CHAPTER IX

GENERAL DISCUSSION
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The present investigation was carried out based on information given by tribal physicians who claim to have treated diseases having symptoms of cancer. The plant material was collected and identified as *Barringtonia racemosa* Roxb by taxonomists of Kerala University, Thiruvananthapuram, Kerala. Accordingly, the plant material was obtained and dried in the shade and various parts were separated and pulverized. Preliminary studies indicated a better activity in the seeds of the plant. The other parts of the plant did not show any activity and hence the work was concentrated on the study of the seeds. The tribal physicians used to give the medicine by preparing the extract of the seed in water, which is actually a mixture of particles and colloidal suspensions. These are given directly the form of a liquid, which is digested and absorbed for the action in the body. Here we find that even though it is not completely soluble it is subjected to digestion with absorption just like any seed containing neutral fats or several other compounds. We have followed cytotoxic studies with the crude extract using very low concentration of 12 μg/ml for both DLA and EAC cell lines. Even the crude extract was showing a very good response in the cytotoxic studies as compared to many of the drugs used as medicines like vincristine. This can give a clear conception regarding better efficiency in cytotoxic studies.

We have tried to purify the *B.racemosa* seed extract and collected 70 fractions. Among these two fractions such as F₅-7:3 (26-29) and F₆-2:8 (47-53) were found to be more cytotoxic against DLA and EAC cells compared to other fractions. It was found that the concentration required for 50% cytotoxic for F₅-7:3 was 4μg/ml were as the other purified fraction F₆-2:8 showed 50% cytotoxicity the concentration of 6μg/ml. This shows that these fractions were purified 10 times. Here, we find that during the purification...
process some factors might have been removed because of which purification is not manifested. It can also be considered that certain co-factors, which are present in the crude extract, might have been lost during the purification process. Here we find a 10 fold activity for DLA and 4 other cell lines it was only a 5 fold activity. We have not purified the active principal as pure single components and therefore it is not possible at present to say whether the toxicity is due to the active principle or some other compound.

Our *in vivo* experiments in the tumour reduction and tumour prevention using DLA & EAC cell lines showed remarkable response in preventing the tumour development and increasing the life span of the animal. The drug at a dose of 12 mg/kg or above showed toxic symptoms. Therefore, the highest dose selected in the *in vivo* experiments was 9mg/kg. At this dose level the drug did not cause any apparent toxic manifestations. The LD$_{50}$ value for a single i.p. dose for male mice was 35mg/kg and that for female mice was almost the same (35mg/kg). This explains the lower concentration used in the *in vivo* experiments like tumour reduction or tumour prevention without any side effects.

We have also carried out the effect of the drug on the growth of different cancer cell lines by cell culture experiments and we find that good response was obtained for these cell lines, showing the inhibition of cell growth. Here we have to assume that the multiplication of the cells were retarded either directly active on nucleic acids or at the level of protein synthesis. It is to be mentioned that all the concentrations used for this experiments were far low (1,2,5&10µg/ml) so that other toxic compounds do not interfere. We have also carried out chromosomal analysis using the blood of normal healthy persons. Specimen blood was collected from normal healthy individual and subjected to clastogenic studies using our drug. The
results are encouraging that at a higher concentration of 400µg/ml it did not exhibit any chromosomal breaks. This shows that in the normal human being there was no toxic effect or any other undesirable effect when healthy human blood was used for anticlastogenic studies. We have not tried this medicine in normal individuals or cancer patients however the anticlastogenic study shows that it may not have a deleterious effect on human beings.

One of the mechanism by which anti cancer drug act is to induce the normal physiological mechanism of apoptosis in DLA cells and this may be one of the mechanism by which the drug acts on cells.

Apoptotic studies also indicate several morphological changes for the cell as a prelude to cell destruction. It is to be emphasized that the concentration used were lower than the required cytotoxic concentration. All this experiments indicate that B.racemosa seed extract have cytotoxic actions and tumour preventive activities. This can be further tried in the human beings also. One of the difficulties with the modern drugs as cyclophosphamide and vincristine frequently used in the treatment of different kinds of cancer can cause toxic symptoms and therefore, only lower concentration could be used. Otherwise, if higher concentrations are used for better activity, it may increase toxic symptoms like hepatic and renal toxicity. Therefore, the present trend is to find out plant products and other drugs as chemo protectors and is given in experimental tumour. Thus, we know that Ixora coccinea, Ixora javanica, Saffron, Saraca ashoka and several plant products have been experimentally proved to posse's chemo prevention(Nair et al 1990; Nair et al 1991a; Varghese et al 1991; Kuttan and Kuttan 1992). In the case of Ixora javanica and Saffron it was found that the toxic metabolite of CYP has been isolated in the urine of experimental
animals while this was absent in the urine of treated animal. Here the plant products have reacted with the toxic metabolite and toxicity is nullified. In our experiments, we have been able to find out the hepatic and renal toxicity developed in the control animals were reduced in the treated animal thereby indicating the potential role of our drug in preventing the toxicity of CYP and vincristine. All this indicates that the drug has not only anticancer property but also possesses chemo protection from the toxic symptoms of CYP and VCR.

Thus, we know that *Ixora coccinea, Ixora javanica, Saffron, Seraca ashoka, Nigella sativa, Viscum album* etc. and several plant products have been experimentally proved to possess chemo prevention Therefore, the dosage of the anticancer drugs could be increased without any side effects. In the case of *Crocus sativus*, it was seen that one of the toxic metabolites of CYP combined with the plant extract and was demonstrated as a nontoxic component in the urine (Nair *et al* 1991b).

In our experiments, we have been able to find out the hepatic and renal toxicity developed in the control animals was reduced in the treated animals thereby indicating the potential role of our drug in preventing the toxicity of CYP and vincristine. Haematological and biochemical parameters were measured. All this indicates that the drug has not only anticancer property but also possess chemo protection from the toxic symptoms of CYP and VCR. Since our drug is a purified extract, it may either contain several compounds and these compounds collectively or individually prevent the cancer development since we have not isolated the active principle. So that at present it is not possible to say what type of compound is present in the purified fraction $F_8$ (2:8). It may be an unsaturated fatty acid as in the case of *Nigella sativa* (Salomi *et al* 1992).
During the study of solid tumour experiments, we have been able to get and interest in the study from the department of Optoelectronics, University of Kerala. They were interested in IR spectroscopy of solid tumour. Therefore, a collaborative study was carried out and the results were very encouraging. The analysis of the spectra of the tumours and non-tumours specimen revealed interesting facts, which are coinciding with the pathological report. In the pathology report, we were able to find out remarkable distinction in having necrosis, fibrosis and non-development of the tumour in the treated case as compared to the untreated tumour animal. The spectral findings are in agreement that during the development of cancer other changes take place due to the formation of cancer. The formation of collagen is affected in having the absence of specific bands indicating hydroxylation reactions. Here, one of the essential step in collagen formation, the hydroxylation of proline to hydroxy proline is not taking place in the case of tumour without drug treatment. However in the treated case simultaneous reduction in the tumour along with hydroxylation reactions occur. This shows that normal synthesis of collagen takes place when the drug is administered. This way of expression definitely indicates the anticancer property of the drug.
SUMMARY AND CONCLUSIONS
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The investigations were carried out to assess the claim of the tribal physicians in curing cancer with the plant *B. racemosa*. They used the seeds for the treatment. However, we have tried the cytotoxic property of different parts of the plant and found the seeds having good activities and a better cytotoxicity in the seed coat. Better extraction was obtained with the solvent methanol and 50% cytotoxicity against DLA and EAC were obtained with a concentration of 12μg/ml. We have been able to get a good response in *in vitro* cell culture experiments. The crude and purified fractions of *B. racemosa* seed extract exhibited a significant growth inhibitory effect against various human and murine cell lines. The *in vivo* experiments in the tumour reducing property was carried out and we find that the treated animals did not develop tumour and continued to live as a normal animal for long time. While the control, animals develop tumour and died within 25 days. Solid tumour experiments were carried out and systematic investigations were done on these specimens. It was found that a remarkable inhibition of DLA induced solid tumour growth formation when the mice was given *B. racemosa* seed extract at a concentration of 9mg/kg for 15 days.

Another function of the anti-cancer drug is to know whether it has any enhancing effect on the normal physiological process of apoptosis. We find here that there is increase of apoptosis in the presence of the drug. The drug has no deleterious effect on chromosome of normal human beings. Thus, the anticlastogenic effect is interesting. The plant product has been investigated to find whether it has any chemo preventive activity against the toxicity of CYP, VCR, and we find a very good response in reducing the toxic symptoms associated with liver and kidney. This can help in increasing the
dosage of the standard drug along with our drug, which is also anticancerous in nature.

Infrared spectroscopic studies of DLA induced solid tumour experiments were also studied in collaboration with department of Optoelectronics, Kerala University and we got interesting result in the non-development of tumour in the treated cases. The spectra indicated the formation of tumour and degeneration of collagen by this change was not there in the treated cases. The pathological investigations revealed necrosis, fibrosis and non-development of tumour in the treated cases.

We have been able to purify the drug by column chromatographic procedure and chemical nature is not known. It is most probably a fatty material association with carbohydrate or proteins. Thus, we are able to show that the drug is effective in preventing the development of experimental tumour in animals. Further study and human trial will be of use in establishing it as a potential anticancer drug.