SUMMARY AND CONCLUSION

Numerous biochemical abnormalities occur in the cancerous conditions. The curative potential of D-Pinitol was evaluated by studying the biochemical and molecular alterations observed in experimental mammary carcinoma in Sprague - Dawley rats and in Human breast cancer cell lines MCF-7 and MDA-MB-231. The results obtained from D-Pinitol treated and untreated groups are compared with their respective controls. The findings of the study have been summarized below.

D-PINITOL INHIBITS 7, 12-DIMETHYLBENZ(a)ANTHRACENE INDUCED MAMMARY CARCINOMA- IN VIVO STUDY

- Body weight and tumour weight were observed in the cancerous conditions. There was a sharp drop in the body weight in the mammary carcinoma bearing rats and the tumour weight was found to be significantly increased in cancer prone animals. On D-Pinitol treatment, a significant increase in the body weight and an observable tumour regression was found.

- Total protein content was found to be decreased in DMBA induced cancer condition. The administration of D-Pinitol normalized the protein content in D-Pinitol treated animals.

- DNA damage is a sensitive indicator in the carcinogenesis. The levels of cellular constituents namely, DNA and RNA have been estimated. In cancer bearing animals, the levels of DNA and RNA were found to be significantly increased whereas it was significantly brought back to near normal levels in D-Pinitol treated animals.

- Enormous production of free radicals is the characteristic property of cancer cells. Lipid peroxidation was increased in cancer bearing animals due to excessive production of free radicals generated by DMBA. On the contrary, enzymic antioxidants such as SOD, CAT and
Schematic representation of effect of D-Pinitol during 7, 12-Dimethylnaphthacene (DMBA)-induced breast carcinogenesis

**Group I**
Vehicle Control (Corn Oil)

**Group II**
Breast Cancer induced with 20 mg/kg/bw DMBA diluted in corn oil (1ml) orally [Tumor control]

**Group III**
After DMBA administration, Breast cancer bearing animals were treated with D-Pinitol (200 mg/kg/bw) orally for 45 days

**Group IV**
D-Pinitol alone (200 mg/kg/bw) orally for 45 days [Drug control]

Normal Breast Tissue

- Body weight 
- Tumor weight

Mammary Carcinoma

- Antioxidants
- Nucleic acid Content
- Glycoproteins
- Membrane ATPase
- Lipid Peroxidation
- Lipid Profile
- Biotransformation enzymes
  - Phase I
  - Phase II
- Hormones
- Lysosomal enzymes
- Tumor Markers
- Bcl-2, NF-κB
- p53, Caspase-3, Bax

7, 12 - DMBA

D-Pinitol
GPx and non-enzymic antioxidants viz. GSH, Vit-C and Vit-E were reduced in DMBA treated animals. Administration of D-Pinitol decreased the LPO levels and increased the antioxidants levels thus indicating the antioxidant properties of the D-Pinitol.

Glycoproteins serve as reliable classical markers in the malignant conditions. The levels of glycoprotein components were elevated in the cancer conditions and brought back to near normal levels in D-Pinitol administration indicating its positive protective effect on the membranes. This shows D-Pinitol has the capacity for retrieving the normal structure, rigidity and function of the damaged cell membranes and the membranes of other subcellular organelles too.

Hyperlipidemia is a secondary complication in the breast cancer. Hyperlipidemia was observed in mammary carcinoma bearing animals when compared to their respective controls. On D-Pinitol treatment, these levels were decreased which indicating the hypolipidemic property of the D-Pinitol.

The lipid metabolising enzymes were sensitively affected in the cancerous condition. The activities of lipid metabolising enzymes were found to be abnormal when compared to control. The activities were brought back to near normal values in the D-Pinitol treatment animals.

Lysosomal enzymes play a significant role in the breakdown of cells and intracellular substances thereby enhancing tumour invasion and the activities of lysosomal enzymes were significantly increased in mammary carcinoma bearing animals. Where as the activities were reverted back to near normal in D-Pinitol treatment which indicating its cytostabilizing property.

Membrane damage is a basic feature of the malignant cells. The membrane bound ATPase activities were decreased which indicating the severity of the disease. These activities were restored to near
normal activites upon D-Pinitol treatment indicating its membrane stabilizing action.

- Phase I biotransformation enzymes such as Cyto -P<sub>450</sub>, Cyto-b<sub>5</sub>, NADPH cyto-C reductase were increased in mammary carcinoma bearing animals due to microsomal damage caused by DMBA induced free radicals. On the other hand, UDPGT and GST were decreased in cancer bearing animals due to involvement in DMBA metabolism. Due to the antigenotoxic effect of D-Pinitol the phase I and phase II biotransformation enzyme activities were modulated towards normal in D-Pinitol treated animals. The inhibition of carcinogen metabolic activation indicates the anticancer activity of D-Pinitol in DMBA induced breast cancer.

- Serum CEA and CA 15-3 are specific marker of breast cancer and were dramatically raised in cancer bearing animals. On D-Pinitol treatment these levels becomes near normal range. Which explains that treatment with D-Pinitol may alleviate cancer initiation and progression in mammary carcinogenesis and possibly acts as anticancer agents.

- Hormones play an important role in the aetiology of breast cancer through the expression of their receptors. D-Pinitol reduces the serum concentration of estrogen and progesterone in group III rats. This indicates D-Pinitol might inhibit the mammary epithelial cell proliferation probably by preventing the over expression of estrogen and progesterone in the mammary tissues of Sprague-Dawley rats.

- D-Pinitol treatment significantly reduces the serum cytokine levels thereby contributing to reduction in tumor burden.

- The study of apoptosis is very important. It has been proved that occurrence of cancer is due to the loss of control of normal apoptosis and the disturbance of balance between cell apoptosis and cell
proliferation. DNA fragmentation pattern was observed in D-Pinitol treated animals indicates, the apoptosis induced by the D-Pinitol. Administration of D-Pinitol reduced the DNA damage.

In breast cancer condition the protein and mRNA expression of p53, Bax and caspase-3 were found to be lowered, contrarily the Bcl-2 and NF-κB protein expression were high. These alterations in the protein and mRNA expression were brought back to near normal in D-Pinitol treated animals when compared to control animals. This shows the apoptotic nature of D-Pinitol.

Histopathological examination was carried out in the control and exprimental animals. The antineoplastic property of D-Pinitol was further supported by the histopathological observations towards the normal architecture of the vital organs such as, breast and liver tissue in the D-Pinitol treated rats.

The ultra structure of breast tissues by transmission electron microscopic studies revealed that the architecture of breast tissues was drastically damaged in breast cancer bearing animals due to the DMBA induced neoplastic condition, which was restored after D-Pinitol treatment. This inevitably indicates the anticancer potential of D-Pinitol.

ANTICANCER EFFECT OF D-PINITOL ON HUMAN MAMMARY CARCINOMA CELL LINES (MCF-7 AND MDA-MB-231) - IN VITRO STUDY

The viability of MCF-7 and MDA-MB-231cells were significantly reduced by D-Pinitol treatment in a dose-dependent manner. Inhibitory effect of D-Pinitol treated MCF-7 and MDA-MB-231cells indicates the cytotoxic nature and antiproliferative property of D-Pinitol.
The lactate dehydrogenase (LDH) was significantly released in D-Pinitol treated MCF-7 and MDA-MB-231 cells shows the cytotoxic nature of D-Pinitol.

The GSH content was significantly decreased in the D-Pinitol treated MCF-7 and MDA-MB-231 cells by inducing intracellular oxidation by induction of apoptosis.

Significant morphological changes were observed in light and fluorescent microscopic studies. This clearly indicates the apoptotic property induced by D-Pinitol in MCF-7 and MDA-MB-231 cells.

Significant mitochondrial membrane potential changes were noticed in D-Pinitol treated MCF-7 and MDA-MB-231 cells, which indicates the activation of apoptosis by releasing the cytochrome C.

DNA fragmentation was observed in D-Pinitol treated MCF-7 and MDA-MB-231 cells. This may be due to the apoptotic activity of D-Pinitol. The apoptosis induced by D-Pinitol indicates the anticancer potency.

Noticeable changes in the intensity (protein and mRNA expression) of apoptotic proteins were found in D-Pinitol treated MCF-7 and MDA-MB-231 cells which clearly indicates the D-Pinitol induces the apoptosis.

It is concluded from the results of both in vivo and in vitro breast cancer studies, that D-Pinitol has been found to exhibit an enhanced antioxidative, hypolipidemic, membrane stabilizing, cytostabilizing and apoptotic properties. It is clear that D-Pinitol has a potency to inhibit growth and progression of cancer cells and also has an ability to protect the cells from cancer. Due to its potent therapeutic property and non-toxic nature it may be considered to be used as a safe pharmacological drug in the field of cancer therapy. Further studies are in progress to delineate the specific molecular mechanism of anticancer properties of D-Pinitol.