ABSTRACT

The continuous efflux of reactive oxygen species (ROS) from endogenous and exogenous sources results in continuous and accumulative oxidative damage to cellular components and alters many cellular functions. Among the biological targets proteinaceous enzymes, lipidic membranes and DNA are most vulnerable to oxidative damages. Ionizing radiation is an important source exogenous ROS and the toxic effect of radiation results mostly from oxidative damages through the generation of several reactive oxygen species such as superoxide, hydrogen peroxide, hydroxyl radicals etc, and the most important sub cellular target is the DNA. Many xenobiotics supplied to living organisms are metabolized inside the body by conjugation with the cellular antioxidant enzymes like GSH, and cause their depletion that lead to oxidative stress.

In the present project, the biological activities of the extracts of the medicinal plants *Acorus calamus*, *Hemidesmus indicus* and *Coscinium fenestratum* and one of the active components of *A.calamus* extract α-asarone was evaluated. The extracts and α-asarone have been examined for their antioxidant potential and found to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, hydroxyl radical, superoxide radical and and ABTS radical (2, 2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) in a concentration dependent manner thus indicating their antioxidant potential. The anti inflammatory activities of all these extracts and compound were also examined and found to be possessed significant anti-inflammatory activity against carrageenan and formalin induced paw edema, hence effective in both acute chronic inflammatory conditions and the effect shown by them might probably due to their significant antioxidant power.

The cytotoxic effects of *C.fenestratum* extract on Hela cells, apoptotic induction on DLA cells, DNA fragmentation assay and cytochrome-c release assay along with in vivo tumor regression activity was studied and it was observed that *C.fenestratum* extract had a marked inhibitory effect on HeLa cells and is concentration dependent. The tumor cells become apoptotic and the cellular DNA was cleaved into multiple...
fragments upon apoptosis induced by C.fenestratum extract. Also it triggered the release of mitochondrial Cyt-C and thereby enhances the activation of caspases 3 and caspases 9. Treatment with the extract at doses of 250 mg/kg body weight for 7 consecutive days after tumor development, showed regression in tumor volume in tumor bearing mice. Thus C.fenestratum extract exhibit a combination of anticancer activities both under in vitro and invivo conditions. The significant antioxidant and anti-inflammatory activity of the extract may also contribute to its significant antitumor property.

The radioprotecting property of the extracts of A.calamus and H.indicus together with α-asarone was examined and the result clearly indicated that both the extract and asarone provide significant protection against radiation induced damages to DNA, the primary target of radiation injury, under in vitro, in vivo and ex vivo conditions. They effectively protect radiation-induced single strand breaks in plasmid pBR 322 DNA. The ability of A.calamus extract, H.indicus extract and α-asarone to protect cellular DNA from γ-radiation-induced damages was studied using alkaline single cell gel electrophoresis or comet assay in human or mouse peripheral blood leucocytes, ex vivo and in murine blood, bone marrow and spleen cells in vivo. The comet parameters of DNA, such as comet tail length, % of DNA in tail, tail moment and Olive tail moment, were found increased in all these cells due to radiation-induced DNA strand breaks. Administration of A.calamus and H.indicus (250 mg/kg body weight) or α-asarone (50 mg/kg body weight) prior to irradiation protected cellular DNA from radiation-induced strand breaks in blood, spleen and bone marrow cells of irradiated mice as evidenced from the decrease in the comet parameters. They also protected membrane lipids ex vivo and in vivo from radiation-induced peroxidative damages which measured as TBARS.

Whole-body exposure of mice to lethal and sub-lethal dose of γ-radiation leads to depletion of tissue antioxidant defence and damages the haematopoietic and gastrointestinal systems in mice. Administration of extracts of A.calamus, H.indicus (250 mg/kg body weight) or α-asarone (50 mg/kg body weight) prior to irradiation significantly prevent the lowering of the antioxidant enzymes and peroxidation of membrane lipids. Moreover radiation induced depletion of total WBC count, haemoglobin, and blood GSH content were found to be reversed by the extracts or α-asarone administration and also enhances the bone marrow cellularity and spleen
colony formation in whole body irradiated mice, indicating the ability of *A.calamus* extract, α-asarone and *H.indicus* extract in stimulating the hematopoietic recovery. In addition they also protected the epithelial cells of the gastrointestinal tract from the radiation-induced structural alterations. The increase in the survival rate and mean body weight of whole body irradiated animals administered with the extracts or α-asarone indicate their ability to protect living system from the deleterious effect of ionizing radiation.

The comet analysis of DNA of whole body irradiated mice blood and bone marrow cells at different time intervals showed a decrease in comet parameters with the increase in time which indicates that the strand breaks in cellular DNA were repaired in a time dependent manner. When extracts or α-sarone was administered to mice following radiation, it was found that repair of cellular DNA damage in whole body irradiated mice is enhanced greatly. Thus in addition to provide protection to the cellular genomic DNA against radiation damages these extracts or asarone also enhances the repair mechanism.

Administration of *A.calamus* extract or α-asarone also protected whole body irradiated mice from genotoxic effects of γ-irradiation as shown by reduction in the number of radiation-induced chromosomal aberrations and frequency of micronuclei formed, indicating the protection of cellular DNA from radiation-induced lesions.

To determine the protective effect of *A.calamus* extract and α-asarone against the oxidative stress induced by some selected xenobiotics, acetaminophen induced hepatotoxicity, doxorubicin induced cardiotoxicity and cisplatin induced nephrotoxicity were studied.

Acetaminophen administration (150 mg/kg body weight) results in manifestation of hepatotoxicity as can be revealed from the elevated levels of serum marker enzymes like SGOT, SGPT, ALP and serum bilirubin and alter the architecture of normal hepatic cells. It also induced oxidative stress as indicated by increased lipid peroxidation in hepatic tissues and significant reduction in tissue GSH, GPx, SOD and catalase. Administration of *A.calamus* extract or α-asarone helps to reverse the levels of GOT, GPT, ALP and bilirubin and enhances the activity of antioxidant enzymes and more protection was observed in pre treated group than the post treated ones.
To find out the cardio protective activity of A.calamus extract and α-asarone doxorubicin (DOXO) an anthracycline antibiotic, induced cardiotoxicity was studied. An increase in oxidative stress, evidenced by an increase in free radicals and lipid peroxidation as well as a decrease in antioxidants, plays an important role in the pathogenesis of DOXO- induced cardiotoxicity. Single i.p injection of DOXO (25mg/kg body weight) significantly elevated serum LDH and CK-MB levels indicative of cardio toxicity. Both pre and post treatments with A.calamus extract or α-asarone significantly ameliorated CK-MB and LDH enzyme activities, increases the total antioxidant activity, reduced the peroxidation of membrane damage and reverse the DOXO induced myocardial degeneration.

Neproprotecting property of A.calamus and α-asarone was also examined against cisplatin (CP) induced renal toxicity in mammalian system. CP-induced nephrotoxicity is closely associated with elevated levels of serum urea and creatinine and an increase lipid peroxidation in the kidney tissues. It also causes severe alterations in renal tissue architecture, depletion of GSH levels and inhibits the activity of antioxidant enzymes in renal tissue. When the animals were treated with A.calamus extract or α-asarone, the serum urea and creatinine levels were brought down significantly and restore the normal renal tissue architecture. In addition the CP-induced oxidative stress was also reduced through the elevation of GSH and other antioxidant enzymes like SOD, GPx and Catalase in the renal tissue and there by counteract the toxic effect of cisplatin in the mammalian system.

In summary the present project revealed the biological potential of the herbal extracts Acorus calamus, Hemidesmus indicus and Coscinium fenestratum and the phytceutical α-asarone against oxidative stress induced damages which mainly caused by some xenobiotics like paracetamol, doxorubicin and cisplatin or by radiation exposures in mammalian system. These extracts or compound offered protection both at cellular and molecular levels. The free radical scavenging property may be the basic mechanism underlying their protecting ability. Thus the potential usefulness of these herbal extracts or phytceutical in ameliorating the damaging effect of ionizing radiation or the side effects of drugs like acetaminophen, doxorubicin and cisplatin was confirmed from the present work.