

VI. SUMMARY AND CONCLUSION

Studies were performed to **evaluate the anticancer properties of *Acorus calamus* L. rhizome using *in vitro*, *in vivo* and *in silico* models.**

Based on the extraction study, the Methanolic Extract of *A. calamus* (MEAC) was used for both *in vitro* and *in vivo* studies as it had the highest yield of 5.00 % w/w.

- Qualitative chemical analysis of the plant extract showed the presence of various types of phytoconstituents like alkaloids, terpenoids, steroids carbohydrate, saponin, flavonoids etc.
- Cytotoxicity studies carried out on DAL cells by trypan blue exclusion method showed a reduction in the viable cell count in MEAC treated group as compared to the control group. Percentage growth of inhibition was found to be increased in a dose dependent manner.
- These cytotoxicity study on MEAC indicated that the calculated LD₅₀ value (Dixons likelihood method) for the oral doses of the MEAC was found to be more than 2000 mg/kg body weight, accordingly 100 and 200 mg/kg body weight were taken as low and high dose of MEAC for the experiment.
- In the tumor growth response study, MEAC significantly reduced tumor volume, packed cell volume and non-viable cell count compared to those of DAL control mice, while viable cell count was found to be increased significantly in the treated groups.
- In the lifespan study, the mean survival time for the DAL control group was 22 days where as it was 25 and 28 days for low dose and high dose of MEAC treated groups. The mean survival time for the group treated with standard drug 5- FU. was 32 days.
- The restoration of body weight to near normal level was observed after treatment with MEAC.

- RBC count and hemoglobin content, which were decreased after DAL inoculation, were found to be significantly restored to the normal levels in the animals treated with MEAC.
- Administration of MEAC significantly reduced the WBC count in both groups (100 mg / kg and 200 mg / kg) of MEAC when compared to that of DAL control mice.
- With regard to Packed Cell Volume (PCV) significant decrease was observed in all the treatment groups compared to that of DAL control animals.
- In DAL control mice, there were significant reduction in antioxidant enzymes, SOD and catalase activity both in liver and kidney. The treatment with MEAC had significantly improved these parameters as the standard drug treated mice.
- The reduced GSH level in DAL control group was restored to the near normal level by treatment with MEAC as well as positive control in liver. But in kidney lower dose was found to be not significant.
- Similarly the reduced GPx level in DAL control group was restored to the near normal values by treatment with MEAC as well as positive control in both tissues and the lower dose was found to be more significant.
- The level of lipid peroxide in liver and kidney tissues were significantly increased in DAL control mice. After administration of MEAC extract, lipid peroxide levels were significantly reduced when compared with DAL control mice.
- With regard to vitamin C & E levels in liver and kidney there was a significant reduction in DAL control animals, which was significantly improved by the treatment with MEAC.
- Estimation of liver functional enzymes such as ALP, SGPT and SGOT in serum of DAL control animals indicated an elevated level when compared to normal animals. The treatment with MEAC had reduced the levels to near normal conditions.

- TP, urea, uric acid, creatinine, cholesterol and TGL were found to be increased in the DAL control mice. The treatment with MEAC had reduced the above parameters and brought to near normal level. The data with respect to HDL indicated a decreased level in the DAL control mice whereas in the treated group the HDL was found to be restored to normal.
- Haematoxylin and Eosin stained section of liver tissue of DAL treated mice showed loss of normal architecture. Lobules show neutrophilic satellitosis with apoptosis. MEAC treated sections of liver tissue revealed manifestation of mild hepatic damage with preserved architecture. There is no fibrosis submassive necrosis and carcinoma.
- In DAL control mice, alterations in glomerular region of kidney was observed and suppuration with collection of foamy macrophages. The animals treated with standard drug, MEAC exhibited regeneration of glomerular region with slightly dilated tubules. No acute tubular necrosis, malignancy or kidney infarct seen. Renal medulla was found to be normal.
- The MTT assay on MCF-7 cells showed growth inhibition in a dose dependent manner when treated with MEAC at concentrations ranging from 18.75 – 300 µg/ml. The IC₅₀ value of MEAC was found to be 52.07 µg/ml.
- When MN formation was analyzed after treatment with different concentrations of MEAC, significant changes in the frequency of MN were detected for 20 µl and 40 µl ($p < 0.01$).
- With regard to mutagen sensitivity, there was a significant variation was observed within the subjects. Among the breast cancer patients all the treatment groups were found to be sensitive whereas the healthy subjects were hyposensitive. The lowest range of mean break / per cell value was observed in the dose range of 40 µg/ µl in both breast cancer and healthy individuals. Between the four treatment groups of both the

breast cancer and healthy animals, dose dependent decrease in the mean break / per cell value was observed.

- The GC-MS study showed 14 volatile organic constituents from the rhizome of *A. calamus*. Major constituents selected as ligands for further studies were (2R) – 2 - [(1S) – 1 – hexadecanoyloxy - 2-hydroxyethyl] – 4 – hydroxyl – 5 – oxo - 2H-furan – 3 - yl, tetradecanoic acid, 2-hydroxy-6-undecylbenzoic acid and linoleic acid.
- In the *in silico* studies to know the interaction profiling with Breast cancer proteins such as BRCA1, BRCA2, PTEN, HER2, ERbB2, ATM and CHEK2. ERBb2 protein exhibited a higher interaction profile and more protein conformational stability with [(2R)-2-[(1S)-1- hexadecanoyloxy-2-hydroxyethyl]-4-hydroxy-5-oxo-2H-furan-3-yl] suggesting that this ligand can act as a potential drug against breast cancer.

This study throws the possibility of MEAC can be developed as a potent anticancer drug.

Suggestions for further research

- The results of the present study can be useful to design and develop novel compounds having better inhibitory activity against breast cancer.
- Long term studies have to be carried about to identify the right dosage and cytotoxic side effects if any.
- The promising ligand identified in the present study could be subjected to further analysis in different model systems so that it could be developed as a drug against breast cancer treatments.

Recommendations

- *A. calamus* is a locally available medicinal plant with tremendous potential and high demand in the world market due to the wide range of pharmacological applications. So this ethnomedicinal plant should be conserved and need to be evaluated for pharmacological studies and

should be given priority to carryout investigations for clinical trials with its bioactive compounds for the management for breast cancer and thereby can avoid the costly cancer therapy.

- Further investigations, including the isolation and purification of the bioactive components in *A. calamus*, are necessary to elucidate the antitumor activities of exact compounds.
- India carries one fourth of world's burden of breast cancer. There is an urgent need to collaborate with health authorities and give high priority for breast cancer screening and early detection.