Summary
SUMMARY

- Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer representing 85% of liver cancers. Other types of liver cancers are cholangio carcinoma, which starts in the cells that line the bile duct, angiosarcoma (or haemangiosarcoma) which starts in the blood vessels of the liver and hepatoblastoma which is very rare and usually affects young children.

- The molecular mechanism of hepato carcinogenesis is very complicated. Cancer cells have defect in regulatory genes that have control over normal cell proliferation and homeostasis due to a progressive accumulation of mutations. The alterations in physiology resulting in cancer namely i) oncogenes ii) tumor suppressor genes iii) suicide genes iv) limitless replication potential v) neo- angiogenesis, and tissue metastases.

- In fact hepato carcinogenesis is a multistep process involving subsequent mutations of genes that control proliferation and/ or apoptosis in the hepatocytes subjected to continuous inflammatory and regenerative stimuli, starting from the initial phases of chronic hepatitis and then of liver cirrhosis.

- Cancer is one of the scenario were too little apoptosis occurs, resulting in malignant cells that do not die. The rebirth of herbal medicine, especially in developed countries, is largely based on a renewed interest of the public and scientific information concerning plants. As plants used in our day today like onion, pomegranate, grape seeds, turmeric etc were found to have anticancer effect, the present study was designed to evaluate the anticancer effect of an edible part of the plant *Ipomoea batatas* and *Solanum tuberosum*.
was taken as the source for the present study. The present study was to evaluate the chemopreventive and therapeutic efficacy of *Ipomoea batatas* and *Solanum tuberosum* by *in vitro* studies against HepG2 cell lines, *in vivo* studies on DEN induced hepatic carcinoma in wistar albino rats and *in silico* analysis.

The salient findings of the study are summarized below

- The phytochemical screening of the tuberous extract revealed the presence of Carbohydrates, Flavonoids, Quinones, Saponins, Coumarins, which were present in high concentration in Aqueous extract of *Ipomoea batatas* and ethylacetate extract of *Solanum tuberosum* when compared to the other extracts.

- Among the four extracts (ethylacetate < ethanol < hydroethanol < aqueous) extracts the aqueous extract of *Ipomoea batatas* and ethylacetate extract of *Solanum tuberosum* was found to contain major phytochemicals.

- Quantitative analysis of secondary metabolites revealed high percentage of coumarins, saponins, flavonoids, and quinones in the aqueous extract of *Ipomoea batatas* and ethylacetate extract of *Solanum tuberosum*.

- The *in vitro* radical scavenging potential of *Ipomoea batatas* and *Solanum tuberosum* were assessed by DPPH, Nitric oxide, Superoxide anione, H$_2$O$_2$ assay. These assays revealed that both the tuberous extracts possessed radical scavenging potential. The antioxidant activity was studied by FRAP and in this both the extracts showed better activity. The above two results strongly support the antioxidant nature of the extract which is the main criterion for the chemotherapeutic drug.
The *in vitro* anticancer potential of the selected extracts were analysed by MTT assay against HepG2 cell lines. The result of this study reveals that *Solanum tuberosum* has higher activity than *Ipomoea batatas*.

The *in vitro* antioxidant and anticancer results were further supported by the *in vivo* efficacy of the *Ipomoea batatas* and *Solanum tuberosum* in DEN induced hepatocellular carcinoma in wistar rats.

HCC rats showed a significant decrease in the body weight. On supplementation of the selected extract the body weight was significantly increased. The supplementation of *Ipomoea batatas* and *Solanum tuberosum* significantly reduced the increased liver weight treatment groups. There were no significant histopathological changes in the organs like heart, kidney, spleen, pancreas and brain in DEN treated rats, extracts supplemented rats compared to the control rats. The cancer incidence was also decreased in extract treated groups when compared to cancer induced group.

Bilirubin level was found to be elevated in the serum of cancer bearing group. This condition was reversed to normal level in the groups treated with *Ipomoea batatas* and *Solanum tuberosum* extracts. This result reveals that selected extract may exert its potential protective role in liver.

Serum total protein and albumin level were decreased in DEN induced group and on supplementation with the extracts the levels of proteins and albumin were restored to that of control group.

HCC bearing rats showed significant increase in serum urea level. On treatment with *Ipomoea batatas* and *Solanum tuberosum* showed as significant decrease in the levels of...
urea in serum. This shows that the selected extracts has an impact on regulation process involved in excretion of urea. There was no significant changes noted in the level of creatinine in control and treated groups.

- The blood glucose level in cancer bearing rats was reduced due to the increased uptake of glucose for energy production by the cancer cells. Upon treatment with *Ipomoea batatas* and *Solanum tuberosum* it was reverted and glucose level was brought back towards the normal range. The extracts may exert its protective role in liver.

- The marker enzymes such as SGOT, SGPT, ALP and GGT activities were significantly elevated and there was a significant decrease in the activities enzymatic of antioxidants such as SOD, CAT, GPx, non enzymatic antioxidant such as glutathione, Vit C, Vit E were observed in HCC treated groups compared to normal. The treatment with *Ipomoea batatas* and *Solanum tuberosum*, significantly reduced the markers enzyme values and restored the cellular redox status of the animals thereby significantly increasing the efficacy of antioxidant defense system. *Solanum tuberosum* showed more significant changes in *in vivo* marker enzymes and antioxidant potential compared to *Ipomoea batatas* extract.

- Lipid peroxides level was found to be elevated in DEN induced HCC rats. These levels were brought back to normal upon administration of *Ipomoea batatas* and *Solanum tuberosum* in treated groups suggesting that the macromolecular damages induced by DEN was mainly mediated through free radicals and the two extract displays its preventive role in inhibiting free radical production.
AFP is a tumor marker and its level in serum is used in the prognosis of liver cancer. Cancer induced groups showed significantly high level of AFP in serum. DEN augmented the level of AFP expression while the Ipomoea batatas and Solanum tuberosum extracts treated groups showed significant decrease in AFP levels in serum. Solanum tuberosum extract showed marked decrease in AFP level compared with Ipomoea batatas treated group and silymarin (drug control) group.

In the present study the blood cells are significantly altered in HCC bearing rats as compared to control rats. The RBC count, HB, HCT, MCV, MCH and MCHC were reduced significantly and WBC was increased in DEN induced liver cancer bearing rats. Treatment with Ipomoea batatas and Solanum tuberosum extracts restored all the above mentioned hematological parameters to near normal values. This indicated that the selected extracts may possess compounds which have protective action on hematopoietic system.

In order to identify the bioactive compound which is actually responsible for the in vivo anticancer effect both the Ipomoea batatas and Solanum tuberosum extracts were subjected to column chromatography. Aqueous extract of Ipomoea batatas and Ethyl acetate extract of Solanum tuberosum gave 75, 70 fractions respectively and was pooled to five active fractions each based on the TLC profile.

The anticancer effect of the extracts on HepG2 cell lines were determined by MTT assay. From the results of MTT assay two fractions namely (ethanolic : ethylacetae) FRACTION III of Ipomoea batatas and (ethanolic) FRACTION V of Solanum tuberosum possessed better anticancer activity against HepG2 cell lines.
As the active fractions were found to contain good anticancer activity its bioactive constituents were identified by subjecting to GCMS and HPTLC analysis.

GCMS analysis of active fraction III of *Ipomoea batatas* revealed the presence of 29 compounds such as 1,2-Benzenedicarboxylic Acid, 4-Mercaptophenol, 2-Formylhistamine, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl etc and the active fraction V of *Solanum tuberosum* showed the presence of 47 compounds such as N-isobutyl-2(E),6(Z),8(E)-Decatrienamide, 9-Hexadecenoic acid, ethyl 9-hexadeconate, hexadecanoic acid, Bis(2-ethylhexyl) phthalate, etc.

HPTLC analysis of active fraction III of *Ipomoea batatas* and the active fraction V of *Solanum tuberosum* revealed the presence of 13 and 7 non volatile compounds, respectively.

DNA fragmentation and flow cytometric analysis by annexin V-FITC/PI of active fractions III of *Ipomoea batatas* and active fraction V of *Solanum tuberosum* stated that both the active fractions induced cell cycle arrest and thus executed cell death by apoptosis and not by necrosis.

Since the antiproliferative activity of the active fractions were confirmed by *in vitro* analysis, the effect of these active fraction on various genes involved in hepatocellular carcinoma were determined.

Both FRACTION III of *Ipomoea batatas* and FRACTION V of *Solanum tuberosum*, down regulted the expression of anti apoptotic genes namely TNFα, TGFβ, NFkB p50, NFkBp65 and Bcl2 and upregulated the expression of Bax, Caspase3 and Caspase9 genes. *Solanum tuberosum* fraction was more effective than *Ipomoea batatas* fraction.
To identify the lead molecules and to study its interaction with the proteins involved in hepatic carcinogenesis, five compounds of both the fractions were subjected to molecular docking studies, with TNFα, TGFβ as protein targets. The drug likeliness (ADMET) property of all the five ligands were determined. From the Dock score it was found that 3,4,5 Trihydroxy benzoic acid ethyl ester of *Solanum tuberosum* fraction possessed the highest dock score with both TNFα, TGFβ proteins.

The outcome of the current research study would provide better insights in understanding the chemopreventive and anticancer role of *Ipomoea batatas* and *Solanum tuberosum* against hepatocellular carcinoma.