5.0 SUMMARY AND CONCLUSION

Huge amounts of money are being spent by foreign countries for the research on medicinal plants for taking patent on a particular plant or a plant product. But no significant work is going on in India even through India has a good heritage of medicinal plants. This lacuna has to be filled up by active research on medicinal plants.

Two medicinal plants namely *Cynodon dactylon* and *Terminalia catappa* were selected for the study as there is no systematic study on the antioxidative, antitumorigenic and immunomodulatory effects in them. *Cynodon dactylon* belongs to the family Poaceae and *Terminalia catappa* belongs to the family Combretaceae.

Tumors are mystifying diseases which remain completely incurable in spite of large number of current research works in this area. The major form of treatment of tumor is surgery and radiation. A chemotherapeutic agent can often provide temporary relief and can prolong life. A successful anticancer agent without causing any damage to normal cells are rare or practically nil. In this context, the use of plant protein in the treatment of tumor is more relevant. Eventhough India is enriched with a wide array of valuable medicinal plants, no comprehensive systematic and controlled survey of antioxidative and immunomodulatory activities have been undertaken so far. So the present research on “Antioxidative, antitumor and immunomodulatory efficacy of protein fraction of *Cynodon dactylon* and *Terminalia catappa* leaves on experimentally implanted ELA cells in Swiss albino mice” was carried out and summarized as follows:

The experiments were carried out in four phases. In the first phase, phosphate buffered saline leaf extracts of *C. dactylon* and *T. catappa* were prepared. Ten different aliquots of PBS extracts were subjected to 10-100 per cent ammonium sulphate saturation to precipitate the proteins, the protein contents were estimated and purified by PAGE. The protein content was found to be more in *C. dactylon* at 60 per cent saturation of ammonium sulphate (9.3mg/g leaf) and in *T. catappa* at 70 per cent saturation of ammonium sulphate (7.5mg/g leaf) than that of the other saturations. The major bands of
these selected protein fractions were eluted, purified and characterized by PAGE and SDS PAGE.

In the second phase, in vitro antioxidative potential of C. dactylonPF and T. catappaPF was evaluated by following the DPPH, NO and H\textsubscript{2}O\textsubscript{2} scavenging activity. Both the protein fractions showed a dose dependent free radical scavenging activity and confirmed their antioxidative potential. In vitro antitumorigenic effect of protein fractions of C. dactyl\textit{on} and T. catappa was assessed by cytotoxic studies using ELA tumor cells. The selected major bands of 60 per cent and 70 per cent ammonium sulphate protein fractions of C. dactyl\textit{on} and T. catappa showed dose dependent cytotoxic effect to ELA tumor cells. From the graphs the 50 per cent effective concentration (EC\textsubscript{50}) was found to be 52 µg of protein and 40 µg of protein of C. dactyl\textit{on} and T. catappa respectively. These EC\textsubscript{50} of C. dactyl\textit{on} and T. catappa were referred as C. dactyl\textit{on}PF and T. catappaPF.

In the third phase, C. dactyl\textit{on}PF and T. catappaPF were evaluated for their in vivo antitumorigenic effect against ELA induced mice and antioxidative efficacy against the standard antioxidant silymarin and by assessing the liver marker enzymes, enzymic, nonenzymic antioxidants and lipid peroxidation status in the liver after 15, 30, 45 and 60 days and the histological analysis after 60 days of the experimental tenure.

Codministration of C. dactyl\textit{on}PF and T. catappaPF to ELA tumor induced mice increased the life span by 200 and 130 per cent respectively. Increase in the life span of ELA tumor induced mice confirmed the antitumor activity of C. dactyl\textit{on}PF and T. catappaPF.

The liver marker enzymes in serum namely GOT, GPT and ALP were analysed in all the experimental animals to assess the normal functioning of the liver. The activities of SGOT and SGPT were found to be significantly decreased by the administration of C. dactyl\textit{on}PF and T. catappaPF to all the experimental groups. Mice administered with C. dactyl\textit{on}PF showed significant decrease in ALP activity in all the treatment periods than that of T. catappaPF when compared to the control. The activities of all the three liver marker enzymes were found to be significantly increased in ELA tumor induced mice. But the coadministration of C. dactyl\textit{on}PF and T. catappaPF to ELA
tumor induced mice showed a significant decrease in the activities of the above enzymes. The above observations of the present study could be attributed to the significant protective effect of the *C. dactylon*PF and *T. catappa*PF and established the normal functioning of the liver.

Knowing the protective role of the *C. dactylon*PF and *T. catappa*PF, the antioxidant potential was followed by assessing the enzymic and nonenzymic antioxidant status in the liver and the rate of LPO in the selected organs such as liver, lung, kidney, spleen, heart and brain of experimental animals. Administration of *C. dactylon*PF and *T. catappa*PF significantly enhanced the activity of CAT and GPx in all the treatment periods when compared to their respective controls. The activity of SOD was significantly enhanced by the administration of *C. dactylon*PF and *T. catappa*PF only after 60 days treatment period. The enhancement of enzymic antioxidants by the administration of *C. dactylon*PF and *T. catappa*PF was more significant than that of silymarin.

In ELA induced mice, coadministration of *C. dactylon*PF and *T. catappa*PF showed significant increase in all the endogenous enzymic antioxidant systems namely CAT, SOD and GPx after 15 days, 30 days, 45 days and 60 days of treatment periods. The *C. dactylon*PF gave more pronounced antioxidative effect than that of *T. catappa*PF and silymarin. Results indicated that CAT and GPx activities were enhanced and proved their significant defense mechanisms by protecting the liver against oxidative challenges induced in the liver of tumor transplanted mice.

Similarly the nonenzymic antioxidants such as Vitamin A, E and GSH levels were found to be increased by the administration of *C. dactylon*PF and *T. catappa*PF. The enhancement of nonenzymic antioxidants by the administration of *C. dactylon*PF and *T. catappa*PF was also more significant than that of silymarin. The ELA tumor induced mice showed significant decrease in the level of nonenzymic antioxidants when compared to the control mice. Administration of *C. dactylon*PF and *T. catappa*PF to the ELA tumor induced mice showed a significant increase in the levels of Vitamin A, E and GSH in all the treatment periods when compared to 15 days treatment period of ELA tumor induced mice.
Lipid peroxidation status as assessed by the levels of lipid peroxides as MDA in 15, 30, 45 and 60 days of treatment period was found to be significantly increased in ELA tumor induced mice in all the selected organs. Increase in the concentration of MDA observed in this study is the index of LPO and cell membrane damage. Thus, it could be suggested that *C. dactylon*PF and *T. catappa*PF would have strengthened the endogenous antioxidant defense from ROS ravage and restored the optimal balance by neutralizing the ROS. Inhibition of LPO by *C. dactylon*PF and *T. catappa*PF was more significant than that of silymarin. *C. dactylon*PF showed significant inhibition of LPO than *T. catappa*PF individually and in tumor induced mice. Administration of *C. dactylon*PF and *T. catappa*PF individually and to the ELA tumor induced mice showed significant decrease in the rate of lipidperoxidation and showed their antlipidperoxidative role.

It is also well documented that the ROS play a major role in tumor progression by cellular damage. In the present study, cellular damage in ELA tumor induced mice was indicated by the significant reduction in the activities of enzymic antioxidants, levels of nonenzymic antioxidants and significant lipidperoxidation rate by the increased lipid peroxide levels. The above study of the enzymic antioxidants activities (CAT, SOD and GPx) and the nonenzymic antioxidants levels (Vitamin A, E and GSH) revealed that *C. dactylon*PF is the most effective which was followed by *T. catappa*PF and silymarin the standard antioxidant. Thus, the use of these protein fractions can be recommended as the standard antioxidant instead of silymarin. Administration of *C. dactylon*PF and *T. catappa*PF to ELA induced mice showed antioxidative and antlipidperoxidative role by the significant increase in the activities of enzymic antioxidant, the levels of nonenzymic antioxidant and significant decrease in the levels of lipid peroxide. All these observations clearly supported the antioxidative and antitumorigenic effect of *C. dactylon*PF and *T. catappa*PF.

Histological observations also suggested the possibility of the *C. dactylon*PF and *T. catappa*PF in conditioning antioxidative potential of the hepatic cells to a state of accelerated regeneration and thus decreasing the leakage of GOT, GST and ALP into the circulation. Histological examination of
the liver of ELA tumor induced mice showed necrosis whereas \textit{C. dactylon}PF and \textit{T. catappa}PF individually and to ELA induced mice showed inhibition of necrosis which also supported their antitumorigenic nature.

In the fourth phase, the immunomodulatory role of \textit{C. dactylon}PF and \textit{T. catappa}PF was evaluated by the administration of the immunosuppressor pyrogallol. Administration of \textit{C. dactylon}PF and \textit{T. catappa}PF along with the pyrogallol significantly increased the weight of spleen and thymus when compared to the pyrogallol administered mice. In ELA tumor induced mice, the weight of spleen was found to be increased significantly than in pyrogallol administered mice. By the coadministration of \textit{C. dactylon}PF and \textit{T. catappa}PF to ELA tumor induced mice, there is a significant increase in the weight of spleen and thymus. This enhancement was due to the immunostimulatory activity of \textit{C. dactylon}PF and \textit{T. catappa}PF. Increase in the number of thymocytes in the epithelial cells would have caused an increase in the weight of thymus by the administration of \textit{C. dactylon}PF and \textit{T. catappa}PF. \textit{C. dactylon}PF and \textit{T. catappa}PF administration would have activated the cells that secrete antibodies which are present in the spleen and thus increases the spleen weight.

Total leukocyte count of pyrogallol treated mice was significantly decreased when compared to PBS control mice. Administration of \textit{C. dactylon}PF and \textit{T. catappa}PF to the pyrogallol treated mice and coadministration to the ELA tumor induced mice recorded significantly increased leukocyte count when compared to pyrogallol and ELA induced mice respectively. In this context, increase in TC by the administration of \textit{T. catappa}PF was found to be more than that of the \textit{C. dactylon}PF.

Administration of \textit{C. dactylon}PF and \textit{T. catappa}PF along with the immunosuppressor pyrogallol showed an increase in the per cent neutrophil adhesion and phagocytic index than that of control group which indicated their immunostimulatory role. The significant increase in TC, per cent neutrophil adhesion and phagocytic index confirmed the cell mediated immune response of \textit{C. dactylon}PF and \textit{T. catappa}PF.

The antibody titre of the pyrogallol treated mice showed 2 and 4 fold decreases whereas \textit{C. dactylon}PF and \textit{T. catappa}PF treated mice revealed a 4
fold and 8 fold increases in the antibody titre respectively when compared to PBS control mice. This augmentation of the humoral response to SRBC indicated an enhanced responsiveness of the macrophages and T and B lymphocyte subsets involved in antibody response. It would be concluded that both the plant protein fractions have the capacity to stimulate humoral immune response and are able to effectively modulate immunological interactions.

The above immunostimulatory role of \textit{C. dactylonPF} and \textit{T. catappaPF} was confirmed by the serum protein profile of PBS, \textit{C. dactylonPF}, \textit{T. catappaPF}, \textit{C. dactylonPF+ELA}, \textit{T. catappaPF+ELA} and ELA treated mice in SDS PAGE, serum protein arc in double immunodiffusion and immunoelectrophoresis. Serum protein profile of the ELA tumor induced mice showed an extra band of which was neutralized by the administration of \textit{C. dactylonPF} and \textit{T. catappaPF} to the ELA tumor induced mice. This neutralization of protein was confirmed by the formation of precipitin arc for the serum of ELA induced mice against \textit{C. dactylonPF} and \textit{T. catappaPF} in double immunodiffusion and immunoelectrophoresis. ILS of mice administered with \textit{C. dactylonPF} and \textit{T. catappaPF} to ELA tumor induced mice might be due to destruction of ELA specific protein by the administration of \textit{C. dactylonPF} and \textit{T. catappaPF}. The tumor specific protein can be isolated and anti antibodies to this tumor induced protein can be prepared which might form the basis for the production of tumor vaccines in future.

The results of the present study indicated the effectiveness of \textit{C. dactylonPF} and \textit{T. catappaPF} in stimulating both humoral and cellular immune responses without any side effects which in turn could effectively stimulate the immunity and could be used as an anticancer agent in the treatment of cancer. The results of the \textit{in vitro} and \textit{in vivo} antioxidative, antitumorigenic and immunomodulatory activity were found to be most effective in \textit{C. dactylonPF} which was followed by \textit{T. catappaPF} and silymarin. Thus, the antioxidative, antitumorigenic and immunomodulatory effect of \textit{C. dactylonPF} and \textit{T. catappaPF} were thus established from the results of the present study.

\textbf{Conclusion}

The results of the study suggested the antioxidative efficacy by enhancing the antioxidative enzymic and non enzymic status, antilipidperoxidative role by
inhibiting the lipid peroxide status, antitumorigenic against ELA tumor by increasing the ILS and immunostimulatory against the immunosuppressor pyrogallol by the favorable enhancement of the humoral and cell mediated immune responses in mice by the administration of C. dactylonPF and T. catappaPF. Thus, C. dactylonPF and T. catappaPF could be exploited as an antioxidative, antitumorigenic and immunostimulatory agents to combat the oxidative degenerative diseases such as atherosclerosis, cancer, multiple sclerosis, aging and arthritis. Further study and human trial will be of use in establishing C. dactylonPF and T. catappaPF as a potential anticancer drug.

**Future Recommendations**

Following studies are warranted to follow the mechanisms responsible for antitumorigenic and immunomodulatory role:

- Evaluation of Xenobiotic enzymes such as GST in phase I and Cyt b5, P450 in phase II.
- Study on the Isoenzymic pattern of the xenobiotic phase I and phase II enzymes in the tumorigenesis protocol.
- Screening of the mechanism of apoptosis by C. dactylonPF and T. catappaPF in different types of cancer cells.
- Counting of T lymphocytes and B lymphocytes.
- Evaluation of antibody response in the presence and absence of immunostimulator and immunosuppressor.
- Isolation of the specific antibody to produce the anti antibodies for the production of vaccine.
- Lymphocyte culture studies to follow the anti-inflammatory role.
- Studies on the mechanisms of immuno-modulation and probable use in immuno compromised individual.
- Attempt on human clinical trials are recommended in near future to authentically project C. dactylonPF and T. catappaPF as a safe potential anticancer drug.