Chapter 8

General discussion, future perspectives and conclusion
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General discussion

From the results and discussions in different sections of this thesis, it would be revealed that we had two distinctive issues to address: (a) to find out if certain homeopathic mother tinctures and their bioactive compounds generally showed anti-cancer potential as tested on certain cancer cell lines, and (b) to find out if some potentized homeopathic drugs diluted beyond Avogadro’s limit could have proven effects at the molecular level and these could elicit demonstrable responses in lower form of organisms or microorganisms like bacteria and virus as well.

Initially, chemopreventive effects of ethanolic extract of the Goldenseal, *Hydrastis canadensis*, on HeLa cells were established with special emphasis on its drug-DNA interaction and apoptosis induction ability. Thereafter, efficacy of the plant alkaloid berberine, one of the major bioactive components of Goldenseal, *Hydrastis canadensis*, was tested on HPV -18 positive cervical cancer cell HeLa, involving certain cellular, viral and epigenetic protocols. Next, anti-cancer effects of palmatine, another major active component of *Hydrastis canadensis*, were evaluated with particular reference to is specific DNA damage responses, if any, in HeLa cells. Overall results were critically analyzed to ascertain if the whole ethanolic extract or a particular bioactive component had the same or greater degree of effect. But...
apparently, no clear-cut conclusion could be arrived at. Both the bioactive ingredients had almost same kind of anti-cancer effect as was found to be caused by the treatment of the crude ethanolic extract of Goldenseal, *Hydrastis canadensis* (mother tincture), although the doses for each varied to some extent. Therefore, the use of the mother tincture, that is, the whole ethanolic crude extract, in the treatment of diseases (including cases with cancer symptoms) by the homeopathic practitioners could be validated as a cost-effective mode of treatment, because separation and identification of the bioactive compounds, involving some cost, were not necessary, for this and the separated and purified ingredients also appeared to have effect of similar magnitude.

However, for formulation of advanced drugs, the information on the ability of different fragments (bioactive ingredients) could help in future drug formulation for a specific type of cancer, after further in-depth studies involving many other cell lines or suitable animal models.

Homeopathy happens to be more than 250 years old now, and more systematic study on different aspects of homeopathy is important, not only because of validation of its scientific use against common diseases, but also for enhancing the possibility if its use in combination with orthodox therapy in case of some dreadful diseases like cancer, at least as a supportive medicine. One singular or unique finding obtained from this study is the ability of certain potentized homeopathic drugs diluted above Avogadro’s limit to modulate gene expressions. It was not only revealed by the Western blot or immunoblot analysis, but also by its ability to modulate certain signal proteins both at protein and gene expression levels. The results obtained by the state-of-the-art technique of global microarray profiling clearly showed evidence of differential gene expressions when compared between the drug fed and the placebo fed series.

The results of the demonstrable effects of the ultra-highly diluted homeopathic remedies in lower organisms certainly had a great impact on understanding its molecular
mechanism; analysis of experiments conducted on the phenotypic evidence using bacteriophage phiX174(ΦX174) as a model (utilizing its special plaque forming ability as an assay method) yielded spectacular result that could help in the understanding of how these ultra-highly diluted homeopathic remedies could influence modulation of gene expressions.

Thus if the whole work presented in the different chapters is critically analyzed for picking out some novel findings, the following salient features would come out, some of which are quite thought-provoking.

In case of crude ethanolic extract of the Goldenseal, *Hydrastis canadensis* the critical points that came out are:

1. Cytotoxicity of *Hydrastis canadensis* on cervical cancer cell line HeLa was assessed, which had not been done before.

2. Molecular mechanisms of *Hydrastis canadensis* induced apoptotic events were elucidated for the first time.

3. *In vitro* interaction between calf thymus DNA and *Hydrastis canadensis*, was studied for the first time.

In case of berberine, the major bioactive component of *Hydrastis canadensis*, certain points come out for critical attention and analysis. These are:

1. Berberine causes disruption of tubulin network and cellular membrane structure, a new finding not studied earlier.

2. It has DNA binding ability causing biologically significant anti-neoplastic effect.

3. Study of DNA binding associated processes indicates its ability to induce epigenetic modifications, not known earlier.

4. Modulation of cellular and viral oncoproteins reveal possible signaling cascade, not studied earlier.

5. Molecular docking analysis revealed certain possible binding sites of berberine, a new finding.

However, it should be pointed out that the results also need to be extended by further studies specifically to elucidate if there is a definite
structure-activity relationship, which may lead to recommendation of berberine for specific clinical application.

Similarly, in respect of palmatine, certain points come out for critical attention and analysis.

1. Mechanism of cellular uptake of palmatine in HeLa cells is fairly revealed from this study—particularly its DNA binding abilities leading to biologically significant anti-cancer potential.
2. Possible signaling events due to DNA binding and DNA damage responses have been elucidated.
3. Molecular docking approach of structure activity relationship revealed possible binding sites, which could render help in pharmacological drug developmental strategy.

Apart from the role of the bioactive ingredients stated above, the present study also revealed the molecular mechanisms of action of the potentized homeopathic remedies through relevant experimental evidences on bacteria and bacteriophage, and also through analysis of global microarray gene profiling, that lent credence to the hypothesis proposed by Khuda-Bukhsh (1997), Khuda-Bukhsh (2003).

**Future perspectives**

In view of the results of our study and that of some other similar interdisciplinary studies, the prospect of using combinatorial therapy (like orthodox medicine plus some CAM) in treating dreadful diseases like cancer is brightening up, which can give the patients a better quality of life as well as a longer life. The emerging knowledge on drug structure and target of drug molecules is becoming more handy in understanding drug-disease combating events that in turn can help develop innovative therapeutic strategies. Therefore, attention is now being paid to the ability of the drugs in terms of their ability of targeting DNA and associated process for more effective targeted cancer therapy in the treatment and management of deadly diseases like cancer. List of such chemo-preventive agents, particularly from the natural and medicinal plant resources is now expanding rapidly for utilization in more effective drug formulation. However,
towards this goal, there are some concerns and some relevant factors are to be given due importance: selection of proper dose and concentration, experimental verification and validation, wiping off side effects or adverse effects, ascertaining feasibility of short-term acute and long-term chronic uses, and due consideration of pharmacodynamic/pharmacokinetic properties. Therefore, these concerns related to either the bioactive part or the whole crude extract, should be carefully tested and properly taken care of before recommending for therapeutic use. On the contrary, with the advanced knowledge of nano-biotechnology, special methods can also be formulated for better adsorption, specificity, dose limitation of the natural products, and drug bioavailability for targeted cancer therapy (Samadder et al., 2013, Bhattacharya et al., 2012).

Thus, future research program may be designed that can also address some other novel and exciting areas in understanding the immunological and epigenetic processes in relation to cancer therapeutics. The major objectives can be proposed to elucidate - (1) the probable physico-chemical interactions between chromatin and immunoglobulins; (2) identifying specific sets of genes associated with epigenetic modifications and immunological defense mechanisms in suitable cancer model (e.g., lung cancer, cervical cancer, glioma etc.); (3) identifying common transcription factors possibly associated with host defense and epigenetic modifications; (4) understanding the underlying mechanism of pluripotency and differentiation in hematopoietic stem cell model in response to these common transcription factors; (5) elucidating host-pathogen interactions (e.g., HPV oncogenic proteins and immunoglobulin molecules); (6) identifying probable gain or loss of function of epigenetic factors and (7) small molecules (e.g., interferons, aptamers, nanoparticles etc.) mediated signal transduction in terms of epigenetic pathway.

Till now, molecular mechanisms controlling transcription through epigenetic changes are poorly understood. The immunological molecules which control host defense mechanisms in complex disorders (e.g.,
cancer) are considered as key aspect in therapeutic application. Considering these issues, the combination of these two events can be a novel strategic approach in future cancer therapeutics. Additionally, the role of transcription factors controlling the pluripotency and differentiation in hematopoietic stem cells in terms of immunology and epigenetic factors can also bring a new direction in cancer stem cell research. The challenging aspects of the project can be—(1) identifying common transcription factors; (2) role of these common transcription factors in underlying mechanism of pluripotency and differentiation in hematopoietic stem cells; and (3) structural elucidation of host-pathogen interaction and chromatin-immunoglobulin molecules interaction.

These future projects can be aimed at through genomic approaches (e.g., global microarray analysis, methylation specific microarray, Next generation sequencing, CHIP- Sequencing approaches etc) to identify sets of target genes, role of transcription factors and to identify tentative DNA-protein interactions. For, any specific loss/gain of gene function to analyze in details, reporter based assay (e.g., Luciferase assay), site directed mutagenesis and in vivo model of knockout or immuno-compromised mice could be rewarding. Finally, the role of any specific signal transduction pathway, and structure-activity relationship study could add a lot in the understanding and effective combating of drug-disease phenomena.

**Conclusion**

In conclusion, it can safely be said that a thorough search of medicinal plants and their ingredients available in the nature’s domain can prove to be rewarding in finding suitable anticancer drugs. These can either be used as a supportive remedy in combination with other orthodox medicine, to ameliorate cancer and give cancer patients a much better quality of life, or further research may also prove that such natural drugs can even ameliorate toxicity of those orthodox medicines, the effective use of which otherwise is precluded because of their tremendous toxicity and side-effects. Hopefully, our present work will open up a conversation between groups engaged in research on orthodox and
unorthodox systems of medicine, to get the best out of both systems in the overall welfare of mankind.