"Summary"
A natural daylight absorbing pigment of particular interest is vitamin B$_2$ or riboflavin which is present in all aerobic cells. The photodynamic action of riboflavin is known to involve the generation of reactive oxygen species. Studies in this laboratory have formerly shown the damaging effect of this sensitizer on several biological molecules. Hitherto the effect of certain biologically relevant endogenous/externally added compounds can inhibit/promote photodamage in living system. In this context an important class of biologically active and commercially valuable compounds which could potentially interact with riboflavin in humans may be the externally administered drugs. Since the vitamin and drug can occupy common locations in a biological system, light mediated interactions between them can initiate desirable/undesirable consequences. With reference to this framework of sensitizer-drug interactions, a recent report from this lab examined the combination of riboflavin and aminophylline. Aminophylline is a routinely used medicine for various respiratory disorders especially asthma. Aminophylline in combination with riboflavin was found to augment degradative capacities of the sensitizer with aminophylline getting oxidized in the process. Chemotherapeutic drugs are an essential category of compounds that need to be probed in this milieu.

The aims of photogenotoxicity studies are to evaluate the 'genetic health' of surviving cells. During the past several years, phototoxicity has been studied at the molecular level. Phototoxic effects span from the cytotoxic-apoptotic effect to the induction of primary DNA damage and a variety of stress acting on the cell. Ultimately, it can lead to the induction of hereditary DNA modification. The development of new drugs has highlighted the necessity to develop the assessment of phototoxicity in the safety evaluation of compounds. The study is proposed to specifically address all the consequences of riboflavin-induced toxicity, the \textit{in vitro} methods that can be proposed and used to screen for toxicity of sensitizer riboflavin and the photosensitization process resulting from the activation of drugs by light. Biological systems and endpoints of interest in that
particular objective are listed. The clinical relevance of interaction mediated consequences of drug and sensitiser is represented in patients undergoing radiation and chemotherapy cumulatively, wherein the potential interactions need to be carefully considered.

Energy in the visible and UVA (315-400 nm) region can be absorbed by endogenous flavins and transferred to biomolecules where it causes damage. Photosensitized damage to biomacromolecules such as DNA and proteins, participates in phototoxicity, photogenotoxicity and solar UV-carcinogenesis. Chemoprevention of photosensitized damage is an important method to curtail the phototoxic effects of endogenous sensitizers. The aforementioned are a few phototoxic effects exhibited by riboflavin, the sensitiser. It is rational to postulate scientifically that an agent rendering protection to the above would be entitled as 'photochemoprotective' and the analysis 'photochemoprevention'.

In the present analysis the impact of photoactivated riboflavin has been elucidated in the presence of chemotherapeutic drugs cisplatin and 5-fluorouracil. Perhaps, the potential properties of both drugs in the presence of riboflavin as a sensitiser and the ongoing impact of interactions on biological molecules have not been investigated. In this study a conscious and planned effort was designed to explore not only the degree of these interactions and understanding the photo mechanism alone but also the concurrent influence on biologically relevant molecules. The thesis is in continuation with our previous drug based study and aims to extend our effort into the sensitiser-drug array of interactions.

In view of this, first chapter of the thesis specifically addresses cisplatin mediated inhibition on photoinduced oxidative damage by the sensitiser, riboflavin. The chapter has been bifurcated to deal with proteins and DNA individually. Binding of the sensitiser to the target biomolecules enhanced with photoactivation. Significantly, the observed binding correlates with firstly, the formation of free radicals and secondly, the degradative efficiency of riboflavin. A prominent decline effect by cisplatin on riboflavin induced structural
alterations, impairment of enzymatic activity and damage to proteins was indicative of curbed photodecomposition. This corresponds to reduced photoexcitation of the sensitizer in presence of this particular platinum drug. The second unit to the first chapter reaffirms the occurrence of an analogous series of inhibition reaction. DNA degradation in cells such as lymphocytes implies genotoxicity by riboflavin. Using a cellular system of lymphocytes isolated from human peripheral blood and alkaline single cell gel electrophoresis, it was confirmed that cisplatin is indeed capable of rendering protection to DNA breakage in cells also. An interesting finding in the provisos of cisplatin was reduced generation of reactive oxygen species in cells. Nevertheless, a principal reason contributive to cytotoxicity of cisplatin is free radical production in normal cells. The concentration of drug utilized in our study is pharmacologically relevant and is used clinically for patients undergoing therapy. Furthermore cisplatin is equally efficient in causing DNA damage in this system. The complex riboflavin-cisplatin reduced the degradation catalyzed by each of them individually. The drug forms a complex with native entity of riboflavin. This complexation plausibly inhibits the photoexcitation phenomenon and reduces the damaging potential consequently.

In the second chapter of the thesis, drug 5-fluorouracil different in class and mechanism of action has been prudently chosen to illustrate the oxidative potential of sensitizer in its presence. The drug behaves in a biphasic manner in case of both proteins and DNA with lower concentrations of the drug inhibiting the photosensitization phenomenon. Intermolecular interaction is ascertained to be the plausible reason for the anomalous pattern displayed by this particular drug at higher concentrations. The inhibition trend was similar with both the target biomolecules. The third and final chapter of the thesis addresses the spectral properties of native and excited riboflavin in the presence and absence of both drugs and vice versa. Cisplatin inhibited the excitation of riboflavin and retained it in its native condition strengthening the behavior to be postulated as
'photochemoprotective'. In addition, 5-fluorouracil is shown to behave in an atypical biphasic manner.

Based on our research on the role of riboflavin as photooxidative stress, we describe here the identification of the platinum drug cis-diaminedichloro-platinum(II) as a chemopreventive agent for photoprotection. The causative role of photoexcited state in photodamage suggests that direct molecular antagonism of photosensitization reactions could be by physical quenchers. This leads to screening of the damaging facet offering a novel chemopreventive opportunity for photoprotection. Akin to studies conducted earlier where compounds have been identified as pronounced quenchers of photoexcited states activity (QPES). QPES compounds antagonize the harmful excited state chemistry of endogenous sensitizers by physical quenching, facilitating the harmless return of the sensitizer excited state to the electronic ground state by energy dissipation. In our investigational consequence cisplatin displayed screening efficacy in riboflavin-sensitized photodamage by complex formation. It is believed that cisplatin is atypical quencher that binds with the sensitizer offering protection at the protein and DNA reaction scheme. Furthermore, photoprotection of human peripheral lymphocytes exposed to photoilluminated riboflavin has been demonstrated. Favorably, undesirable aspect of cisplatin is suppressed substantially by this interaction. This preventive mechanism may be used for the novel chemoprevention of phototoxicity, photo-genotoxicity and solar-UV carcinogenesis.