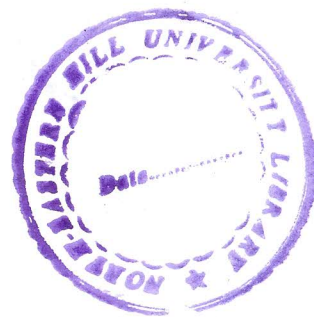


**STUDIES ON THE EFFECT OF CISPLATIN  
ON MITOCHONDRIA IN DALTON'S  
LYMPHOMA - BEARING MICE**



By

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SUBMITTED IN FULFILMENT OF THE REQUIREMENT OF  
THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

OF

**NORTH-EASTERN HILL UNIVERSITY**  
SHILLONG - 793 022, INDIA

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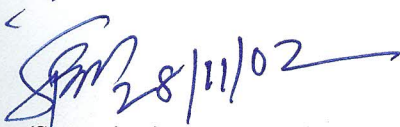
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## Declaration

I, Arpaia Kharbangar, do hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other university/Institute.

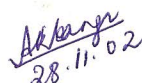
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# **ACKNOWLEDGEMENTS**

*First and foremost I would like to render my heartfelt gratitude to God for the knowledge imparted to us in his never-ending ways.*

*I wish to express my sincere thanks and gratitude to :*

*My supervisor, **Prof. S. B. Prasad**, Department of Zoology, North-Eastern Hill University, Shillong 793022, India, for his ever presence and valuable suggestions without whose help nothing could have been possible.*

*The Head of Department of Zoology, NEHU, Prof. B.B.P.Gupta and his predecessors Prof.K.Chaterjee and Prof B.K. Sharma for giving a conducive atmosphere for the completion of my endeavour.*

*The Head, RSIC and his team for their help and facilities regarding transmission electron microscopic studies and Prof B.B.P. Gupta for the Oxygen electrode facilities.*

*The photographers, Mr A.Shadap and Mr B.K. Das for their endurance to bring out the best.*

*Mr. A. Nongrum and Mr. N. Khongmen for their help in typing out the thesis and the financial support from the North Eastern Council, Govt. of India.*

*Not to forget, I take this opportunity to thank Dr. D. Khyriam, Bonnie, Lotha, Sam, Dr. C. Syngai, Kavita, Aparajita, Sanjeev, Freddy, Carina, Zaiba, Larisha, Pradeep, Bidhyadhar, Quendarisa, Upal, Mumubai, Jenny, Shanwell, Omarlin, Nonnie, Indira, Chumbeni and others who have never let me down with the best they can probably give.*

*Finally, I would like to thank my parents, my husband, Hilson, my daughter, Ibanmer, my sisters, Sarabha, Daphira, Daphikir and Bameda, my brother, Phyrnai, my niece and nephews, Mattibabha, Lamphang and Damebanrap, my family members and other well wishers and dear ones for standing with me through the thickest and thinnest of times.*

*I thank all the persons who had helped me in the experimental work and/or preparation of this thesis, but due to my oversight their names have not been mentioned here.*

*Arpaia*  
28.11.02  
(Arpaia Kharbangar)

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## ABBREVIATIONS

BSA	Bovine serum albumin
DL cells	Dalton's lymphoma cells
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
EDTA	Ethylene diamine tetraacetic acid
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GST	Glutathione-s-transferase
i.p.	Intraperitoneally
LPO	Lipid peroxidation
MDH	Malate dehydrogenase
Mt	Mitochondrial
NPSH	Non-protein sulfhydryl
O.D.	Optical density
PBS	Phosphate buffer saline
ROS	Reactive oxygen species
SDH	Succinate dehydrogenase
SOD	Superoxide dismutase
TSH	Total sulfhydryl

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# INTRODUCTION

## *i) CANCER*

The multiplication of cells in the body is carefully regulated. In an adult, some cell types e.g. nerve cells do not divide while others like skin and progenitors of the blood cells divide throughout the life in order to replace billions of cells that die everyday. Thus, a very carefully controlled programme exists to determine the multiplication of different types of cells in the body.

Eukaryotic cell division occurs in four well defined phases: synthesis or S phase, gap 2 or G2 phase, mitosis or M Phase and gap 1 or G1 phase. After passing through mitosis and into G1, a cell either continues through another division or ceases to divide, entering a quiescent phase (Go) that may last hours, days or the lifetime of the cell. When a cell in Go begins to divide again, it re-enters the division cycle through the G1 (Hunt and Nasmyth, 1997). The cell cycle is controlled by a family of protein kinases that are the heterodimers with a regulatory subunit, cyclin and a catalytic subunit, cyclin-dependent protein kinase (CDK). The cell division is also regulated by a family of extracellular growth factors (Weinberg, 1996).

If occasionally, the exquisite control mechanisms of regulating cell multiplication break down, a cell begins to grow and divide in an uncontrolled manner. Descendants of such cells inherit the propensity to proliferate without responding to regulation and expand indefinitely to develop as a lump, which is commonly referred to as a tumor. Defects in the synthesis, regulation or recognition of growth factors may also be involved in developing a tumor (Rubin, 1985).

Tumors are strictly defined as neoplasm, although the term tumor may be applied to any swelling (Vincent, 1985). The terms neoplasm and tumor are commonly used interchangeably (Friedberg, 1986). Tumors violate the basic homeostatic principle of

the body and ideally fall into one of the two categories, the slowly growing 'benign' and the rapidly growing 'malignant' forms which are invasive, disseminating and show metastasis (Vincent, 1985). The spread of tumor from the primary organ or tissue in which neoplasm initially occurs to secondary sites is called metastasis (Fidler and Hart, 1982).

The development of malignant tumor is a multistep process characterised by a progression of genetic alterations in a single line of cells. Various cancer causing agents are called carcinogens. These carcinogens could be (i) physical agents (ultraviolet rays,  $\gamma$ -rays, X-rays) (ii) chemicals (Benzpyrene, aflatoxin B1, benzanthracene, methylcholanthrene, lead, carbon tetrachloride, asbestos) and (iii) viral agents (Rous, sarcoma virus, polyoma virus, simian virus 40, adenovirus, Epstein barr virus etc) (Fearon, 1997).

In the carcinogenesis two categories of genes (tumor suppressor genes and oncogenes) may be implicated. Tumor suppressor genes (about 20 in human) normally act as cell's brakes. They encode proteins that restrain cell growth and prevent cells from becoming malignant. The transformation of a normal cell to a cancer cell is accompanied by the loss or decrease of function of one or more tumor suppressor genes. Most of the proteins encoded by tumor suppressor genes act as negative regulators of cells proliferation which may be as transcription factors (p53 and WT1), cell cycle regulators (RB and p16), components regulating signalling pathways (NF1) and components regulating RNA polymerase II elongation (VHL). Thus, their elimination contributes and promotes uncontrolled cell growth (Haber and Harlow, 1997).

In contrast to tumor suppressor genes, oncogenes (Greek; onkos, a tumor) encode proteins that promote the loss of growth control and conversion of a cell to a malignant state. Oncogenes are generally derived from proto-oncogenes which are genes that encode proteins having a function in the normal cell. These oncogenes products act in many

ways, for example, i) as growth factors or their receptors e.g. SIS oncogenes derived growth factor (PDGF), erbB oncogenes which directs the formation of a receptor, ii) as cytoplasmic protein kinases e.g. RAF that heads the MAP kinase the primary growth controlling signalling pathway in cells, iii) as nuclear transcription factors e.g. myc oncogene and iv) as the products that inhibits apoptosis. The ras oncogene (Hunter, 1997).

Malignant tumors are commonly referred to as cancers. The word cancer (= crab) suggests its capacity to reach out and cling tenaciously to adjacent tissues. Cancer is considered to be a dynamic developmental disorder and a disease of failed differentiation (Rubin, 1985). Cancer cells have unlimited life span, require no growth factors and exhibit anchorage independence for growth. Cancer cells are rounded/convex shape, show reduced adhesion to substratum with the loss of contact inhibition of movement and multilayering in culture. Cancer cells also acquire the ability to produce proteolytic enzymes, altered antigenicity, increased negative surface charge, disorganised cytoskeleton etc (Hynes, 1979).

Cancers are generally classified into three broad groups: carcinomas and leukemia/lymphomas (Cairns, 1986). About 85% of cancers are carcinomas, tumors that arise from endodermal or ectodermal tissues such as skin or the lining of internal organs and glands, colon, breast, prostate, ovary, lungs etc. (~5%) arise less frequently and are derived from mesodermal connective tissues such as bone marrow, fat and cartilage. The leukemias and lymphomas are cancers of haematopoietic cells.

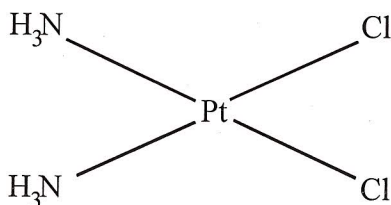
Although the idea about cancer dates back to the late 17<sup>th</sup> century (Currie, 1982) and in spite of a lot of scientific advancements, cancer remains an unpredictable and fearful disease. Considerable efforts have been directed towards improving the diagnosis and treatment of cancer. Surgery, chemotherapy and radiotherapy

are the three main ways of widely accepted treatment for cancer. In chemotherapy, cisplatin plays a pivotal role and can be used singly or in combination with radiotherapy and/or surgery in the treatment of many cancers.

## ii) CISPLATIN

Rosenberg et al. (1965) while studying the effect of electric fields on bacterial (*Escherichia coli*) growth, noted that the bacterial growth continued but cell division was inhibited. The inhibition of cell division was attributed to the formation of amminechloro compounds from platinum electrodes and ammonium chloride in the growth medium and it was subsequently identified as *cis*-diamminedichloroplatinum (II) (Rosenberg et al., 1967). It is now commonly known as cisplatin.

The cell division inhibiting property of cisplatin evoked to study the antitumor activity of cisplatin and it was recognized as a potential antitumor agent (Rosenberg et al., 1969). Now cisplatin has been established to be a potent antitumor drug against a wide spectrum of experimental tumors such as leukemia L1210, DMBA mammary carcinoma, Rous sarcoma, Dunning ascites leukemia, Walker 256 carcinoma (Kociba et al., 1970; Sarna and Sodhi, 1978; Rosenberg, 1985) and also in human malignancies such as ovarian and testicular tumors, bladder carcinoma, head and neck cancer (Pil and Lippard, 1997; Lebwohl and Canetta, 1998).



**Structure of Cisplatin**

