CHAPTER – VII
SUMMARY AND CONCLUSION

Summary

In this chapter, the analysis of the study on “The effect of selected protein isolates on the induced hepatocellular carcinoma and myocardial infarction” is summarized experiment wise, and in conclusion, certain findings are highlighted.

a) Amino Acid Composition - (Experiment Ia)

From the comparative analysis of amino acid content in candidate proteins, it was observed that glutamic acid was the major amino acid in all the five proteins. Arginine was the second highest amino acid in garlic and coconut protein and the fourth most abundant amino acid in soy protein. Aspartic acid was the second highest in amino acid content in soy protein and in whey protein, whereas it was the third highest in garlic and coconut protein. Leucine was the third most abundant amino acid in soy protein and it was fourth highest in garlic and coconut.

Arginine was reported to have preventive effects against various cancers. Most of the animal proteins which were proven to be hypercholesterolemic had a lysine: arginine ratio nearly about 2. In our analysis we found that lysine:arginine ratio in garlic protein (0.43), coconut protein (0.26) and soy protein (0.79) was very low.

b) Peptide Fragments - (Experiment Ib)

MALDI-TOF MS of the various protein samples revealed the presence of certain useful peptides among the soy, garlic and coconut protein fed animals. Peptides of Mz. around 5000 Daltons were identified only in these three groups. The control diet protein showed the presence of a few peptides which are known to have roles in embryonic development and in aspects of tumor progression. In coconut protein a peptide with ACC no. A5CSL0 has been correlated with repression of a number of important genes involved in DNA repair, especially in SOS response. Q9BYE9 is another peptide, from coconut protein sample, which can cause contact inhibition in the surface of epithelial cells. Some peptides identified through protein
prospecting bearing resemblance to the 3 high-intensity casein peptides showed that they have protective, and growth promoting effects.

However, we need further studies to make any effective inference.

c) Antibacterial activity - (Experiment II)

From the present study we have found that certain protein isolates effectively inhibited the growth of certain pathogenic bacteria by forming a clear zone of inhibition. Among the protein isolates studied, the effective antibacterial ones were garlic protein and soy protein followed by whey, casein and coconut protein. They possibly contain the antimicrobial peptides. Positively charged residues such as Lys and Arg and substantial hydrophobic residues (30% or more) are reportedly found in these peptides. AMPs are generally cationic (i.e., the net charge at neutral pH varies from +2 to +9) and amphipathic, which enables the peptides to interact with and disrupt lipid membranes. AMPs target the cell membrane of invading microorganisms, leading to cell lysis and death. It may be inferred from the present study that the presence of antimicrobial peptides in proteins manifest in them the ability to inhibit the growth of bacteria.

d) Safety of the protein isolates - (Experiment III)

Protein foods vary in clinical allergy significance. In the present study a minimal increase in the titers of IgE was observed in soy, coconut and casein groups, and a highly significant elevation in garlic and whey protein treated animals compared to control mice. The level of increase varied with different protein isolates depending upon their antigenic nature to cause hypersensitivity reactions.

Their growth and behavior pattern was not disturbed. In the present study a significant rise in the value of WBC count was observed in garlic protein fed animals.

As the present investigation is only a preliminary type and as there are only limited studies involving the use of proteins causing allergic reactions, further insight are necessary to prove the actual mechanism of action of various proteins in elevating WBCs and related allergic reactions.
e) Hepatocellular Carcinoma - *(Experiment IV)*

Hepatocellular carcinoma is a major form of cancer in humans. Although varied causes are cited for its incidence in human populations, the entire mechanisms of its incidence and progression are yet unclear. Liver cell necrosis and formation of cancerous foci may decrease the functioning of the liver.

Existing literature points out that no allopathic medicinal regimen has yet been conclusive in offering complete and decisive cure for HCC. As preventive medicine is contemplated to be better, than addressing the problem of cure after these diseases occur and as this disease is silent in its progression until signs and symptoms are clinically established in patients, we resorted to experiment administration of various protein isolates in suitable animal models which mimicked experimentally the pathology and the symptoms of the disease.

In an earlier study in our laboratory, garlic protein as a post treatment was employed after the onset of hepatocellular carcinoma. And we found that there was practically no effect (unpublished). These results also prompted us to try pretreatment with various proteins.

The summary of our observations and findings is as follows:

1. The growth rate, after consuming the protein from various sources, as calculated by gain of body weight was similar in all groups, upto 3 weeks from the start of the study. From the 4th week onwards, variation in their growth rate was observed, such that the body weight gain in garlic protein, coconut protein and casein-fed mice was found to be higher than that for soy and whey protein-fed mice. Whey as the primary source of protein lead to reduction in body weight due to its effective inhibition of fat accumulation in adipocytes. A thermogenic utilizing effect caused a reduction in body weight of the soy protein fed animals.

2. A dramatic decline in the concentration of AFP both in serum and liver of the soy, coconut protein and garlic protein fed groups was observed. The whey and casein protein fed animals, although had a significant decrease in serum and liver AFP values, when compared with the induced HCC group, this decline was in no way closer to the animals treated with soy, coconut protein and garlic protein.
3. The restoration of TNF-α level to near normal range was the highest following pretreatment with soy protein, and a little lesser than that in coconut protein and garlic protein-fed animals.

4. Increased intracellular concentrations of polyamines reflected the rate of tumor proliferation in the DEN induced group. Soy protein and coconut protein-fed animals had the lowest ODC activity in serum and liver tissue, followed by the garlic, whey and casein protein-fed animal groups. The fundamental concept of polyamine metabolism in tumours is that the transformed tumor cells have a greater requirement for polyamine synthesis for continuous growth, when compared to normal tissues, which have a lesser requirement for polyamines, because of their controlled growth behavior.

5. A dramatic decline in GGTP activity both in serum and liver of the soy, coconut protein and garlic protein fed groups was observed. The whey and casein protein-fed animals had, although had a significant decrease in serum and liver GGTP values, when compared with the induced HCC group, this decline was in no way closer to the animals treated with soy and coconut protein. Pretreatment with protein isolates brought the activities of these enzymes to normal, indicating its protective effect during cancer.

6. The animals fed with soy protein and coconut protein registered the remarkable decrease in liver function enzyme levels followed by garlic, whey and casein-fed groups of animals.

7. In the soy protein treated group the activity of SOD, CAT and GPx was the highest and their serum level was almost identical and statistically insignificant to that of the control level, thus making soy protein the anticarcinogenic premium protein. Garlic and coconut protein pretreated animals also had significantly higher activity of these antioxidant enzymes.

8. Restoration of most of the normal hepatocyte architecture with regular dark nuclei was observed in groups treated with soy protein, garlic protein and coconut protein and few hepatic vacuolations, dilated sinusoids and dysplasia were seen in groups pretreated with whey protein and casein. Thus, we may infer that protein pretreatment decisively inhibits weight loss, restore the near normal level of HCC markers of the liver and liver-specific enzymes,
and prevents the fall of antioxidant serum and tissue enzymes. Soy, coconut and garlic protein feeding are more protective than whey or casein administration.

**f) Cardiovascular disease - (Experiment V)**

Cardiovascular disease is often insidious, and hence referred to as a ‘silent killer’. Stress, genetic profile, dietary habits and lifestyle changes contribute to the incidence of CVD. In our study, we have assessed the role of dietary protein feeding in alleviating the symptoms and the biochemical changes that prevail during the occurrence of CVD. We used mice as an appropriate model by inducing experimental myocardial infarction with the isoproterenol and characterized the protective effects of protein from various dietary sources by evaluating their efficacy against that of the control animals.

The following is a summary of our observations and findings:

1. Significant decrease in final body weight was observed in whey protein-fed animals. The final body weight of animals pretreated with soy-protein was found to be lower than the garlic protein, coconut protein and casein treated animals. Glycomacropeptide is present in whey and this may be responsible for the weight reduction in animals.

2. Soy protein and garlic protein seem to have a highly protective influence on the heart tissue with reference to the level of the enzyme creatine kinase in the serum and the cardiac tissue. The other three protein isolates, in the order coconut, whey and casein had lowered cardiac injury and hence had a relatively a lower serum CK values than the ISO induced animals.

3. Pretreatment with soy and garlic protein maintained the activities of marker enzymes in serum and heart close to near normal levels. Also, pretreatment with coconut protein, whey protein and casein showed a moderate effect on the activities of these enzymes in both serum and heart homogenate of the animals from their respective groups. As already discussed, the positive role of soy protein may be attributed to its hypolipidemic action, that of coconut to a high content of arginine, that of garlic to its low lysine: arginine ratio and that of whey protein and casein to the antithrombotic peptides.
4. The AST and ALT values (as percent of control) both in serum and heart tissue suggest that the rate of recovery from myocardial infarction was impressive in the mice administered soy, garlic and coconut protein treated groups. The results of this study indicate a cardio protective effect of these three protein isolates and that may be attributed to high arginine content in these protein isolates.

5. Soy protein, garlic protein and coconut protein pretreatment has been found to prevent the loss of the activity of SOD and CAT followed by ISO administration and also been able to scavenge superoxide radicals and thus reduce myocardial damage caused by free radicals. Pretreatment with whey protein and casein showed a minimal increase in the activities of SOD and CAT.

6. In the present study, soy protein, garlic protein, and coconut protein treated animals showed a significant decrease level of lipid peroxide.

7. Histoarchitecture was normal in soy protein pretreated animals. Animals pretreated with coconut protein showed mild myocytic necrosis with moderate infiltration of leucocytes. Garlic protein pretreated mice exhibited decreased degree of necrosis (mild) and less infiltration of inflammatory cells. Occasional areas of focal myonecrosis were observed.

Thus it is obvious that soy protein, garlic protein and coconut protein are good dietary regimens for the prevention of CVD because they show minimal indications of tissue damage and changes in levels of biochemical markers of disease. Other proteins are not as efficient as the aforementioned protein isolates.

**Conclusion**

On the basis of aminoacid composition, casein being a complete protein with all essential amino acids, promote and sustain growth, whereas whey protein may be useful in weight reduction. Soy, garlic and coconut protein stand out for their high content arginine and the low lysine/arginine ratio; and these features gain them a therapeutic value.

From the perspective of the antimicrobial activity soy protein stands out, followed by garlic and whey.

Even if a bioactive molecule is of great therapeutic value, it should be agreeable with the whole system, or else, it may end up with a nuisance value.
Looking from the angle of safety, the coconut protein scored high followed by casein. Soy and garlic protein, though prove to be superior, due caution should be exercised by taking into consideration the negative effect, if used unchecked. Whey protein causes allergic reaction, if used in large amounts, hence, judicious use in weight reduction may be appropriate.

With these basic attributes to these proteins, in the order soy protein, coconut protein and garlic protein are efficient in preventing the onset of hepatocellular carcinoma like conditions. However, they are found only to be preventive, but not curative, meaning that if administered after the occurrence of cancer, they may not be effective.

However, with reference to situations like myocardial infarction, soy protein was found to be very effective, followed by garlic, coconut protein, whey and casein even as a curative. The influence was minimal with casein and whey.

Hence, these protein isolates coconut, garlic and soy protein may be considered as worthy candidates for formulations as nutraceuticals in the prevention of cancer and cardiovascular diseases.