu et al., 1963). The analysis of LDH isoenzyme spectrum proves to be very informative tool giving an answer to numerous questions and makes it possible to reveal the unnoticed feature that is specific of all types of human neoplasm, both benign and malignant tumors of most diverse localization as well as leukemic blast cells. This feature shows a sharp decrease in the activity of LDH isoenzyme I. Secondly, on the basis of such analysis it is possible to distinguish reliably a malignant neoplasm from a benign neoplasm, especially by employing LDH V / LDH I coefficient which may be of prognostic value (Shapot, 1980).

A change in the topographical pattern is a common feature associated with the process of malignant transformation and such change may contribute to the pathological outcome of cancer with regards to the process of neoplastic invasion. Many structural and functional properties of malignant cells are related to changes in the cell surface, cell membrane (Hynes, 1979). The presence of cell-cell contact has been described in solid tumor and in few ascitic tumors (Hayashi & Ishimaru, 1981). The pattern of cell distribution cell-cell association and the effect of metal-based drug may provide an

insight in the mechanism of growth inhibition of lymphoma cells. Scanning Electron Microscope (SEM) studies gives major clue on the pattern of cell surface topography and cell-cell association with reference to the effect of cisplatin on ascitic Dalton's lymphoma cells (Prasad & Arjun. 1991). Cisplatin treatment showed significant changes in the arrangement, movement of ruffles and blebs over the cells (Prasad & Arjun. 1991). The direct disintegration and breaking in the plasma membrane was more pronounced after treatment of cisplatin within 6 to 8 days.

Thiosemicarbazone and their metal chelate are well known for their biological activity and more particularly for their antineoplastic activity. The cisdichloro diamine Pt (II) and bis (thiosemicarbazonate) Cu (II) complex and even Cu and Fe complexes of thiosemicarbazone have been studied extensively. Various copper complexes have been seen to possess cytotoxicity and antineoplastic properties as mentioned elsewhere. Therefore, it has been selected a copper based complex having thiosemicarbazone for evaluating their antineoplastic activities in mice bearing Dalton's lymphoma.

Aims and objectives:

Thus the present investigation has been aimed at:

- To synthesize BRH_2 a copper based drug.
- To assess the effect of BRH_2 on Dalton's lymphoma using certain biomarkers like lactic dehydrogenase.
- To find out the alteration, if any, in the level of Zn, Cu, Se.
- To find out the effect of BRH_2 on the surface topography of Dalton's lymphoma cell.
- To find out the histological changes of host liver, spleen and kidney.