Chapter 9

Summary and conclusion
*Phyllanthus amarus* is being used in the traditional Indian medicine for the last 2000 years for several disease conditions. Presently research on *P. amarus* is going on in several laboratories in India, Germany, Australia, USA etc. The major activity of this plant is in conditions involving liver and this medication is frequently prescribed in jaundice. It has been shown to be useful to reduce the HbsAg antigen found in human HIV carriers (Unander and Blumberg, 1992). *P. amarus* inhibited hepatitis B virus polymerase activity and decreased episomal hepatitis B virus DNA content and suppressed viral release into the culture medium (Lee et al; 1996). In chemically induced liver toxicity models *P. amarus* significantly protected the liver tissue (Prakash et al; 1995). Phyllanthin, a diaryl butane lignan, isolated from *P. amarus* showed a significant protection against CCl$_4$ induced elevation in transferase levels and significantly increased protein level (Syamasundar et al; 1985). It also showed antigenotoxic properties (Gowrishanker and Vivekanandan; 1994). It is also prescribed for gonorrhea, diabetes, ulcers of skin, sores, swelling and use topically as poultice in itching. An infusion of this plant is used in the treatment of chronic dysentery. Recently the extract as well as isolated compounds from *P. amarus* were found to inhibit the replication of human immunodeficiency virus.

*P. amarus* extract was studied in our laboratory for its inhibition of liver carcinogenesis produced by N-nitrosodiethylamine (NDEA) and 20 methyl cholangthrene (20MC). *P. amarus* was found to inhibit the liver carcinogenesis induced by NDEA and more significantly the
extract was found to have a significant activity on the tumour bearing animals. These indicates that *P. amarus* contains materials that could inhibit events in carcinogenesis and produces an inhibition of the tumour cell proliferation. In fact *P. amarus* was found to inhibit the topoisomerase I and II and cdc25 tyrosine phosphatase enzyme indicating its effect on the cell cycle enzymes.

*P. amarus* was found to have a significant anti-oxidant activity and it could inhibit lipid peroxidation, superoxide radicals as well as scavenge hydroxyl radical production *in vitro*.

In the present study we have looked into several of pharmacological activities of *P. amarus* which was not looked into earlier and give a rational explanation of these activities. We have looked into the inhibition of mutagenicity induced by sodium azide (NaN₃), N-methyl N'-nitro N-nitrosoguanidine (MNNG) and 4-nitro-O-phenylenediamine (NPDA) using Ames *Salmonella typhimurium* assay. The extract at concentration of 1mg/ plate could significantly inhibit mutagenesis. It was also found to inhibit mutagenicity induced by 2-acetamidofluorene (2-AAF) and aflatoxin B₁ to *Salmonella typhimurium*. These mutagens needed activation by microsomal enzymes and *P. amarus* extract seems to inhibit the activation and thereby inhibit mutagenicity. Moreover the urinary mutagenicity induced in rats by administration of benzo [a] pyrene was found to be significantly inhibited by the oral administration of *P. amarus*.
We have also tested anti-carcinogenicity of *P. amarus* carcinogenesis model induced by 7, 12 dimethylbenz(a)anthracene (DMBA) as initiator and croton oil as promoter. It was found that skin papilloma produced by DMBA and croton oil application was significantly inhibited by topical application of *P. amarus*. It was also observed that prior treatment of *P. amarus* before DMBA application was also found to inhibit the papilloma formation indicating that *P. amarus* could inhibit the initiation of carcinogen by DMBA as well as produce an inhibition of tumour cell promotion produced by croton oil.

*P. amarus* treatment was also found to significantly inhibit the stomach cancer induced by N-methyl N’-nitro N-nitrosoguanidine (MNNG). This was reflected in the reduction in the number of tumours in the *P. amarus* treated group as well as the onset of induction of tumour. It was observed that while in the untreated group all the animals had stomach tumour after MNNG treatment, only 44% of the animals produce stomach tumour in the *P. amarus* treated groups. This was also reflected in the levels of γ-glutamyl transpeptidase, cytosolic glutathione- S-transferase, tissue glutathione and cytosolic glutathione reductase activity values which were altered by the MNNG treatment. *P. amarus* also showed reduction in the elevated number of AgNOR dots and clusters induced by MNNG administration. These results compliment to the reported results on the inhibition of carcinogenicity by *P. amarus* treatment.

We have also studied the effect of *P. amarus* on the induction of diabetes produced by alloxan. Administration of alloxan has been
shown to produce damage to βcells of pancreas and the onset hypoglycemia in the animals. It has been shown that alloxan induces its effect through the free radical mediated oxidation. Since *P. amarus* has been shown to have significant anti-oxidant activity the effect of *P. amarus* in alloxan induced diabetes was studied in animals. Administration of *P. amarus* along with alloxan was found to significantly reduce tissue injury produced by alloxan with a subsequent decrease in blood sugar. The effect was also seen in the normal animals by administration of *P. amarus* indicating the effect of *P. amarus* may not be specific to its anti-oxidant activity but the reduction in hyperglycemia is caused by another mechanism which is not known at present. Administration of alloxan to animals was also found to produce tissue injury to organs other than to pancreas. It produces hepatic injury as seen by elevation of ALP, GPT as well as renal injury as seen in blood urea nitrogen and creatinine. The alloxan also produces significant damage to hematological system as seen from the decrease in total WBC count. Administration of *P. amarus* was found to reverse toxicity induced by alloxan as seen decrease in ALP, GPT, creatinine and blood urea nitrogen and there was an elevation of total WBC. *P. amarus* alone did not produce any toxicity to liver or kidney or the hematological system.

Inflammatory process is the net result of oxidant activity in the tissue produced by oxidative burst of macrophages. The role of *P. amarus* in reducing inflammation was studied using two different models. In the first model inflammation was produced by
carrageenan, a sea weed which has been shown to produce significant paw oedema. Administration of *P. amarus* was found to significantly inhibit the paw oedema induced by carrageenan indicating that *P. amarus* could act as significant anti-inflammatory agent. *P. amarus* was also tested for its activity on gastric inflammation produced by the administration of alcohol. Metabolism of alcohol has been shown to produce intermediate oxygen radical which causes injury to stomach and produces an inflammatory response. Severe inflammation produced by intragastric administration of alcohol was found to be significantly inhibited by subsequent administration of *P. amarus*. Administration of alcohol was found to produce intraluminal bleeding, increased ulcer index, and mortality. This was reduced by simultaneous administration of *P. amarus*. Glutathione levels in the stomach mucosa, which was decreased by alcohol administration, was found to be elevated by the *P. amarus* treatment.

Even though *P. amarus* has been shown significant anti-viral activity, we could not come across literature on its antibacterial and anti-fungal activity. We checked these activities using *in vitro* experiments. Addition of *P. amarus* to the growth medium was found to inhibit growth of both gram positive such as (*Staphylococcus aureus*) and gram negative such as (*Escherichia coli*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Proteus vulgaris*). However the observed activity was not significant. At concentration of 2mg produced 15-38% inhibition of the growth of the organism was observed during 24 hours. *P.*
*amarus* also showed its activity on the growth of *Aspergillus parasiticus* as seen from the weight of mycelium. Addition of *P. amarus* was found to significantly inhibit the production of aflatoxin by *Aspergillus parasiticus*. The aqueous and alcoholic extract of *P. amarus* was found to produce more significant inhibition of aflatoxin production compared to hydrophobic solvents.

We have done some preliminary study on the chemistry of active ingredient in *P. amarus*. Most of the anti-oxidant activity was in aqueous and alcoholic extracts and extracts with non-polar solvents produced only lowered activity. Moreover the yield of alcoholic and aqueous extracts were significantly higher compared to those produced by non-polar solvents. We have tried to purify active material by column chromatography as well as with thin layer chromatography. We have also used anti-oxidant activity as the marker during purification. During silica gel column chromatography most of the anti-oxidant activity was found with methanol, ethyl acetate and acetone, indicating the polar nature of active ingredient. TLC of various fractions indicated that the activity is mainly seen in the polyphenolic fractions (ferric chloride positive bands) indicating that most of the activity of *P. amarus* is due to polyphenols present in the extract.

Several active compounds have been identified in *P. amarus* extracts. Most common among them are lignans like phyllanthin and hypophyllanthin (Somanabandhu et al., 1993), flavonoids like quercetin, astragalin (Nara et al., 1977), ellagitannins like amarinic acid
(Foo., 1995) as well as gallotannins like amariin and geraniin, corilagin, 1,6-digalloylucopyranoside, and rutin and quercetin-3-O-
glucopyranoside (Foo., 1993), and phyllanthusiin D (Foo and Wong., 1992). Aqueous as well as alcohol based P. amarus extracts potentially inhibited HIV-I replication in HeLaCD4+ cells with 50% effective concentration (EC50). A gallotannin enriched fraction showed enhanced activity (0.4µg/ml), and the purified gallotannins geraniin and corilagin were most active (0.24µg/ml) (Notka et al., 2003). Anti-viral agents: repandusinic acid (Ogata et al., 1992) and niruiside (Qian-Cutron et al., 1996) isolated from P. amarus were shown to inhibit HIV transcription in tissue culture.

Hydrolysable tannins such as amariin and geraniin, corilagin, 1,6-digalloylucopyranoside, as well as rutin and quercetin-3-O-
glucopyranoside, are reported to be present at very low concentrations in P. amarus extract. Some of the hydrolysable tannins in P. amarus are reported to inhibit protein kinase such as CDPK and PKC at very low concentrations (Polya., 1995). In fact kinase inhibition by the gallotannins was the lowest recorded in the literature. This indicated that P. amarus and its active ingredients can significantly interfere with signal transduction and would be responsible for many of the observed activities. This needed a thorough study using molecular biology techniques.