Title: EVALUATION OF CYTOTOXIC POTENTIAL OF CURCIN PROTEIN FROM *Jatropha curcas* L.

SUMMARY

Overall aim of the study was to elucidate cytotoxic potential of curcin protein isolated from *Jatropha curcs L.* on panel of five human origin cell lines. To achieve this aim the seeds of Jatropha were collected from locally grown plant in Pune, Maharashtra. The total protein was extracted from defatted skinned seeds of it. The protein precipitated by Ammonium Sulphate precipitation method. The highest amount of protein was precipitated in 60% of ammonium sulphate saturation. The FTIR analysis of 60% saturation of protein confirmed the presence of protein in the given crude extract. The estimation of isolated protein in crude extract resulted the yield in the range of 0.88 mg/ml to 71.44 mg/ml with maximum yield of 71.44 mg/ml obtained in 60% saturation. According to previous reports the toxic protein present in *Jatropha curcas L.* is lectin and hence haemagglutination assay was used as gold standard during isolation of curcin protein at every step. In the study 60% crude protein fraction showed positive agglutination for B^{+ve} blood group hence it proved to be galactose specific lectin. During purification of curcin protein SDS-PAGE gel electrophoresis of proteins derived from *Jatropha* showed a single intense band in 60% fraction comparable to the band of purified protein. This affinity chromatography technique was used for the first time to purify curcin protein in a single step. Curcin being a lectin binds to galactose i.e. carbohydrate adsorbent during its purification by column chromatography. The 20 mM galactose solution was used as eluent during purification. The protein concentration of purified protein was found to be 1.884 µg/ml from total protein isolated as 71.44 mg/ml. The efficiency of column

was found to be good depending on the therotical plates calculated as 762.64 much higher than expected above 100. The Molecular weight calculated from the graph was found to be 28344 Dalton which is approximately 28.2 kDa similar to previously recorded. The curcin protein sugar specificity was validated by sugar inhibition assay, galactose sugar inhibited haemagglutination activity at concentration less than 50 mM which is more than 200 mM for other sugars .These findings affirmed the galactose specificity of curcin and was employed for its single step purification by affinity chromatography.

To further confirm the presence of curcin protein HR-MS analysis was carried out and based on m/z values obtained the MASCOT PMF showed its similarity to ricin A and proricin. Ricin A chain shares similarity to amino acid composition to curcin protein.(Fig.3.15)

The *in silico* studies of curcin protein using BLAST showed similarity with Ricin A chain with more than 100% query coverage. Ricin A chain was used as a template for curcin structure prediction for its interaction with cell surface receptors like Her receptor. Taken together, the results indicated that curcin binds to preferentially to active site of Her receptor making it possible for entry of protein in cancer cells. This curcin protein and its receptor interaction establish its effectiveness in developing new anticancer drug with selective in its action.

Finally the cytotoxicity of curcin protein was investigated on normal and cancer cells. As curcin protein is lectin galactose specific in action cancer cells were showed more cytotoxicity due to expression of galactosyl transferase enzymes on their surface. The panel of cell line which includes ZR-75-1, A579, a375, HL60, HepG2 were used to study the anticancer activity of curcin protein. The cytotoxicity of curcin protein was evaluated by MTT assay

which was comparted with PBMC cells. The curcin protein exhibited cytotoxic effect on A375 and ZR-75-1 cell line with IC_{50} value of 6.4 µg/ml and 8.6 µg/ml, respectively which was further confirmed by fluorescent staining of cells. Curcin protein kills the cells by degrading the ribosomes and thereby inhibiting the protein synthesis. The curcin protein exhibits the N-glycosidase activity, cleaving the 28s rRNA and reducing the growth of cancer cells.

In conclusion, this study established the specificity of curcin lectin with galactose sugar moieties and its utilisation in curcin purification by affinity chromatography. It was further elucidated the cytotoxicity of curcin protein for cancer cells. Hence, the knowledge provided by the study surely a step forwarded in removing the toxic protein curcin from the seed cake to facilitate its use as manure, animal feed as well as in targeted drug delivery system.