SUMMARY

Majority of anticancer drugs used for cancer chemotherapy are highly toxic thereby restricts their wider use. These drugs are not very selective to cancer cells and showed parallel toxicity toward continuous and rapidly growing normal cells. Most of the anticancer drugs exert their cytotoxic effect by inducing apoptosis and therefore apoptotic pathway have often implicated in chemotherapy regiment. In this context cisplatin is amazing drug in the category of transition metal complexes but it induces numerous untoward side effects. Cancer chemotherapy seeks new approaches to develop precise anticancer drugs which selectively kill cancer cells by inducing apoptosis without affecting normal cells of body. For precise cancer therapy, selective complexes and their anticancer activities towards specific type of cancer are required and anticipated to have a lower systemic toxicity. Among the several non-platinum metals that are currently being investigated for their anticancer activity, Ru, Rh and Ir complexes occupy a prominent position. On the basis of above facts it worthy to undertake this study on anticancer activity of novel Ru, Rh and Ir arene complexes (20 complexes of three different series) and chapter wise summary is mentioned below:

In vitro cytotoxicity and antiproliferation studies were performed on different types of cancer cell lines and normal cells and antitumor activity of selected complexes was evaluated in vivo tumor model. The present study demonstrated that Ru-arene complexes D3 and D13 exhibit higher and preferential cytotoxicity against HeLa cells with least IC$_{50}$ value among all five cancer cell lines studied. However both
complexes did not exert substantial cytotoxicity in normal cells and showed 2-3 fold higher efficacies in killing cancer cells particularly in HeLa cells. Antiproliferative efficacy of complexes was also studied in same cells (HeLa) and found to be increased as exposure time increases with decreased IC$_{50}$ value. However, both complexes did not exert substantial cytotoxicity on normal cell lines and human PBMCs also. On the other hand Cisplatin exert more cytotoxicity on normal cells (HaCaT) as compared to cancer cells. Along with this in vivo study reflected the antitumor property (survivability and tumor regression) of both complexes in DL bearing mice. D3 and D13 at MTD significantly increased the longevity of tumor bearing mice and reduced the tumor growth rate as compared to control and Cisplatin treated groups.

Apoptosis inducing property and effect of both complexes on cell cycle distribution were established in accordance with array of morphological changes ranging from membrane blebbing to formation of apoptotic bodies and followed by DNA fragmentation assay. Morphological investigation confirmed the cell shrinkage, membrane blebbing, nuclear condensation and DNA fragmentation in treated HeLa cells which indicate the apoptotic induction property of complexes. Increased number of cell death by apoptosis seems to main mechanism behind anticancer property of both complexes. Further, the presence of the prominent laddering pattern of DNA fragments on agarose gel confirms apoptosis. Another important hallmark of apoptosis is the externalization of Phosphatidyl Serine (PS) in the plasma membrane was also detected by flowcytometry as large number of treated HeLa cells was found to undergo apoptosis. Apoptotic induction and cell cycle regulation are intimately
related to each other and anticancer property of drugs may be a synergistic effect or result in which cell cycle was affected along with apoptosis induction and together play chemotherapeutic role against cancer. Further, through cell cycle analysis, it can be confirmed that both complexes at 24 h blocked cells in G2/M phase with concomitant increase in Sub G1 phase population of cells in a dose dependent way. Treatment with D3 dramatically increases the population of cells in S phase and promoting delay in cell cycle progression of the HeLa cells. Further mitochondrial aggregation around the nucleus in HeLa cells in treated groups indicate that mitochondrial dynamics were affected with complexes in a dose dependent manner and involved in the mechanism of apoptotic pathway. However mitochondria are often depicted as discrete entities uniformly scattered in the cytoplasm of healthy (control) cells. In this study, both Ru-arene complexes significantly increased intracellular ROS level which may trigger the activation of downstream signals for apoptosis such as activation of related genes and caspases.

Western blot analysis, semi-quantitative RT-PCR and real time qPCR indicated that both Ru-arene complexes induce apoptosis in HeLa cells in a background of elevated p53. In addition, treatment of HeLa cells with Ru-arene complexes slows down cell division. The mitochondria-mediated apoptotic pathway appears to be central to the signaling of cell death in mammalian cells. The activity of pro-apoptotic and antiapoptotic Bcl-2 family members, such as Bax and Bcl-2 itself, has been highlighted as important for the mitochondrial apoptotic pathway. Following the treatment of HeLa cells with Ru-arene complexes, a significant increase in the expression of Bax and decrease of Bcl-2 was observed. This altered ratio of pro-
apoptotic and antiapoptotic factors favors the promotion of apoptosis. Both Ru-arene complexes reduced the expression of Bcl-2 by which the concentration of Bcl-2 dimers as well as ratio of Bcl-2/Bax did not increased and increases the susceptibility of cells to apoptosis and cell cycle delay. Consistent with our results, analysis of mRNA expression revealed that the Ru-arene complexes caused a rapid and significant increase in the expression of caspase-3, which corroborated the caspase activity in apoptosis. Taken together, our all resulted data indicate that both Ru-arene complexes increased the ROS level consequently all cascading activities (mitochondrial aggregation, p53 activation through Bax, Bak and Bcl-2 regulation) were start and the activation of caspase-3 is crucial for the execution of apoptosis in HeLa cells.
CONCLUSION

In conclusion, on the basis of short term cytotoxicity *in vitro*, the selected two complexes D3 and D13 were used for comprehensive study for anticancer potential. Further, both complexes have been found to be more active in HeLa cells as reflected from their IC$_{50}$ values and antiproliferative efficacy in comparisons to other cell lines. The interesting observation was that both the complexes did not exert substantial cytotoxicity on viability of normal cells. Further, the in vivo studies reflected that both the complexes significantly increased the longevity of tumor bearing mice as compared to control and cisplatin treated groups. Further investigation on their anticancer properties revealed that both the complexes preferred apoptotic mode of cell death and also have significant inhibition on cell cycle proliferation. Mitochondrial aggregation around the nucleus and increased level of intracellular ROS indicated the involvement of mitochondria in apoptotic induction. Increased intracellular ROS may trigger the activation of downstream signals for apoptosis such as activation of related genes and caspases. Elevated expression level of p53, Bax, Bak, Caspase-3 and down regulation of antiapoptotic Bcl-2 gene have further been established by Western blot analysis, semi-quantitative RT-PCR and real time qPCR indicated that both Ru-arene complexes induce apoptosis in cancer cells. These studies provides deep insight and understanding toward the cytotoxicity, antiproliferation, mode of cell death, cell cycle delay properties of selected Ru-arene complexes on specific cancer cells. Preferential and selective toxicity with apoptotic mode of cell death are necessary for the
development of potential anticancer agents for specific type of cancer. Further, the results from the present study suggest that these compounds merits as a new anticancer drugs. Rigorous screening and inclusive testing along with modifications may further improve the selectivity and anticancer activity of the resulting new drugs.