



Summary and Conclusion

Cancer is a genetic term for a wide variety of malignant neoplasm that may result in deleterious effects on the host due to their invasive and metastasizing nature. The role of free radicals and active oxygen in the pathogenesis of human diseases including cancer has been recognized. The generation of ROS can be exploited in the treatment of cancer. Currently there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine. As plants produce significant amount of antioxidant to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Several medicinal plants and their constituents probably exert their chemopreventive effect, by scavenging reactive oxygen species and improving the antioxidant defence system. Drug discovery from medicinal plants has played an important role in the treatment of cancer and most clinical applications of plant secondary metabolites and their derivatives over the half century have been applied towards combating cancer.

The present investigation comprises of three phases.

In Phase I, the root of *Coleus forskohlii* was collected from Tamil Nadu Agricultural University, Coimbatore. They were shade dried and powdered. The powder was extracted with different solvents such as petroleum ether, chloroform, acetone and methanol. The *Balb/C* mice weighing between 25-30g were procured from N.G.P College of Pharmacy, Coimbatore. The root extracts of *Coleus forskohlii* was administered in 4 different doses of the extracts (25, 50, 500, 2000mg/Kg body weight) to the mice and the acute toxicity of the mice was studied by observing for 72 hours. The DLA cells were obtained from Amala

Cancer Research Institute, Thrissur. The mice were induced with DLA cells and treated with the extracts to evaluate the antioxidant activity of the extracts against DLA tumor. The enzymic antioxidants like catalase, superoxide dismutase, glutathione -S- transferase, glutathione peroxidase and glutathione reductase and non enzymic antioxidants such as glutathione and ascorbic acid were assessed.

In Phase II the extract which had shown better antioxidant activity was selected and was used to treat mice with the DLA cells. Then the hematological parameters such as hemoglobin content, WBC count, lymphocyte count, monocyte count and differential cell count were determined. The body weight, the survival time and the histopathological analysis were carried out to evaluate the antitumorigenic effect of *C.forskohlii*.

In Phase III, the radical scavenging effect of the *Coleus forskohlii* root extract was analysed. The methanol extract of the roots of *Coleus forskohlii* was screened for the presence of phytochemicals. Then it was fractionated by solvents of increasing polarity and each fractions were subjected to cytotoxic assay and MTT assay. The fraction which was found to have the cytotoxic activity was subjected to GC-MS, to findout the constituents present in it. The fraction which showed better cytotoxic activity was subjected to TLC and HPLC analysis, to confirm the active constituent responsible for anticancer activity.

The significant findings of the study are

- The results of the acute toxicity indicated that the petroleum ether, chloroform, acetone and methanol extracts were devoid of acute toxicity at the dose levels of 25,50,500 and 2000µg /ml. The mice ingested with 25mg/Kg of body weight showed restlessness for the

first 3 hours and then died at the 11th hour, which indicated that the dose was toxic at 25 mg/kg body weight. No adverse effects were observed with all the other groups of mice. The LD₅₀ of the test substance as per OECD guidelines falls under category 5 (LD₅₀ 500 to 2000 mg/kg of body weight). High concentration of the substance was not found to be toxic to the mice. 200mg/kg body weight was the dose selected for the present study.

- Lipid peroxidation, which involves a series of free radical mediated chain reaction processes, is found to have significantly increased in the mice with DLA tumor compared to the untreated control which is found to have decreased in the DME treated mice.
- The *in vivo* antioxidant analysis in the mice revealed that the enzymic antioxidants such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase activities were found to have decreased in the DLA treated mice compared to the untreated control. The oral administration of the root extracts of *Coleus forskohlii* has significantly increased enzymic antioxidants. Among the extracts, the methanolic extract showed the maximum enzyme activities.
- The non enzymic antioxidants level such as glutathione and vitamin C was found to be decreased in the mice with DLA tumor compared to the untreated control. The levels were raised significantly in the mice, treated with the methanol extract.
- The liver marker enzymes such as ALP, ACP, SGOT, SGPT , LDH were found to have increased in the serum, indicating the

liver damage and it is reduced significantly in the methanol extract treated mice which is on par with the standard and the untreated control.

- As the methanolic extract showed better activity compared to the other extracts of the roots of *Coleus forskohlii*, it was selected for studying the influence of this extract in the mice with DLA tumor for haematological parameters.
- Among the hematological parameters, hemoglobin is found to have decreased in the mice with DLA compared to the untreated control. It was brought back to normal in the methanol extract treated mice.
- The WBC count, differential cell count and monocyte and lymphocyte count were found to have increased in the DLA control mice compared to the untreated control and it got reduced significantly when the mice were treated with the methanolic extract of *Coleus forskohlii*.
- The body weight, the survival time and the histopathological studies were carried out in DLA induced mice and the methanolic extract treated mice and compared with the untreated control and the standard. The body weight got increased in the DLA induced mice compared to the untreated control and it was reduced in the DME treated mice as comparable with standard drug. The survival time of the DLA induced mice was found to be reduced compared to the untreated control and the extract treated mice showed an increase in the survival time which is on par with the standard drug treated mice.

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- The histology of the untreated mice showed a healthier liver tissue whereas in the DLA induced mice liver sections show a perivenular and periportal mixed inflammatory infiltrate composed of lymphocytes within the hepatocytes indicating the clean pathology of the liver. The liver sections of the methanolic extract treated mice showed liver tissue with a few collections of lymphocytes in between the hepatocytes. Reductions in lymphoid cells show no specific pathology.
 - The phytochemical screening showed the presence of tannins, saponins, flavonoids and terpenoids.
 - The root extract of *Coleus forskohlii* was found to be effective in the inhibition of superoxide generation, nitric oxide generation, scavenging DPPH free radicals and hydroxyl radicals at a dose dependent manner.
 - Among the different solvent fractions, the chloroform fraction of the methanolic extract showed the cytotoxic activity against the DLA cells, which was confirmed by MTT assay.
 - The HPTLC assay performed with the chloroform fraction of methanolic extract showed the presence of terpenoids.
 - The GC-MS of chloroform methanolic fraction showed the presence of Alpha-Cubebene, Alpha-Cedrol, 8β(H),14-β-ethyl podocarpane and ferruginol.
 - The further fractionation of chloroform methanol extract of *Coleus forskohlii* with different combinations of (50:50, 60:40, 70:30 and 80:20) chloroform and methanol and was subjected to

cytotoxicity studies and the 70:30 fraction of methanol chloroform extract was proved to be cytotoxic and the same fraction of the extract is used for analysing in TLC and HPLC.

- The TLC showed the presence of 2 spots with one corresponding to forskolin and the other spot was confirmed by HPLC which showed the presence of forskolin and ferruginol.
- The outcome of this study confirms the protective effects of the *Coleus forskohlii* roots against DLA tumor. The administration of methanolic extract of *Coleus forskohlii* has exhibited significant antioxidant, antitumor activity which might be due to the presence of diterpenoids, forskolin and ferruginol.

Recommendations :

The clinical efficacies of the plant preparation have to be validated. The mechanism of action of the active ingredients must be identified to get a complete picture of the antioxidative and the antitumor activity of the plant. A further elucidation of both the known and yet to be identified natural antioxidants of *Coleus forskohlii* clubbed with the newly emerging technology, metabolomics, could help cancer prevention and cure.