GENERAL DISCUSSION, SUMMARY AND CONCLUSION

In the present study diethyl ether and methanol fraction of *Piper nigrum* and water extract and water- methanol and methanol fractions of *Piper betle* showed protective effects in all different parameters used. Phytochemical analysis by using different analytical methods was carried out on the crude extract and solvent fractions of these plants. There are reports on the investigation of chemical constituents of *Piper betle* and *Piper nigrum*.

Nalina and Rahim (2007) analyzed the constituents of *Piper betle* leaves using TLC and gas chromatography and mass spectrometry, and the analysis showed the presence of hydroxychavicol, fatty acids (steiric and palmitic acids) and hydroxyl fatty acid esters (steiric, palmitic and myristic acids) as main components. They studied the antibacterial effect of crude aqueous extract *Piper betle* against *Streptococcus mutans*. And according to them the antibacterial activity was due to the combined effect of different chemical constituents present in the *Piper betle*. There is lot of controversies regarding the use of *Piper betle* for medicinal purpose, because the *Piper betle* as a part of quid has been implicated in oral cancers. However, the use of *Piper betle* leaf was known for centuries for their curative properties as mentioned earlier. In Chinese folk medicine betel leaves are used for the treatment of various disorders and claimed to have detoxification, antioxidation and antimutation properties (Nikhil kumar et al., 2010).

Amonkar et al (1986) for the first time showed the nonmutagenic property of betel leaves and the presence of hydroxychavicol (a phenol)

in *Piper betle* leaves with antimutagenic properties. This was the turning point in *Piper betle* research which established that *Piper betle* per se do not contribute to the oral cancer. Later several medicinal properties have been attributed to *Piper betle* including anticancer effects and some of the activities have been patented also (Nikhil kumar et al., 2010). An herbal composition (containing *Piper betle*) for treating CD33 acute and chronic myeloid leukemia and the method thereof, have been patented (USA/ 2007/ 7306817). In our study although we observed the antitumor activity the effect was minimal. Probably the selected doses contain low amount of proven bioactive compounds with antitumor effects. However, with crude extract and fractions we cannot increase the dose because many of these natural compounds have optimum dose levels and increasing the dose may not have beneficial effects rather sometimes it may have negative effects. Many of the plant extracts have prooxidant effects at higher doses. This prooxidant effect could interfere in the antitumor activities (Uma Devi et al., 1995).

Piper betle showed good antioxidant and anticlastogenic activity. Nikhil kumar et al. (2010) reviewed the antioxidant activity of *Piper betle*. The *Piper betle* crude extract and hydroxychavicol showed significant *in vitro* and *in vivo* free radical scavenging activities. According to one study the overall activity of *Piper betle* was superior to tea (Bhattacharya et al., 2007). Rathee et al (2006) reported the antioxidant activity of *Piper betle* leaf extract and its constituents.

According to this study the plant phenolics in general are highly effective free radical scavengers and antioxidants. Therefore, the antioxidant activities of plant/ herb extracts are often explained with their total phenolic content with good correlation. In our study we observed presence of phenolic contents in its crude aqueous extract as well as its solvent fractions.

Black pepper (*Piper nigrum*) contains many bioactive constituents including piperine, limonene, pinene, and terpinolene. It also contains safrole, a known carcinogenic substance whose presence is likely mitigated by the other constituents in black pepper such as limonene. Piperine has been shown to possess antioxidant activity. Piperine showed protective effect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species and hydroxyl radicals. Piperine was found to act as a hydroxyl radical scavenger at low concentrations, but at higher concentrations, it activated the fenton reaction resulting in increased generation of hydroxyl radicals. It also acts as a powerful superoxide scavenger and it inhibited the lipid peroxidation (Mittal and Gupta, 2000). The antimetastatic potential of piperine was demonstrated in a mouse model of lung metastasis from melanoma. In mice given piperine, there was a significant reduction in the size and number of lung metastasis enhancement of longevity of the treated mice. In cultured human colon cancer cells, piperine reduced the proliferation of cells at doses that are roughly equivalent to those obtained from frequent

consumption in the diet. Piperine is well known to affect the bioavailablity and pharmacodynamics of many drugs and botanicals (Kaczor, 2010).

Many studies have attributed the anticlastogenic, antitumor and antioxidant properties of *Piper nigrum* to its component piperidene/ piperine. Lim et al (2009) identified the alkaloids like pallitarine, piperidene, piperine and pellitorine in *Piper nigrum* and *Piper betle*. Reshmi et al (2010) isolated the alkaloidal constituent piperidene from *Piper nigrum*. *In vitro* antitumor activity of this compound was determined by using MTT assay in HEp2 cells. It showed about 52% of inhibition in HEp2 cells, indicating its antiproliferative activity. Kumar et al (2010) reported the antioxidant of piperine. The activities of enzymatic antioxidants such as SOD, catalase, glutathione peroxidase were assayed in erythrocytes, liver and heart. They also studied the level of non- enzymatic antioxidants such as GSH, vit C and E. Heart tissues in these groups were subjected to histopathological examinations. Piperine protected the heart from histological damage and also showed antioxidant activity. The authors correlated the protective effect of piperine and heart tissues to its antioxidant activity. In our study also we observed the protective effects of *Piper nigrum* and *Piper betle* fractions against the radiation induced lipid peroxidation, reduced level of glutathione and radiation induced sickness, neurological degeneration and mortality.

The constituents of the *Piper* species have also been attributed towards providing protection against radiation injury to the cells and the antioxidant systems in the body. Jagetia et al., (2006) evaluated the radioprotective effects of Liv 52 and assayed the lipid peroxidation and glutathione content.

Summary and Conclusion

- Present investigation is divided into two parts, that is, protective effect of extract/ solvent fractions of *Piper nigrum* and *Piper betle* against e- beam radiation (4 and 14 Gy) and cyclophosphamide (50 mg/kg. b. w).

- To assess the protective effects, anticlastogenecity, antioxidant, hematological, histological, behavioral and physiological studies were carried out.

- For anticlastogenecity studies bone marrow and peripheral blood micronucleus tests were used. For biochemical studies, SOD, GSH, catalase and lipid peroxidation assays were carried out. In hematological studies total WBC and total RBC count was done. Mouse liver and intestine were used to assess the degree of histological damage induced by positive agent and the protective effect of the test compounds. The level of radiation injury was determined by assessing the, hair loss, facial edema, weight loss and diarrheic conditions in the experimental animals. The extent of radiation effect on the behavior and the protective effect of the test compounds was estimated by using radial, open field and elevated plus maze tests.

- For antitumor studies Ehrlich's Ascites Carcinoma was used as the tumor model. *In vivo* antitumor test was performed using the different test compounds in Swiss albino mouse. The

cytotoxic/ antitumor effect of the test compounds was studied *in vitro* by using trypan blue dye exclusion test and MTT assay.

- The selected doses of e- beam radiation and CP showed clastogenicity in the in vivo system. The extract/ solvent fractions of *Piper species* significantly reduced the clastogenic effects of the anticancer drugs cyclophosphamide and electron beam radiation (4 and 14 Gy). Here the methanol fraction of *Piper nigrum* and water- methanol fraction of *Piper betle* showed effective anticlastogenic action.
- The e- beam radiation significantly reduced the levels of SOD, GSH and catalase, while there was significant increase in the level of lipid peroxidation. Antioxidant molecule / enzymes were significantly increased by the extract/ solvent fractions. Methanolic fraction of *Piper nigrum* and water- methanol and methanol fractions of *Piper betle* significantly enhanced the SOD, GSH and catalase level in animals treated with radiation. The peroxidation process was significantly reduced in combined treatment groups as evidenced by the decreased level of MDA level with an increase in GSH level. This shows that the test compounds protected the cells by potentiating the antioxidant defense system.
- In hematological studies, the extract/ solvent fractions improved the total WBC and RBC count, which was reduced by radiation treatment. The pre- treatment with test compounds

reduced the tissue damage as observed in the histological sections of liver and intestine.

- Pre- treatment with the extract/ solvent fractions also reduced the radiation induced mortality along with reduction in the rate of hair loss, facial edema and diarrheic conditions in the test animals.
- Behavioral assessment of the test animals revealed significant improvement in the behavioral changes induced by radiation.
- In the *in vivo* antitumor studies the extract/ solvent fractions displayed moderate antitumor effect.
- In the *in vitro* antitumor studies the extract/ solvent fractions showed significant tumor cell inhibitory effects. As revealed by the IC_{50} values, methanol fraction of *Piper nigrum* and water-methanol fraction of *Piper betle* showed better tumor inhibitory effects with Ehrlich's ascites carcinoma cells.
- The preliminary phytochemical analysis followed by thin layer chromatography displayed the presence of carbohydrates, flavonoids, Alkaloids, steroids, flavones, phenols in *Piper nigrum* and *Piper betle*. Literature survey pertaining to the chemical constituents of *Piper nigrum* and *Piper betle* provided the information on the presence of piperine and piperidine as major components in *Piper nigrum*, whereas chavicol, chavibetol and hydroxychavicol were found to be the major components in *Piper betle*. Some studies have revealed the protective and antioxidant effects of these compounds in different test systems.

However, in our study we used crude form of the extracts which also showed the protective effects in the *in vivo* system and antitumor effect in *in vitro* systems. The protective effects imparted by these compounds may not be due to a single component but may be due to the synergestic/ additive effects of different components present in the extract/ solvent fractions of *Piper nigrum* and *Piper betle*. Further studies are needed to isolate and evaluate the major protective components present in these plants.