9.1. DISCUSSION:

Plants provide several classes of compounds with immense therapeutic potential. Some of these compounds are not likely to be synthesised in the laboratory. The above experiments clearly establish the anticancer property of *Saraca asoca* (Ashoka) bark, flower and leaves.

The active principle was cytotoxic to various tumour cells (*in vitro*) at very low concentrations. The growth of K-562, KB, CHO and Vero cells in culture experiments were significantly inhibited by the active principle. The cytotoxic studies using human lymphocytes and leukaemia cells showed that normal lymphocytes were insensitive to the drug(s) as compared to leukaemia cells.

Results of thymidine incorporation assay showed that the possible mechanism of the action of the drug at the site of DNA level.

*In vivo* studies showed that the active principle of Ashoka bark or flower significantly inhibited the development of Dalton's lymphoma ascites and Sarcoma-180 ascites tumours in Swiss albino mice. Ashoka bark extracts significantly increases the life span of already developed tumours suggesting its action on developed tumours. EAC cells showed a late development of tumour and subsequent death of mice. These observations led to the conclusion that the action of
active principle is species specific. Moreover, the oral administration of active principle of Ashoka bark or flower extract inhibited the growth of solid tumours initiated by DLA or S-180 suggesting the penetrating power of the drug(s) after absorption and transport through the blood stream.

Chemical carcinogenesis can be induced either by a single protocol as in the case of methylcholanthrene or two stage protocol as in the case of DMBA/Croton Oil. The experiments have conducted with both these techniques and the efficacy of the active principle in reducing chemically induced tumours in mice has also been evaluated. The oral administration of the drug(s) significantly inhibited the tumour diameter and growth of soft tissue sarcomas induced by 20-methyl cholangthrene. It was seen that the topical application of 'Ashoka' bark or flower (drugs) could effectively inhibit the growth of papillomas besides reducing the number of papillomas/mouse. This was further established by pathological examination which shows an improvement in convoluted and involuted pattern and drug induced phase reversal supporting the preventive action of the drug.

The co-administration of active principle reduced the cyclophosphamide or cisplatin induced toxicities. The role of 2-mercaptopropionylglycine against the cyclophosphamide toxicity was investigated (228,229) and the results are on similar lines. Cisplatin - toxicity - induced weight loss was not altered by the
flower extract. Similar results were obtained during the study of chemoprotection of *Nigella sativa* extract towards CTX- toxicity in mice (232). But then the active principle of Saffron could improve the cisplatin - toxicity - induced weight loss in mice as the protective effect shown by the bark extract of *Saraca asoca*.

The chemoprotective effect brought about by Ashoka bark or flower may be by the mechanism of scavenging of free radicals formed by the activation of cisplatin and cyclophosphamide besides the increase in glutathione levels resulting in the detoxification mechanism.

Hyperthermia in trimodality treatment using Ashoka bark or flower and cisplatin or cyclophosphamide enhanced the antitumour effect on S-180 solid tumours. This clearly established the superiority of hyperthermia along with drug in complete remission of solid tumour as a fact to be considered as prospective for clinical application.

The chemical analysis of the active compound from Ashoka bark indicated the presence of (-) - Epicatechin, proanthocyanidin B2 and new proanthocyanidin while in the case of flower it was (-) - Epicatechin, leucopelargonidin 3-0-β-D-glucoside and dihydro chalcone. The above effect like cytotoxicity, antitumour, inhibition of chemical carcinogenesis, chemoprotection and enhancement of antitumour activity in presence of hyperthermia may be due to the presence of the above compounds. However another point of interest
is that Ashoka bark extract is more active than other parts of plant like flowers or leaves. This may be due to either different compounds or more number of compounds in greater concentration are found in bark. Presently we do not know whether the effect observed is due to the action of one component present in Ashoka bark or flower or to a combination effect. The exact nature of the compounds or the definite mechanism of action of these compounds in producing the above effects needs further experimentation.
9.2. SUMMARY:

*Saraca asoca* is a plant used in the Indian system of medicine for various types of diseases.

Initial cytotoxic studies revealed better extraction with Ethylacetate in the case of bark, 95% methanol in flowers and 90% acetone in leaves of *Saraca asoca* and this concentrated solution was found to be cytotoxic against Dalton's lymphoma ascites, Ehrlich ascites carcinoma, S-180 cell lines and P 388 lymphocytic leukaemia.

This active fraction were then further purified using paper, column and thin layer chromatography techniques. The active compounds were eluted, evaporated lyophilised and stored in a powder form at 4°C.

The effect of the active principle were further studied against growing cell lines like CHO, Vero, K562 and KB cell lines and have been found to be effective thus establishing the anticancer effect on these cell lines. Cytotoxic studies were detected by trypan blue exclusion method. Studies using normal lymphocytes and leukaemic cells indicated that acute lymphoblastic leukaemia cells were highly sensitive while normal lymphocytes were the least sensitive to drug(s) action. Thymidine incorporation studies showed that the mechanism of action of the drugs at the site of DNA synthesis, since the concentration require to produce cytotoxicity
was much higher than that for the inhibition of thymidine incorporation.

In vivo antitumour studies of Ashoka bark or flowers using DLA, ECA and S-180 showed good effect with DLA and S-180 while little effect was obtained in the case of Ehrlich ascites carcinoma (EAC) tumour cell line. With regard to solid tumours experiments on hind limbs, Ehrlich ascites carcinoma did not respond while better response was obtained in DLA and S-180. Another exciting observation was the mode of treatment namely oral feeding of the drug which clearly showed the penetrating power of the drug after absorption and transport through the blood stream.

The experiments with DMBA on two stage carcinogenesis reveal the presence of negligible number of papilloma as against 2.5 per mice in the controls. This was further established by pathological examination which supported the preventive effect of the drug. Methylcholanthrene induced soft tissue sarcoma could also be prevented, using oral feeding of Saraca asoca bark or flower extract. Thus the chemical carcinogen could be prevented by the active principle of bark or flower of Saraca asoca.

The active compound from Ashoca bark or flower could effectively modulate the toxicities induced by the administration of cisplatin or cyclophosphamide. The co-administration of the drug (Saraca asoca bark or flower) did not alter the anticancer activity of cisplatin or cyclophosphamide. Leucopenia, fall in haemoglobin
levels, azotaemia and elevations in serum enzyme levels were prevented. A substantial increase in life span was also observed.

Trimodality therapy using Ashoka (bark or flower) extract along with cisplatin or cyclophosphamide and hyperthermia restricted the growth of subcutaneously transplanted seven day old Sarcoma-180 tumour significantly. Drug induced toxicities like leucopenia etc., were prevented. Renal or hepatic toxicity was not elevated in presence of Saraca asoca bark or flower and hyperthermia.

Isolation and characterisation of the active principle from the flowers indicated the presence of flavanoids and have characterised the presence (-) - Epicatechin, leucopelargonidin 3-0-B-D-glucoside and a dihydro chalcone in the flowers while from the bark isolated 3 compounds, (-) - Epicatechin proanthocyanidin B2, and a new compound. This compound was proven to be a new proanthocyanidin. However the exact identity of these compounds have been well established on comparison with authentic samples and co-chromatography.