CHAPTER - 6

SUMMARY
6.1 Introduction

The imbalance between reactive oxygen and antioxidant defense system due to free radicals, may increase the oxidative burden and lead to the damage of macromolecules such as DNA, carbohydrates or proteins. Such processes are thought to play a role in pathological processes of various diseases. Plants having vitamins (C, E, caretenoids, etc.), flavonoids (flavones, isoflavones, flavonones, anthocyanins and catechins), polyphenols (ellagic acid, gallic acid and tannins) possess remarkable antioxidant activity. Antioxidant activity is neither restricted to a particular part of the plant nor the specific families.

In nature there are wide variety of naturally occurring antioxidants which are different in their composition, physical and chemical properties, mechanisms and site of action, such as:

**Enzymes:**
- Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx)

**High molecular weight compounds:**
- Proteins like albumin, transferin, ceruplasmin

**Low molecular weight compounds:**
- Tocopherol, quinines, bilirubin and some polyphenols

**Minerals:**
- Selenium, copper, manganese, zinc and chromium

**Vitamins:**
- Vitamins A, C and E
Plants as antioxidants:
There are many Medicinal Plants with antioxidant property. Screening of plants is done by measuring the antioxidant activity through various in vitro models like DPPH method, Nitric oxide method (Joharapurkar et al., 2003), DMPD method, ABTS method, ORAC method, TBARS assay etc. and via various in vivo models using rats or mice.

An antioxidant is any substance that, when present at low concentrations significantly delays or prevents oxidation of cell content like protein, lipids, carbohydrates and DNA. Antioxidants can be classified into three main types: first line defense antioxidants, second line defense antioxidants and third line defense antioxidants.

Antioxidant plays an important role against free radical mediated oxidative stress. Decrease of body’s natural antioxidants, release free radicals, generated in biological systems that cause oxidative stress in tissue, resulting in lipid peroxidation, have been demonstrated by numerous studies. Once natural antioxidants defense system become weak immediately there is biochemical lesion in many metabolic pathways accompanied by structural damage of tissue (Sood et al., 2004), disturbances in glutathione metabolism (Vijayalaxmi et al., 1994), imbalance of enzyme of carbohydrate metabolism, alteration in enzyme yielding system and electron transport (Raghu, 1992), alteration in general cell metabolism and membrane transport leading to decrease of all the macro and micronutrients (Okada, 2007).
6.2 Methodology

The antioxidant property of three Medicinal plants, *Achyranthes aspera*, *Sphagneticola trilobata* and *Chrysanthemum* was evaluated in H$_2$O$_2$ induced lymphocytes of *Oryctolagus cuniculus*. The flower, leaf and root parts of above all plants were used for the experiment. The respective extracts of the different parts was prepared in Methanol and Chloroform separately, by using Soxhlet Extraction apparatus.

For *in vitro* evaluation of antioxidant property of all extracts of all three plants, blood sample was collected from *Oryctolagus cuniculus* L. following density gradient method, and was washed in phosphate buffer saline, and was processed for lymphocyte separation, and then cultured with DMEM and 10% fetal calf serum at 37°C and 5% CO$_2$ for 18 hours in humidified CO$_2$ incubator. After incubation, cell was exposed to oxidative stress with 100µM H$_2$O$_2$ for 2 hours to generate oxidative stress. Thereafter treated lymphocytes were incubated with 5µl/ 10µl / 20µl plant extracts/ 10,000 cells. The treated lymphocytes were also parallel incubated with 5µl/ 10µl/ 20 µl Melatonin/ 10,000 cells. Control set was parallely maintained.

The data obtained was statistically validated by using ANOVA and conclusion was inferred for effect of extracts of all parts of plants in both Chloroform and Methanol was comparatively analysed with Melatonin.

6.3 Experimentation and Observations

- Five samples were processed during the experimentation respectively, to evaluate the activity of each of the enzymes considered for antioxidant
assay (MDA, SOD, CAT, GSH and GPx), for flower, leaf and root of *Sphagnetica trilobata, Achyranthes aspera* and *Chrysanthemum*.

- This was maintained for each of the plant part extract prepared in each of the two solvents (Methanol & Chloroform) separately, and Melatonin as well for comparison.

- Under the subscribed protocol of the “Experimental design”, Group I with only cultured lymphocytes, was taken as the “Control set”, and Group II represented the H$_2$O$_2$ induced oxidative stressed lymphocytes.

- Experiment was proceeded with the pretreatment of H$_2$O$_2$ induced lymphocytes with successively increasing dosage of the plant extract (5µl/10µl/20µl). Observations were made for each of the five samples for enzymatic parameter.

- The parallel set of experiment was maintained with the five samples, for the evaluation of antioxidant activity of each of the five enzymes, with the exposure to melatonin in increasing dosage (5µl/10µl/20µl).

- Observations were made for the antioxidant activities in terms of MDA, SOD, CAT, GSH and GPx respectively and separately, and compared with that of the standard & established antioxidant, melatonin.

- Lymphocytes were separated from blood samples of *Oryctolagus cuniculus* and then processed for *in vitro* culture in our Tissue Culture Lab, and Melatonin was obtained from Loba.
After comparative enzymatic assay for Control, H$_2$O$_2$ induced lymphocytes, and plant extract exposed oxidatively stressed lymphocytes, data was statistically (ANOVA) validated for inference.

6.4 Our Findings

Our findings are summarized as mentioned below:

(A) Free radical scavenging property of *Sphagneticola trilobata* in comparison to *Melatonin*

Evaluation of antioxidant property of *Sphagneticola trilobata* in comparison with Melatonin has revealed the fact that plants having very high potential of free radical scavenging ability besides established antioxidant feature of Melatonin.

In Methanolic extract of flower the antioxidant features indicated by MDA, GSH, SOD, CAT and GPx was found satisfactory but comparatively melatonin was found more suitable.

The Chloroform extract of flower of *Sphagneticola trilobata* have also revealed the free radical scavenging property indicated by the activities of MDA, GSH, SOD, CAT and GPx, but the scavenging potential of melatonin was found greater than Chloroform extract of flower.

The Methanolic extract of leaf of *Spahngenticola trilobata* as an antioxidant examined by activities of MDA, GSH, SOD, CAT and GPx was found very effective even higher than established antioxidant melatonin. Thus Methanolic extract of leaf of *Sphagnosticola trilobata* was found suitable for pharmacological application.
The Chloroform extract of leaf of *Sphagneticola trilobata* also showed high level of antioxidant property in terms of activities of MDA, SOD, CAT and GPx. But activity of GSH was comparatively found higher under influence of melatonin rather than leaf extract. Thus both, Methanolic and Chloroform extract of leaf of *Sphagneticola trilobata* was found suitable for pharmacological application.

The root extract of *Sphagneticola trilobata* in Methanol also showed antioxidant property, but comparatively it was found poor to melatonin in terms of GSH, SOD, CAT and GPx, except MDA whose activities was found more suitable under the influence of root extract than melatonin.

But the root extract of *Sphagneticola trilobata* in Chloroform was found highly effective in antioxidants in comparison to melatonin in terms of activities of MDA, GSH, SOD, CAT and GPx. Thus Chloroformic extract of root is suitable for pharmacological application as potent antioxidants.

**(B) Free radical scavenging property of *Achyranthes aspera* in comparison to *Melatonin***

The extract of flower of *Achyranthes aspera* in Methanol was also examined for its antioxidant feature, and was compared with melatonin. The free radical scavenging ability of flower extract in Methanol was found more suitable for GSH, SOD, CAT and GPx activity than the melatonin but amleorative effect of MDA activity was found more suitable with Melatonin than flower extract, even though, the extract was found suitable for pharmacological application.
The extract of flower of *Achyranthes aspera* in Chloroform was also evaluated to test oxidative stress and was compared with melatonin. Experiment showed that activities of MDA, GSH, SOD, CAT and GPx were found active in reducing oxidative stress under exposure of both extract and melatonin, but it was more significant under influence of melatonin rather than extract.

The Methanolic extract of leaf of *Achyranthes aspera* was also evaluated and compared with melatonin in terms of activities of MDA, GSH, SOD, CAT and GPx, and it was found that both extract and melatonin having ability to scavenge free radicals, but it was found greater under exposure of melatonin than the Methanolic extract of leaf of *Achyranthes aspera*.

The leaf extract of *Achyranthes aspera* in Chloroform was evaluated for antioxidant feature and compared with efficacy of melatonin. But in this set of experiment it was found that amleorating effect of melatonin on the activity of MDA, GSH, SOD, CAT and GPx were more effective than the effect of leaf, although it was also found effective but comparatively poor than melatonin.

The root extract of *Achyranthes aspera* in Methanol was also evaluated for antioxidant property and was compared with melatonin. The amleorative effects of enzymes, MDA, GSH, CAT and GPx were found more effective under exposure of Methanolic extract of root in comparison to melatonin although SOD was more active with melatonin. Thus, this extract was also found suitable for pharmacological application.

The root extract of *Achyranthes aspera* in Chloroform was evaluated for its antioxidant feature in terms of MDA, GSH, SOD, CAT and GPx, and compared with effect of melatonin. Both, extract and melatonin were found effective for
amleorative effect of MDA, GSH, SOD, CAT and GPx, but it was found more significant with root extract in comparison to melatonin. Finally, the root extract of *Achyranthes aspera* in Chloroform was found suitable for pharmacological application.

(C) **Free radical scavenging property of *Chrysanthemum* in comparison to Melatonin**

The Methanolic extract of flower of *Chrysanthemum* was used to evaluate antioxidant property in terms of MDA, GSH, SOD, CAT and GPx, and compared with effect of melatonin. It was found that activities of above mentioned all of the enzymes was recovered by both flower extract as well as melatonin, but the recovery process was more significant in flower extract than melatonin. Thus flower extract in Methanol of *Chrysanthemum* was found suitable for pharmacological application.

The flower extract of *Chrysanthemum* in Chloroform was examined to evaluate oxidative stress in Lymphocyte cells and was comapared with effect of melatonin. To test the free radical scavenging activity of MDA, GSH, SOD, CAT and GPX enzymes were evaluated under influence of both melatonin and extract. The activities of above all enzymes were found more amleorative than melatonin, although melatonin was also found effective; thus the flower extract of *Chrysanthemum in Chloroform* may be suitable for pharmacological application.

The Methanolic extract of the leaf of *Chrysanthemum* also showed antioxidant property, but comparatively the effect of melatonin was found much effective
than the root extract because all enzymes, MDA, GSH, SOD, CAT and GPx showed maximum amleorative effect with melatonin exposure.

The leaf extract of *Chrysanthemum* in Chloroform was also used to examine free radical scavenging ability in terms of MDA, GSH, SOD, CAT and GPx, and was compared with melatonin. Although, both extract and melatonin were found effective in reducing oxidative stress, but melatonin was found more effective in comparison to leaf extract.

The Methanolic extract of root of *Chrysanthemum* showed antioxidant property, but comparatively the effect of melatonin was found much effective than the root extract because all enzymes, MDA, GSH, SOD, CAT and GPx showed maximum amleorative effect with melatonin exposure.

The root extract of *Chrysanthemum* in Chloroform was also used to evaluate free radical scavenging ability in terms of MDA, GSH, SOD, CAT and GPx. The activites of MDA, GSH, CAT and GPx confirmed free radical scavenging ability in both extract in melatonin, but comparatively melatonin was found more suitable than root extract. Only the SOD activity was found more amleorative under influence of root extract of *Chrysanthemum* in Chloroform.