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Academic profile:

2003- Feb. 2006	PhD in Life Sciences, Jadavpur University, Kolkata, West Bengal, 700032, India **Research Topic: "**Molecular mechanism of apoptosis in tumor cells induced by Diospyrin (D1), a plant derived bisnapthoquinonoid compound and its derivative (D7) in combination with radiation"
2001-2003	MSc Biotechnology with Distinction A.P.S University, Rewa, MP, India
1997 - 2001	BSc with Biotechnology, Zoology, Chemistry Ranchi University, Ranchi, Jharkhand, India

Awards and Fellowships:

- Qualified **C**ouncil of **S**cientific and **I**ndustrial **R**esearch National Eligibility Test for Lecturership – 2003
- Qualified Graduate Aptitude Test (GATE) – 2004.
- Awarded Department of Atomic Energy (BRNS) fellowship.

Summary of Ph.D. work

Guide	**Co Guide**
Dr. Banasri Hazra	Dr. K.P.Mishra
Dept of Pharmaceutical technology	Head, Radiation Biology and Health Sciences Division
Jadavpur University	Bhabha Atomic Research Centre
Kolkata- 700 032	Mumbai- 400 085

1. Enhancement of the tumour inhibitory activity, *in vivo*, of diospyrin, a plant-derived quinonoid, through liposomal encapsulation
[**Toxicol. lett, 157, 109-117(2005)]**.

Diospyrin, a bisnaphthoquinonoid plant product, shows inhibitory activity against murine tumour *in vivo* and human cancer cell lines *in vitro*. Efforts have further been made to obtain synthetic derivatives of diospyrin with the objective of improved therapeutic effects. With the goal to reduce the toxicity towards normal cells and enhance the efficacy to tumour cells, diospyrin was encapsulated in liposomal vesicle and its anti-tumour potential was observed on the growth of Ehrlich ascites tumour in Swiss mice. It was found that the longevity of the tumour-bearing mice was significantly enhanced by treatment with liposomal diospyrin as compared with the free drug. Biochemical assays of liver function enzymes, viz. LDH, AP, GOT and GPT in serum of the tumor-bearing mice showed substantial alterations in the activity of these enzymes. These parameters were, however, restored to near normal level when the drug treatment was given encapsulated in a liposome. Histopathological studies on the liver tissues indicated a near normal pathological status in the treated animals despite being challenged by tumour cells. This study on diospyrin has shown, for the first time, an enhancement of its antitumour effect *in vivo* through liposomal encapsulation.

Relevant skills: Induction and propagation of tumors in Swiss mouse models, Liposome Preparation and characterization, animal handling, estimation of enzymes, UV/Visible/Fluorescence spectroscopy

2. Cytotoxicity of diospyrin and its derivatives in relation to the generation of reactive oxygen species in tumour cells, *in vitro* and *in vivo* (Communicated)

Diospyrin (D1) could inhibit the growth of Ehrlich Ascites Carcinoma (EAC) *in vivo.* Hence, derivatives of D1 were synthesized, some of which were cytotoxic to human cancer cell lines. Presently, D1 and its alkyl ethers were evaluated in relation to their cytotoxicity, and the capacity to generate reactive oxidative species (ROS) in tumour cells. D1 was isolated from *Diospyros montana* Roxb, and converted to alkyl ethers (D2 and D7), and were assessed against EAC cells *in vivo*. Evaluation of cytotoxicity in EAC and MCF-7, a cancer cell line, was carried out by MTT assay. ROS generated in MCF-7 cells in presence of the quinonoids was determined fluorimetrically. Cyclic voltammetry (CV) was performed to assess the redox behaviour of these quinonoids. Our results showed the inhibitory activity of various diospyrin derivatives in the order D7>D2>D1 in EAC cells. This order was corroborated by the findings from MTT assay on EAC and MCF-7 cells, and fluorimetric estimation of ROS generation and lipid peroxidation. Thus this finding indicates that D1, a hydroxynaphthoquinonoid, when converted to its alkyl ether, could generate more ROS and showed enhanced cytotoxicity against tumour cells *in vitro* and *in vivo*.

Relevant skills: Maintenance and preservation of mammalian cell lines, determining cyototoxic effects of drugs by MTT assay, evaluating ROS levels and lipid peroxidation using fluorescent dyes.

3. Molecular mechanism of apoptotic death in tumor cells by diospyrin and its derivative: the role of mitochondrial pore transition and oxidative stress (Communicated)

This study was aimed to evaluate anticancer properties of plant-derived bioactive bisnapthoquinonoid, diospyrin (D1) and its diethylether derivative (D7) in terms of induction of apoptotic death in human breast cancer cell line, MCF-7. Mechanism of drug induced apoptosis in MCF-7 cells have shown that cytotoxic effect of these quinonoids was mediated by generation of oxidative stress in the tumour cells, D7 being more effective than D1. Both the compounds were found to produce reactive oxygen species (ROS), mainly involving mitochondrial dysfunction concomitant with its swelling and membrane potential alteration ($\Delta\psi_m$) which correlated with the opening of the mitochondrial permeability transition pore. The induced effects were found reversed by the inhibitors of permeability transition pore complex. The drug-induced apoptotic cell death was further characterized by measuring the peroxidation of mitochondrial cardiolipin, which resulted in the release of cytochrome *c* in the cytosol. The cytochrome *c* release was found to be dependent on mitochondrial membrane pore transition as well as on the intracellular oxidative status. It is further shown that apoptosis induced by D1 and D7 involved release of mitochondrial apoptosis inducing factor, which suggested the caspase independent pathway for cell death. In addition, the notably lower cytotoxicity of D1 and D7 to normal cells as compared to tumour cells may provide an opportunity to develop new strategy for efficient treatment of cancer patients in the clinic.

Relevant Skills: Cell culture technology (MCF-7, HaCaT), Western blotting, Auto-radiography, ELISA, Fluorescence microscopy, Flow cytometry

4. Calcium induced apoptosis by diospyrin diethyl derivatives in tumor cells (Under preparation)

Mitochondrial membrane permeabilization is regarded as an important hallmark of early apoptosis. Induction of the permeability transition pore (PTP) and elevation of the cytosolic calcium concentration may play a role in this process. Elevation of the cytosolic calcium is used as general signaling mechanism, which activate different process in a single cell system. Breast adenocarcinoma cell was used as a model for study of calcium signaling induced by diospyrin derivative (D7), a synthetic bisnapthoquinonoids. Our results indicated that D7 cause sustain release of calcium in concentration dependent manner. Moreover the release calcium is found to be cytosolic. This release of calcium is associated with the irreversible loss of mitochondrial membrane potential as detected by JC-1 .This loss of potential is found to be calcium dependent. To find out the factor involve in the calcium signaling, our study with the specific inhibitor of Phopholipase C (PLC) and with an antioxidant showed that the release of calcium is function of oxidative stress and PLC. In further calcium dependent proteases study showed that this D7 cause activation of calpain (a calcium dependent proteases) and calcineurin (a calcium dependent metalloenzyme) protein and this activity is reversed by NAC. Thus our results indicated that oxidative stress generated by D7 is an important factor for calcium signaling induced by these quinonoid. .

5. Modulation of radiation response by diospyrin derivative: *In vitro* and *In vivo* study (under preparation)

The rationale for combining antitumor agent with radiation in cancer therapy can be considered at several levels and this combination may provide general therapeutic advantage because of the differential toxicity associated with each treatment modality. Further underlying mechanism of action for antitumor drug suggest the potential for synergy with radiation deriving from physical interaction between drug and DNA as well as differential expression of genes. In this study, we examine the capacity of Diospyrin derivative (D7) to modulate radiation response in human tumor cell lines, in vitro (HT1080) and in vivo (mouse fibrosarcoma) and explore the potential mechanism underlying these interactions. Cell was exposed to various doses of radiation (2 & 5Gy) + pretreatment with various concentration of D7 and then clonogenic study was done by staining with crystal violets. Whole cell lysate were evaluated for NFkB and PARP studied. Caspases study was done for apoptosis. Results indicated that D7 sensitize the radiation effect by regulating the NFkB gene and cause caspase dependent apoptosis.

Relevant skills: EMSA (Electro Mobility Gel Shift Assay), Radiation techniques

List of Publications:

1. Hazra. B., Kumar.B, Bishwas.S, Pandey.B.N. and Mishra. K.P., **Enhancement of the tumour inhibitory activity, *in vivo*, of diospyrin, a plant-derived quinonoid through liposomal encapsulation** [**Toxicology letters**, 157, 109-117(2005)].

2. Hazra. B., Sharma. M.D, Kumar. B, Basu. S, Pandey.B.N., and Mishra. K.P., **Cytotoxicity of diospyrin and its derivatives in relation to the generation of reactive oxygen species in tumour cells, *in vitro* and *in vivo*** (**Accepted in Chemotherapy**)

3. Kumar.B, Kumar.A, Pandey. B.N., Mishra.K.P, and Hazra.B, **Molecular mechanism of apoptotic death in tumor cells by diospyrin and its derivative: the role of mitochondrial pore transition and oxidative stress (Communicated)**

4. **Calcium induced apoptosis by diospyrin diethyl derivatives in tumor cells (**MS under preparation)

5. **Modulation of radiation response by diospyrin derivative: *In vitro* and *In vivo* study (**MS under preparation**)**

REFERENCES

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