



## *Discussion*

## 5. DISCUSSION

Cell metabolism continuously produces reactive oxygen species (ROS) as by products of respiration and other metabolic activities (Park *et al.*, 2004a). A growing body of evidence suggests that free radicals play an important role in the development of tissue damage and pathological events in living organisms (Peuchant *et al.*, 2004). They are also involved in the pathogenesis of over 50 human diseases (Moskovitz *et al.*, 2002).

In contrast to the biological potential of ROS formation, the cells have developed a complex defense system, which acts through the enzymatic activities and protection by the low molecular weight antioxidants. Another form of protection is the use of synthetic and natural compounds that show antioxidant effect on the cell. Antioxidants act as reducing agents (free radical terminators), metal chelating and singlet oxygen quenchers (Vertuani *et al.*, 2004).

Most of the antioxidants used in therapy are derived from natural sources. About 28% of the drugs approved by the FDA between 1981 and 2002 are either natural products or chemicals derived from them (Clardy and Walsh, 2004).

Herbs have been utilized to treat acute and chronic disorders for thousands of years. Various medicinal properties have been ascribed to natural herbs (Ivanova *et al.*, 2005). Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite (Dasgupta and De, 2004; David *et al.*, 2004; Gulcin *et al.*, 2004b). Endogenous antioxidants in medicinal herbs may play an important role in the antioxidative defense against oxidative damage, possibly protecting the biological functions of cells (Gunther, 2004).

One such medicinal plant is *Triticum aestivum*, which is commonly called wheat grass. Wheat is a wild grass native to arid countries of Western Asia. Owing to the presence of unique elastic protein complex, it is the only grain suitable for leavened bread and thus may be regarded as an important part of daily diet (Mazza, 2003).

Research reports have been presented about the antioxidant potential of wheat bran (Iqbal *et al.*, 2007) and wheat flour (Yu *et al.*, 2004). However, there is lack of scientific report regarding the efficacy of the grass against oxidative damage. With this backdrop, the present study was undertaken to analyze the antioxidant ability of the plant under oxidatively stressed conditions. The observations made and the results obtained are discussed in this chapter with reference to the relevant published literature.

## PHASE I

In the initial phase of the analysis, the *Triticum aestivum* leaves, at different time points of growth, were analyzed for the levels / activities of non-enzymic and enzymic antioxidants. The different time periods selected were 4, 8 and 12 days after sowing (DAS), in order to find out whether any difference in the antioxidant content observed in the different stages of growth. The results showed that the 4<sup>th</sup> day leaf extract possessed the maximum activities / levels of enzymic and non-enzymic antioxidants followed by 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

The plants could not be effectively used beyond 12 DAS, as they matured and had a fibre content that decreased palatability, as reported by a majority (28 out of 30) volunteers. Since the 4<sup>th</sup> day plant showed better antioxidant content than the later time points, an attempt was also made to test an earlier time interval of 2 DAS. However, the seeds had just sprouted 2 DAS and were very small for analysis. Therefore, the study was conducted only on 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>th</sup> day leaves.

Plants have been considered as valuable sources of medicinal agents for the treatment of many diseases. Several epidemiological studies have indicated that a high intake of plant products is associated with a reduced risk of a number of chronic diseases such as atherosclerosis and cancer (Gosslau and Chen, 2004).

*Triticum aestivum* leaves were found to be a rich source of enzymic antioxidants. The results showed that the 4<sup>th</sup> day leaf extracts possessed maximum activities of all the enzymic antioxidants tested compared to the 8<sup>th</sup> and 12<sup>th</sup> days. Many studies have been reported on the antioxidant status of young and old leaves. Severino *et al.* (2007) have reported that the older leaves of *Trifolium repens* and *Centaurea jacea* had lower antioxidant concentrations and were prone to ozone injury than younger leaves. Diehn *et al.* (1993) had reported that the enzyme peroxidase is developmentally regulated in the leaves of soybean plant.

Endogenous H<sub>2</sub>O<sub>2</sub> production may result in a programmed down-regulation of ascorbate peroxidase activity in gladiolus tepal flower senescence, while GR activity was found to depend upon age (Hossain *et al.*, 2006).

Thus, it is evident that the antioxidant capacity depends upon the plant age and the content also varies with the plant. In the present study, the activities of enzymic antioxidants of *Triticum aestivum* were found to be maximum in the 4<sup>th</sup> day leaf extract followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

Numerous plant products have been shown to have antioxidant activity (Scartezzini and Speroni, 2000; Koleva *et al.*, 2002) and the antioxidant vitamins, flavonoids and polyphenolic compounds of the plant origin have been extensively reported as scavengers of free radicals and inhibitors of lipid peroxidation (Tapiero *et al.*, 2002).

Our results showed that the non-enzymic antioxidant levels were maximum in the 4<sup>th</sup> day leaf extracts followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

Considerable evidence from several epidemiological studies suggests that lycopene has anti-carcinogenic, anti-atherogenic potential, the effects of which have been attributed primarily to antioxidant properties (Omoni and Aluko, 2005).

In accordance with our results, Cano *et al.* (2006) had reported that five days old *Avena sativa* (oat) and *Triticum aestivum* (wheat) leaves exhibited higher hydrophilic antioxidant activity and ascorbic acid level than the 10 and 20 days old leaves under de-etiolation process and light stress. Sharma and Hall (1996) have reported that the 12 days old sorghum plants showed better protection against photoinhibition than the 30 days old leaves and the carotenoid zeaxanthin is newly synthesized in the young leaves under these conditions. Kakani *et al.* (2004) had reported that the photosynthetic rates of 30 days old cotton leaves were reduced significantly when compared with 12 days old leaves upon UV-B exposure.

In *Triticum aestivum* leaves, the levels of non-enzymic antioxidants observed at all the three time points of growth were found to be higher in the 4<sup>th</sup> day leaf extract followed by the 8<sup>th</sup> day and 12<sup>th</sup> day leaf extracts, except for chlorophyll, which is present in high concentrations in the 12<sup>th</sup> day plant.

The results of the present study show that the leaves of *Triticum aestivum* at their early stages of growth are good sources of antioxidants.

## PHASE II

In this phase, a variety of cell free systems and *in vitro* assays were used to characterize the radical scavenging and antioxidant activities of the *Triticum aestivum* leaf extracts.

*In vitro* systems are easier, faster and more cost-effective compared to traditional bioassays *in vivo*. The testing of the antioxidant activity of the compounds *in vitro* is useful, because if a substrate is poorly effective *in vitro*, will not be better under *in vivo* conditions (Aruoma, 2003).

## RADICAL SCAVENGING ACTIVITY

The antioxidant activity of plant origin is dependent on the type and polarity of the extracting solvent as well as on the test system and the substrate to be protected by the antioxidant (Kang and Lee, 2001).

Solvent extraction is frequently used for the identification and isolation of the antioxidants. The extraction yield and antioxidant activity of the extracts are strongly dependent on the solvent, due to the different antioxidant potential of compounds with different polarity. For these reasons, comparative studies for selecting the optimal solvent providing maximum antioxidant activity will be highly useful (Kang and Lee, 2003).

In our study, *Triticum aestivum* leaves were serially extracted into the solvents of increasing polarity and were tested for their ability to scavenge superoxide and nitric oxide radicals. Along with the solvent extracts, a fresh juice (crude homogenate in water) of *Triticum aestivum* leaves was also tested for its antioxidant activity against  $\text{SO}^\bullet$  and NO generation *in vitro*.

The leaf extracts of *Triticum aestivum* at the three time points selected were found to be effective in scavenging superoxide and nitric oxide radicals with the maximum effect being shown by the fresh juice of the 4<sup>th</sup> day plant. This was followed closely by the methanol, ethylacetate and water extracts. Petroleum ether and benzene extracts were less effective in scavenging superoxide and nitric oxide radicals.

The primary free radical in most biological systems is the superoxide anion ( $\text{O}_2^{\bullet-}$ ). Although  $\text{O}_2^{\bullet-}$  itself is quite unreactive compared to the other radicals, the biological system convert it into more reactive species, like  $^\bullet\text{OH}$  radicals (Winterbourn and Kettle, 2003).

Many reports in the literature associate the  $\text{SO}^\bullet$  scavenging activity of plants and their components with strong antioxidant activity. The fresh juice of the 4<sup>th</sup> day plant exhibited a better superoxide radical scavenging activity followed by the fresh juice of the 8<sup>th</sup> and 12<sup>th</sup> day extracts of *Triticum aestivum*, all of which were higher than the scavenging activity of the individual solvent extracts.

High radical-scavenging activity against superoxide anion and hydroxyl radical-scavenging activity have been reported in water extracts of poropolis (Nagai *et al.*, 2003). The aqueous extract of *Phyllanthus fraternus* has been shown to render protection against superoxide generation (Sailaja and Setty, 2006). Siddhuraju and Becker (2003) have reported that an aqueous, methanol and aqueous-ethanol extracts of freeze-dried leaves of *Moringa oleifera* were capable of scavenging peroxy and superoxide radicals.

The methanolic extracts from the leaves of *Rhazya stricta* exhibited antioxidant property as reflected by the capacity to scavenge  $\text{SO}^\bullet$  radical (Iqbal *et al.*, 2006). A hot water extract of *Smilax glycyphylla* effectively quenched the chemically generated superoxide anions (Cox *et al.*, 2005).

The methanolic extracts of yellow gentian (*Gentiana lutea L.*) leaves and roots possess antioxidant properties which was expressed by their ability to scavenge superoxide radicals (Kusar *et al.*, 2006). The ethanolic extracts of many edible Thai plants significantly inhibited  $\text{O}_2^{\bullet-}$  generation (Jiwajinda *et al.*, 2002).

Mansour *et al.* (2007) has reported that the aqueous, petroleum ether, chloroform, ethylacetate and methanol extracts prepared from powdered *Acacia salicina* leaves were active in inhibiting  $\text{SO}^\bullet$  radical production in a xanthine / xanthine oxidase system, while the petroleum ether extract alone was effective at inhibiting nitroblue tetrazolium reduction by the superoxide radical in a non-enzymatic  $\text{SO}^\bullet$  generating system.

The phenolic compounds 3-hydroxyphloridzin, 3-hydroxyphloretin and quercetin isolated from a 70% aqueous acetone extract of the leaves of *Malus doumeri* var. *formosana* (Leu *et al.*, 2006) and pedalitin isolated from the dried leaves of *Rabdosia japonica* (Masuoka *et al.*, 2006) exhibited very strong superoxide radical scavenging activities. The hydroalcoholic extracts from white tea, *Morinda citrifolia* and wheat sprouts exhibited maximum superoxide scavenging activity (Calzuola *et al.*, 2006).

In the present study, the fresh aqueous juice of the 4<sup>th</sup> day plant showed maximum superoxide radical scavenging followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts of *Triticum aestivum*. This observation indicates the strong antioxidant activity of *Triticum aestivum* grass.

Nitric oxide (NO) plays a role in the modification of the trans-membrane transport of ions and signal transduction pathways that regulate cell functions (Paige and Jeffrey, 2007).

Effective inhibition of NO accumulation represents a beneficial therapeutic strategy (Park *et al.*, 2004b). Among all the extracts tested, the fresh juice of the 4<sup>th</sup> day plant of *Triticum aestivum* were able to inhibit the NO generation to a significant extent.

Several plant extracts have been reported to exhibit NO scavenging effects. The ethanolic extract of the *Annona squamosa* leaves showed maximum NO scavenging effects (Shirwaikar *et al.*, 2004). The nitric oxide scavenging effect of the ethyl acetate fraction of *Opuntia humifusa* was higher than that of the methanol, hexane, chloroform, butanol and water fractions (Cho *et al.*, 2006).

The extracts of *Saussurea costus* (Pandey *et al.*, 2005) and *Cytisus scoparius* (Sundararajan *et al.*, 2006) showed maximum scavenging of DPPH, superoxide and nitric oxide radicals.

The polyherbal drugs like abana, chyavanaprasha, triphala, geriforte, septilin and mentat exhibited dose dependant NO scavenging activities (Jagetia *et al.*, 2004). Also, methanolic extracts of the polyherbal formulations Chandraprabha Vati and Maha yogarajan guggulu were found to be good scavengers of nitric oxide (Bagul *et al.*, 2005).

The herbs *Rubia cardifolia*, *Fagonia critica* and *Tinospora cordifolia* (Rawal *et al.*, 2004) as well as the methanolic extracts of *Artemisia iwayomogi* (Kim *et al.*, 2004) exhibited strong scavenging properties against reactive nitrogen species. The ethyl acetate fraction of the dried fruit rind of *Phyllanthus emblica* showed strong NO scavenging activity *in vitro* than the water and hexane phases (Kumaran and Karunakaran, 2006).

In the present study, the fresh juice of the 4<sup>th</sup> day leaves of *Triticum aestivum* showed significant inhibitory effect on NO generation followed by the fresh juice of the 8<sup>th</sup> and 12<sup>th</sup> day leaves of *Triticum aestivum*.

Further to this, the solvent extracts and fresh juice of *Triticum aestivum* leaves were analysed for the scavenging effects on another popular free radical, namely, DPPH. The DPPH<sup>•</sup> radical is frequently used to determine the radical scavenging activity of natural products (Pukalskas *et al.*, 2005). This compound is characterized as a stable free radical by its property of the delocalisation of the electron pair over the molecule as whole, thus the molecules do not dimerise as would be the case with most other free radicals (Molyneux, 2003).

The results obtained from the rapid dot plot assay revealed that among all the other extracts tested, the aqueous extract followed by methanolic and ethyl acetate extracts and the fresh juice of the 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts of *Triticum aestivum* exhibited maximum DPPH quenching capacity. It is evident from this observation that *Triticum aestivum* leaves at all the time points tested

possess active components that are predominantly polar in nature, which seem to contribute to the radical scavenging activity against superoxide and nitric oxide.

Since the fresh juice of the leaves of *Triticum aestivum* at different time points selected showed maximum inhibitory effect against the radicals tested, it was used for the subsequent analyses.

As a first step, the crude aqueous homogenate was tested against the DPPH radical scavenging effect in a qualitative spectrophotometric assay. The results revealed that the fresh aqueous extracts of the 4<sup>th</sup> day leaves of *Triticum aestivum* exhibited maximum DPPH scavenging effect followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

In agreement with our results, the aqueous extracts of the *Piper betle* leaves were found to possess maximum DPPH scavenging activity (Dasgupta and De, 2004). The ethanolic extract of the leaves of *Triticum aestivum* exhibited a growth dependent increase for a period of 15 days in DPPH scavenging activity, while the aqueous extract did not exhibit this trend (Kulkarni *et al.*, 2006b). Dorman and Hiltunen (2004) have reported that the ethyl acetate-soluble fraction of the herb *Satureja hortensis* was the most effective fraction in scavenging DPPH and hydroxyl radical.

The water extract of the herb *Artemisia campestris* (Aniya *et al.*, 2000), aqueous extracts of leaves of Siamese neem tree (*Azadirachta indica* A.Juss Var. *siamensis* Valetton) (Sithisarn *et al.*, 2006) and the methanolic extracts from *Leea indica* and *Spermacoce articularis* (Saha *et al.*, 2004) showed strong DPPH radical scavenging activity.

The aqueous, ethyl acetate, total flavonoids, oligomer fraction and the methanolic extracts from the leaves of *Myrtus communis* (Hayder *et al.*, 2004) and the chloroform and ethyl acetate leaf extracts of *Bauhinia monandra* (Argolo *et*

*al.*, 2004) exhibited scavenging activity towards the DPPH radical. The bearberry-leaf extract (*Arctostaphylos Uva-Ursi*) exhibited the highest antioxidant activity towards the scavenging of DPPH compared to other species such as *Glycyrrhiza lepidota*, *Echinacea augustifolia* and *Equisetum spp.* the other species examined (Amarowicz *et al.*, 2004).

In the present study, the 4<sup>th</sup> day leaf extract of *Triticum aestivum* showed the maximum DPPH scavenging effect followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

The crude homogenate was then tested against the *in vitro* generation of hydroxyl radicals. The hydroxyl radical is one of the most reactive free radical species known with damaging effects to almost every biological molecule found in living cells (Castro and Freeman, 2001). It is a highly reactive oxygen centered radical, which attacks proteins, DNA, PUFA in membranes and most biological molecules in contact (Aruoma, 1999).

In the present study the *Triticum aestivum* leaf extracts were capable of protecting 2'-deoxy-D-ribose from oxidative degradation by scavenging hydroxyl radicals. The 4<sup>th</sup> day leaf extract was more effective than the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts in this respect.

Many studies on the hydroxyl radical scavenging properties of plants are reported in the literature. The aqueous extract of *Tinospora cordifolia* inhibited Fenton reaction and radiation mediated 2-deoxyribose degradation in a dose dependent fashion (Goel *et al.*, 2002). The 70% aqueous acetone extracts of ten Taiwanese native plants effectively inhibited the formation of  $\cdot\text{OH}$  radicals (Hou, *et al.*, 2003). Pedrosa (2006) had reported that the crude extract and hydroalcoholic fraction of *Ouratea parviflora* leaves exhibited a strong concentration dependent inhibition of hydroxyl radicals.

The aqueous extracts of *Teucrium polium* (Ljubuncic *et al.*, 2006) and *Magnifera indica* (Pardo-Andreu *et al.*, 2006) were shown to be potent inhibitors of  $\bullet$ OH radical. The crude methanolic extracts of celery (*Apium graveolens*) leaves and roots were found to be good scavengers of  $\bullet$ OH radicals (Popovic *et al.*, 2006).

The polyherbal formulations, Aller-7 (D'Souza *et al.*, 2004) and hydroalcoholic extract of geriforte (Jagetia and Baliga, 2004) exhibited concentration dependent scavenging activities towards biochemically generated hydroxyl radicals.

It is evident from our results that the 4<sup>th</sup> day leaf extracts of *Triticum aestivum* possess maximum hydroxyl radical scavenging activity followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts which shows its antioxidant potential. In light of the available literature these findings gain significance in establishing the antioxidant potential of the leaves.

Following this, the fresh juice of *Triticum aestivum* leaves were investigated for their H<sub>2</sub>O<sub>2</sub> scavenging activity. The results obtained revealed that the maximum scavenging of H<sub>2</sub>O<sub>2</sub> was mediated by the 4<sup>th</sup> day leaf extract of *Triticum aestivum* followed closely by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

Several studies have been reported on the H<sub>2</sub>O<sub>2</sub> scavenging activity of the plant extracts. The water and ethanolic extracts of *Ocimum basilicum* had effective DPPH radical scavenging and hydrogen peroxide scavenging activities (Gulcin *et al.*, 2007). The polyphenolic compound rosmarinic acid identified in an aqueous extract of peppermint leaves (*Piperitae folium*) exhibited strongest hydrogen peroxide scavenging activity (Sroka *et al.*, 2005).

An aqueous extract of a popular Taiwan folk medicine was capable of scavenging H<sub>2</sub>O<sub>2</sub> in a dose-dependent manner (Wang *et al.*, 2004). The crude extract of *Leontice smirnowii* and the monodesmosides of *Leontice smirnowii*

tuber were effective in scavenging H<sub>2</sub>O<sub>2</sub> (Gulcin *et al.*, 2006). A polysaccharide isolated from *Ocimum sanctum* (Subramanian *et al.*, 2005) possessed promising activity in scavenging H<sub>2</sub>O<sub>2</sub>.

Gulcin (2005) has reported that both the water and ethanolic crude extracts from black pepper (*Piper nigrum*) seeds exhibited strong antioxidant activity as reflected by their effectiveness in scavenging H<sub>2</sub>O<sub>2</sub>. The 80% ethanolic extracts from garlic showed strong scavenging activity against H<sub>2</sub>O<sub>2</sub> (Sato *et al.*, 2006).

In the present study, all the leaf extracts of *Triticum aestivum* analysed showed scavenging of H<sub>2</sub>O<sub>2</sub>, with the 4<sup>th</sup> day leaf extract showing maximum activity.

Our results, thus, demonstrate that the *Triticum aestivum* leaf extracts possess remarkable activities/levels of both enzymic and non-enzymic antioxidants (as indicated in the results of the first phase). They also exhibit strong radical scavenging effect against a battery of radicals tested and were also effective in scavenging H<sub>2</sub>O<sub>2</sub>. The maximum effect was mediated by the 4<sup>th</sup> day leaf extracts of *Triticum aestivum*. It can be concluded from these results that *Triticum aestivum* leaf extracts can effectively mitigate the oxidative stress and free radical mediated pathological events.

#### **EFFECT OF *Triticum aestivum* LEAF EXTRACTS ON THE OXIDATIVE DAMAGE TO BIOMOLECULES**

Having ascertained the antioxidant potential of the leaves, the ability of the leaf extracts to protect cellular biomolecules against oxidative damage was tested. Though oxidants can attack and cause deleterious effects on a wide variety of cellular components, their primary target is identified as lipids in the membrane and their ultimate target, the DNA. Hence, in the present study, the effect of the leaf extracts against oxidative damage to these biomolecules was studied.

## EFFECT OF *Triticum aestivum* LEAF EXTRACTS ON THE LIPID PEROXIDATION IN MEMBRANE MODELS

Polyunsaturated fatty acids are probably the most susceptible targets to free radical attack. The reaction of free radicals with the membrane lipid components leads to lipid peroxidation. This process can eventually cause increased membrane permeability and cell death (Rakonczay *et al.*, 2003).

In the present study, the extent of inhibition of *in vitro* lipid peroxidation by the leaf extracts of *Triticum aestivum* in three different membrane preparations was analyzed. The membrane models used were RBC ghosts (plasma membrane preparations, liver homogenate (mixture of plasma membrane and internal membranes) and liver slices (intact cells). The *Triticum aestivum* leaf extracts, at all the time points studied rendered strong protection to all the membrane model systems used.

The effect elicited by the aqueous extract of the 4<sup>th</sup> day leaves was more pronounced in all the systems studied, followed by the 8<sup>th</sup> and 12 day leaf extracts. This observation reveals that the phytochemical antioxidants of the leaf extracts are very effective in protecting the internal membranes and the plasma membranes from oxidative damage.

The extent of protection rendered by the leaf extracts of *Triticum aestivum* was much better in the liver homogenate than in the other two models. This implies that the phytochemical antioxidants are very active in the internal membranes to prevent the oxidative damage. This observation also throws insight into the mechanism that may be operating in eliciting the protection observed. Better LPO inhibition was observed in the homogenate than the plasma membrane. However, the same extent was not observed in the intact cells. This suggests that the components of the leaf extracts work in conjunction with some endogenous component in the tissue, which is exposed when the cells are

homogenized. It is also possible that the components in the leaf and the proposed endogenous component do not interact effectively in the intact cell. This could either be due to the internal factor being compartmentalized or due to the fact that permeability factors are involved, which are overcome when the tissue is homogenized. More studies are needed to probe this suggestion.

Several plants have been shown to inhibit lipid peroxidation in various systems. The polyphenols in the leaves of *Artemisia princeps* pamp inhibited lipid peroxidation of erythrocytes by hydrogen peroxide ( $H_2O_2$ ) and lecithin peroxidation by the interaction of hemoglobin and  $H_2O_2$  (Toda, 2005). Jose and Janardhanan (2000) have reported that the ethyl acetate and methanol extracts of *Pleurotus florida* exhibited potent hydroxyl radical scavenging and lipid peroxidation inhibition activities.

Fenugreek seed polyphenols prevented oxidative hemolysis and lipid peroxidation induced by  $H_2O_2$  *in vitro* in human erythrocytes (Kaviarasan *et al.*, 2004). The methanolic extract of the plant *Hedyotis corymbosa* significantly reduced the accumulation of lipid peroxides *in vitro* in a dose dependent manner in rat liver homogenate (Sadasivan *et al.*, 2006).

*In vitro* lipid peroxidation in a liver homogenate can proceed in a non enzymatic way. The process is induced by ascorbate in the presence of  $Fe^{2+} / Fe^{3+}$ , and it has been reported that  $Fe^{2+}$  and ascorbic acid stimulated lipid peroxidation in rat liver microsomes and mitochondria. 18 $\beta$ -glycyrrhetic acid inhibited  $FeCl_2$ -ascorbic acid-stimulated lipid peroxidation in liver homogenate (Jeong *et al.*, 2002).

Szachowicz-Petelska *et al.* (2005) have reported that the ingestion of green tea with ethanol partially prevented changes in structure and function of erythrocyte membrane caused by chronic ethanol intoxication in rats. The administration of the plant extracts of *Piper cubeba*, *Physalis angulata* and *Rosa*

*hybrida* significantly lowered the TBARS, a marker of lipid peroxidation in the plasma of rats compared with control group (Choi and Hwang, 2005).

The crude extract and hydro-alcoholic fractions from leaves of *Ouratea parviflora* showed strong inhibitory activity towards lipid peroxidation induced by tert-butyl peroxide in rat liver homogenate (Carbonari *et al.*, 2006). The increase in TBARS generation in rat liver caused by hepatotoxic agents were reverted back to normal by the administration of the butanol extract of *Glycosmis arborea* (Gomes *et al.*, 2003).

In the context opened by the above studies, the fact that the crude aqueous extracts of the 4<sup>th</sup>, 8<sup>th</sup> and 12 day leaf extracts of *Triticum aestivum* were effective in protecting both the plasma membrane and the internal membrane against damage, gains a lot of significance.

#### **EFFECT OF *Triticum aestivum* LEAF EXTRACTS ON THE OXIDATIVE DNA DAMAGE**

DNA of eukaryotic cells, including vascular cells is under constant attack of chemicals, free radicals, or ionizing radiation that can be caused by environmental exposure, by-products of intracellular metabolism, or medical therapy. The damage may be either limited to altered DNA bases and abasic sites or extensive like double-strand breaks (DSBs) (Hartgreaf *et al.*, 2006).

In the present study the protective abilities of the 4, 8 and 12 day leaf extracts of *Triticum aestivum* against oxidative damage induced in purified DNA preparations as well as the DNA within intact, live cells were tested. Commercially available, purified DNA samples belonging to different hierarchies of evolution were employed. The DNA samples used were  $\lambda$  DNA (linear, phage DNA), pUC18 plasmid DNA (circular, bacterial DNA) and herring sperm DNA (genomic haploid DNA from higher eukaryote).

H<sub>2</sub>O<sub>2</sub> exposure significantly damaged all the DNA samples tested. However, *Triticum aestivum* leaf extracts significantly reduced the extent of DNA damage in the different types of DNA. In the case of pUC18 DNA the damage was not reduced significantly by the *Triticum aestivum* leaf extracts at the doses tested. This implies that the source of the DNA has a profound influence on the activity of *Triticum aestivum* leaf extracts. The effect of the leaf extracts has to be further probed using various doses of the extracts.

Many studies have shown that natural antioxidants in plants are closely related to their biofunctionalities, such as the reduction of chronic diseases like DNA damage, mutagenesis, carcinogenesis, etc. and the inhibition of pathogenic bacterial growth, which are often associated with the termination of free radical propagation in biological systems (Jayaprakasha *et al.*, 2002; Zhu *et al.*, 2002).

Carotenoids strongly inhibited DNA strand breaks caused by peroxy nitrite generated from 3-morpholinopyridone in pUC18 plasmid DNA (Muzandu *et al.*, 2006). Sharma *et al.* (2000) have reported that the aqueous extracts of chilli, black pepper and turmeric reduced the radiation- induced degradation of plasmid pUC18 DNA. Hydroxy chavicol, a phenolic compound present in *Piper betle* inhibited pUC18 plasmid DNA damage by hydroxyl radicals (Chang *et al.*, 2002).

The fermented papaya preparation derived from *Carica papaya* Linn significantly reduced the oxidative DNA damage caused by H<sub>2</sub>O<sub>2</sub> exposure in rat pheochromocytoma cells (Aruoma *et al.*, 2006). Pal and Pal (2005) have reported that the crude turmeric extracts offered protection against the X-ray induced DNA damage of lambda cells.

The crude leaf extracts of *Triticum aestivum* at all the time points selected rendered good protection against oxidative DNA damage in purified DNA samples. Following this, the protective effect of these leaf extracts against H<sub>2</sub>O<sub>2</sub> induced DNA damage was followed in intact cells using the single cell gel

electrophoresis (comet) assay. The cells used were KB (oral carcinoma) and Hep2 (laryngeal carcinoma) cells.

The *Triticum aestivum* leaf extracts were very effective in reverting the DNA damage induced by H<sub>2</sub>O<sub>2</sub> in both the cells. *Triticum aestivum* leaf extract treatment by itself, also effectively decreased the extent of the DNA damage when compared to controls implying the ability of the extracts in protecting cells from baseline DNA damage.

Comet assay is a quick and versatile method for assessing DNA damage in individual cells. It allows the detection of single and double DNA strand breaks, as well as the presence of alkali labile sites (Frenzilli *et al.*, 2006).

Several plant extracts have been shown to revert the oxidative DNA damage. The *Polygonum aviculare* extract showed DNA protective effects by inhibiting hydroxyl radical induced DNA strand scissions (Hsu, 2006). The ethanolic extract of *Satureja hortensis* (Mosaffa *et al.*, 2006) and Hsian-tsoa extract (*Mesona procumbens* Hemsl) (Yang, 2006) decreased the DNA damage in the lymphocytes, induced by H<sub>2</sub>O<sub>2</sub> in the comet assay.

Grape seed polyphenols and bearberry strongly protected U937 cells against H<sub>2</sub>O<sub>2</sub> and tert-butylhydroperoxide induced DNA damage in the alkaline single-cell gel electrophoresis assay (O'Brien *et al.*, 2006). The supplementation of anthocyanin/polyphenolic-rich fruit juice decreased the oxidative damage to DNA in healthy probands in the comet assay (Weisel *et al.*, 2006).

The procyanidin B4, catechin, gallic acid, polyphenols from grape seeds at low concentrations prevented the oxidative damage to cellular DNA induced by H<sub>2</sub>O<sub>2</sub> in the mice spleen cells, as evaluated by the comet assay, but at high concentrations they were suggested to have an opposite effect in rendering protection to DNA (Fen and Lou, 2004).

The results of the present study show that the leaves of *Triticum aestivum* leaves were able to protect DNA against oxidative damage. The leaf extracts by themselves reduced the basal DNA damage. Thus, it can be concluded that the consumption of *Triticum aestivum* leaf extracts will render maximum protection against DNA damage.

#### **EFFECT OF *Triticum aestivum* LEAF EXTRACTS ON CELL VIABILITY**

In the present study, the *Triticum aestivum* leaf extracts were tested for their effect on the extent of survival of KB (oral carcinoma) and Hep2 (laryngeal carcinoma) cells, challenged with H<sub>2</sub>O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> exposure caused a remarkable decrease in the viability of KB and Hep2 cells and the addition of *Triticum aestivum* leaf extracts increased the cell viability.

In accordance with our results, Park *et al.* (2006b) have reported a marked decrease in the viability of H9C2 (cardiomyoblast) cells on H<sub>2</sub>O<sub>2</sub> exposure, which was significantly prevented by treatment with Samul (polyherbal medicine) extract. *Ginkgo biloba* extract totally prevented the cell death and growth inhibition induced by H<sub>2</sub>O<sub>2</sub> in C6 glioma cells (Ahtiok *et al.*, 2006). Pu-erh tea extracts increased the viability of human fibroblast HPF-1 cells against hydrogen peroxide induced damage under normal cell culture conditions (Jie *et al.*, 2006).

*Pinus sylvestris* L. and *Plantago lanceolata* L. extracts effectively inhibited NO production in murine macrophage cell line J774A without cytotoxic effects as tested by MTT assay (Vigo *et al.*, 2005). An ethanolic extract of *Garcinia mangostana* showed the most potent anti proliferative activity against SKBR3 human breast adenocarcinoma cell line, as evidenced by the MTT assay (Moongkarndi *et al.*, 2004). The traditional Chinese medicine, Shengmai San pretreatment (Wang *et al.*, 2003) and protocatechuic acid, a phenolic compound (Guan *et al.*, 2006) enhanced the viability of H<sub>2</sub>O<sub>2</sub> exposed PC12 cells as determined by the MTT assay.

Many herbals and phytochemicals have been reported to exert cytoprotective effect (Horakova *et al.*, 2003; Sowmyalakshmi *et al.*, 2005). In the present study, H<sub>2</sub>O<sub>2</sub> exposure caused a reduction in the viability of both KB and Hep2 cells. The viability was increased in the groups co-treated with H<sub>2</sub>O<sub>2</sub> and *Triticum aestivum* leaf extracts. The treatment of *Triticum aestivum* leaf extracts alone were found to decrease the viability of both KB and Hep2 cells. These results suggest that *Triticum aestivum* leaves may be used as a supportive supplement to decrease the toxicity of anticancer drugs.

### **EFFECT OF *Triticum aestivum* LEAF EXTRACTS ON THE ANTIOXIDANT EFFECT OF GOAT LIVER SLICES SUBJECTED TO OXIDATIVE STRESS**

The results obtained thus far revealed that the leaves of *Triticum aestivum* were very effective in protecting the cellular biomolecular targets from oxidative damage. As a next step, the influence of the leaf extracts was tested on the antioxidant status of cells maintained in their tissue architecture. In order to facilitate the exposure of oxidants and the plant extracts, thin slices of the tissue were made. These studies were conducted using the goat liver slices.

Liver is composed predominantly of parenchymal cells (hepatocytes) that carry out most of the specialized function of this organ. Traditionally, testing of drug candidates are being performed using animal models – an expensive and slow process. Nowadays the use of *in vitro* hepatocyte models for drug metabolism and hepatotoxicity studies are rapidly increasing (Brandon *et al.*, 2003). These *in vitro* models play an important role, especially in the earlier part of the pre-clinical studies.

The liver slice is a microcosm of the intact liver consisting of highly organized cellular communities in which the different cell types are subject to mutual contact. Precision-cut liver slices have proved useful for several

pharmacological and toxicological investigations since the model permits the maintenance of normal lobular architecture and cell-cell interactions within their original matrix. Thus, this *in vitro* model may have an advantage over isolated, cultured hepatocytes due to the preservation of tissue architecture. Since their first use for *in vitro* investigations (Warburg, 1923), precision-cut liver slices have been increasingly used in both descriptive and mechanistic studies for xenobiotic and liver specific toxicity (Lerche-Langrand and Toutain, 2000; Onderwater *et al.*, 2004).

In the present study, employing the precision-cut liver slices generated from goat liver as a tool, the protective effects of the *Triticum aestivum* leaves *in vitro* against hydrogen peroxide induced toxicity was evaluated. Enzymic and non-enzymic antioxidants were analyzed in the liver slices after incubation with the oxidant in the presence and the absence of leaf extracts for one hour.

## **ENZYMIC ANTIOXIDANTS**

The enzymic antioxidants analyzed were SOD, CAT, POD, GST and GR.

## **SUPEROXIDE DISMUTASE**

The administration of H<sub>2</sub>O<sub>2</sub> to goat liver slices significantly lowered the activity of SOD, whereas, the *Triticum aestivum* leaf extracts significantly elevated the activity of SOD, with the 4<sup>th</sup> day leaf extract showing maximum effect. Superoxide dismutases (SODs) are the major antioxidant enzymes that inactivate superoxide and thereby control oxidative stress as well as redox signaling (Hu *et al.*, 2007).

SOD functions in the cell as one of the primary enzymatic antioxidant defenses against superoxide radicals (Powers and Lennon, 1999). Increases in SOD enzyme activity corresponds with enhanced resistance to oxidative stress (Fielding and Meydani, 1997).

Shahjahan *et al.*, (2004) have reported that the administration of *Solanum trilobatum* plant extract led to the recovery of the decreased SOD, CAT and GPx activities during CCl<sub>4</sub> exposure to near normal in the liver of rats. The glycoproteins isolated from *Rhus verniciflua* Stokes (RVS) (Ko *et al.*, 2006) and *Ulmus davidiana* Nakai (Ko and Lim, 2006) imparted a protective effect on CCl<sub>4</sub> induced liver injury in mice by significantly increasing the SOD, CAT and GPx activities.

A polyherbal formulation (Himoliv) has been reported to restore the activities of SOD by the reversal of CCl<sub>4</sub> induced changes in the liver of rats (Bhattacharyya *et al.*, 2003). The aqueous extract of the roots of *Decalepis hamiltonii* inhibited the ethanol-induced oxidative stress in the liver of rats by increasing the activities of SOD, CAT, GPx, GR and GST in a dose dependent manner (Srivastava and Shivanandappa, 2006).

Our results show that *Triticum aestivum* leaf extracts effectively improved the activities of superoxide dismutase in oxidatively stressed groups which indicates their antioxidant potential.

## CATALASE

In the present study, during H<sub>2</sub>O<sub>2</sub> intoxication the catalase activities were found to be significantly reduced. This may be due to the decreased capacity of the cells for detoxifying H<sub>2</sub>O<sub>2</sub> and this may be linked to a decrease in catalase activity. However, on administration with the *Triticum aestivum* leaf extracts the CAT activities significantly increased.

Catalase is an enzyme present in the cells of plants, animals and aerobic bacteria (Mates *et al.*, 1999). Catalase is located in the cell organelle called peroxisome. The enzyme very efficiently promotes the conversion of hydrogen peroxide to water and molecular oxygen (Hua *et al.*, 2007).

Cancer cells are nearly always low in MnSOD and catalase (CAT) activity, and usually low in CuZnSOD activity (Oberley and Oberley, 1994). The pretreatment with the *Passiflora alata* leaf extract provided significant protection to the liver as evidenced by the increased catalase and superoxide dismutase activities in rats treated with CCl<sub>4</sub> (Rudnicki *et al.*, 2006). The administration of the *Croton cajucara* leaf extracts increased GPx and catalase activities in paraquat treated liver of rats (Tieppo *et al.*, 2006).

Sanmugapriya and Venkataraman (2006) have reported that aqueous extract of *Strychnos potatorum* Linn. seeds and the seed powder restored the reduced SOD, CAT and GPx activities to normal caused by the CCl<sub>4</sub> toxicity in the liver of rats. The oral administration of the polyherbal formulation HP-1 prevented the depletion of SOD and CAT activities and brought them back to normal levels in the primary mono layer culture of rat hepatocytes subjected to CCl<sub>4</sub> intoxication (Tasaduq *et al.*, 2003).

Our results clearly demonstrate that the administration of *Triticum aestivum* leaf extracts significantly increased the catalase activity in H<sub>2</sub>O<sub>2</sub> exposed liver slices indicating their antioxidant potential. The effect elicited by the 4<sup>th</sup> day leaf extract was effective in this regard.

## **PEROXIDASE**

Treatment of liver slices with H<sub>2</sub>O<sub>2</sub> caused a significant reduction in the activity of peroxidase. This may be due to the depletion of its co-substrates (GSH and NADPH). The adverse effects of H<sub>2</sub>O<sub>2</sub> were reversed by the administration of *Triticum aestivum* leaf extracts.

GPx, a selenoenzyme present in the cytosol and mitochondrial matrix, removes H<sub>2</sub>O<sub>2</sub> (Abuja and Albertini, 2001), thereby protecting the cells against oxidative damage. Naik *et al.* (2004) have reported that in the presence of

curcumin, ethanol failed to alter the activities of SOD, CAT and POD and the activities remained unchanged in the liver slice culture model *in vitro*.

Rajasha *et al.* (2006) have reported that flax seed significantly elevated the hepatic SOD, CAT and peroxidase activities during CCl<sub>4</sub> intoxication. Lee *et al.* (2006a) and Liu *et al.* (2006) have reported that *Phyllanthus* extract and *Ginkgo biloba* methanolic extracts significantly increased the GPx activities upon CCl<sub>4</sub> intoxication in the liver and plasma of rats.

Our results show that *Triticum aestivum* leaf extracts can bring about an improvement in the activities of SOD, CAT and POD indicating their antioxidant potential.

## **GLUTATHIONE REDUCTASE**

Treatment of the goat liver slices with H<sub>2</sub>O<sub>2</sub> caused a significant decrease in the activities of glutathione reductase. The toxic effects of H<sub>2</sub>O<sub>2</sub> were effectively counteracted by the addition of *Triticum aestivum* leaf extracts.

Glutathione reductase plays an important role in GSH metabolism in which the enzymatic activity is regulated in response to stress. Low glutathione reductase activity may also contribute to the lower content of GSH in the tissues (Rogers *et al.*, 2004).

Plant extracts have been reported to stimulate the glutathione reductase activities to counteract oxidative stress. The *Ginkgo biloba* extract (He *et al.*, 2006) and the methanolic extract of *Coscinium fenestratum* stem powder (Venkumar and Latha, 2004) retrieved the decreased activities of glutathione reductase towards near-normalcy in the plasma and liver of rats intoxicated with CCl<sub>4</sub>. Adamska *et al.* (2003) has reported that the ethanol extract and the flavonoid isolated from the leaves and stems of *Aquilegia vulgaris* ameliorated the

antioxidant enzymes such as SOD, GPx and GR to normal, preliminarily enhanced by CCl<sub>4</sub>.

In the present study, the *Triticum aestivum* leaves improved the GR activities, which indicates that the levels of GSH in the cell will be maintained. The effect of the 4<sup>th</sup> day leaf extract was more pronounced followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

### **GLUTATHIONE S-TRANSFERASE**

The activities of GST were significantly decreased upon H<sub>2</sub>O<sub>2</sub> exposure and were restored to normal levels by the administration of *Triticum aestivum* leaf extracts.

Glutathione transferases are a multi-gene family of enzymes responsible for the metabolism of a wide range of both endogenous and exogenous substrates. These polymorphic enzymes, which form part of an adaptive response to chemical and oxidative stress, are widely distributed and ubiquitously expressed and are subject to regulation by a number of structurally unrelated chemicals (Henderson and Wolf, 2005).

The GSTs are a family of phase II detoxification enzymes. They can catalyse glutathione conjugation with various electrophiles. In most cases, the electrophiles are detoxified by this conjugation, but in some cases the electrophiles are activated (van Haften *et al.*, 2003; Mahajan and Atkins, 2005).

*Piper betle* leaf extract attenuated the total glutathione S-transferase activity and GST alpha isoform activity and protected the liver from the damage induced by CCl<sub>4</sub> in rat (Young *et al.*, 2006). The activities of glutathione disulfide reductase and glutathione S-transferase which normally decreased in CCl<sub>4</sub>-injured rat hepatocytes were significantly preserved by the treatment with the

phenylpropanoids isolated from the roots of *Scrophularia buergeriana* (Lee *et al.*, 2002).

Thus, the results obtained in the enzymic antioxidants reveal that H<sub>2</sub>O<sub>2</sub> exposure to the liver slices elicited an oxidative stress as evidenced by the decreased activities of the enzymic antioxidants. The oxidative stress is mitigated by the *Triticum aestivum* leaf extracts efficiently by increasing the activities of enzymic antioxidants.

## NON-ENZYMIC ANTIOXIDANTS

Cells continuously produce free radicals and reactive oxygen species (ROS) as part of metabolic processes. These free radicals are neutralized by an elaborate antioxidant defense system consisting of non-enzymatic antioxidants. The non-enzymic antioxidants analyzed in the present study were vitamin C, vitamin E, vitamin A, GSH and protein thiols.

### VITAMIN C

H<sub>2</sub>O<sub>2</sub> treatment caused a significant reduction in the levels of vitamin C. This reduction was effectively reversed by the presence of the leaf extracts of *Triticum aestivum*.

Vitamin C, which includes ascorbic acid and its oxidation product-dehydroascorbic acid, has many biological activities in the human body (Linster and van Schaftingen, 2007). Ascorbic acid is able to scavenge the superoxide and hydroxyl radicals, as well as regenerate  $\alpha$ -tocopherol (Davey *et al.*, 2000). Block *et al.* (2004) have found that vitamin C can reduce levels of C-reactive protein (CRP), a marker of inflammation and possibly a predictor of heart disease.

Weng *et al.* (2007) have reported that oral administration of vitamin C and vitamin E significantly reduced the generation of ROS and effectively inhibited

the Microcystin-LR induced hepatocyte apoptosis and liver injury in mice. Dietary ascorbic acid is able to protect against high fat diet effects, reducing the increase of body weight, total body fat and enlargement of different adipose depots induced by the cafeteria diet without affecting food intake in rats (Campion *et al.*, 2006).

Superoxide generated by xanthine oxidase / hypoxanthine enhanced the norepinephrine-induced contraction of arteries with endothelium from rats, which was reversed by vitamin C (Miyagawa *et al.*, 2007). *Withania somnifera* root powder administration significantly elevated the decreased activities of SOD, CAT and vitamin C levels in the liver of hypercholesterolemic rats (Visavadiya and Narasimhacharya, 2006).

Thus the toxicity of CCl<sub>4</sub> was effectively counteracted by the administration of *Triticum aestivum* leaf extracts, with the 4<sup>th</sup> day leaf extract showing the maximum effect.

## VITAMIN E

The significant reduction in the levels of vitamin E upon H<sub>2</sub>O<sub>2</sub> treatment, was restored to near normalcy by the leaf extracts of *Triticum aestivum*. The 4<sup>th</sup> day leaf extract was more effective in this regard.

Vitamin E belongs to a group of lipid soluble antioxidants. The biological activity of vitamin E exhibits tocopherols and tocotrienols, especially  $\alpha$ -tocopherol. The predominant reaction responsible for tocopherol antioxidant activity is hydrogen atom donation, where a tocopheroxyl radical is formed (Lampi *et al.*, 2002). Vitamin E shows protective effects against the coronary heart disease due to inhibition of LDL oxidation (Stampfer and Rimm, 1995).

Vitamin E analogs, epitomized by alphan-tocopheryl succinate belong to the group of “mitocans” (mitochondrially targeted anti-cancer drugs), since they are

selective for malignant cells, cause destabilization of their mitochondria and suppress cancer in pre-clinical models (Neuzil *et al.*, 2007).

Ajith *et al.* (2006) have reported that the ethanol extract of *Zingiber officinale* alone and in combination with vitamin E partially ameliorated cisplatin-induced nephrotoxicity by preventing the cisplatin-induced decline of renal antioxidant defense system or by their direct free radical scavenging activity in mice.

Vitamin E effectively counteracted the bleomycin induced pulmonary fibrosis in the lungs of rats (Dede *et al.*, 2006). The ethanol induced decrease in the levels of vitamin E in the heart of rats was counteracted by the administration of ursolic acid. Thus it is perceivable that the increase in vitamin E levels by the leaf extracts of *Triticum aestivum* proves its antioxidant effect.

## VITAMIN A

Vitamin A levels in the goat liver slices decreased significantly upon H<sub>2</sub>O<sub>2</sub> assault. *Triticum aestivum* leaf extracts were effective in restoring the vitamin A levels to near control. The 4<sup>th</sup> day leaf extract was found to be more effective than the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts of *Triticum aestivum*.

Retinol and retinyl esters are precursors of retinoic acid, the most active form of vitamin A and a ligand for retinoid receptors. Retinoic acid plays an important role in controlling cell growth, cell differentiation and apoptosis as well as carcinogenesis and is of potential clinical interest in cancer chemoprevention and treatment (Altucci and Gronemeyer, 2001).

In support of our results, the vitamin A levels were significantly reduced by CCl<sub>4</sub> administration in rats (Mac Donald-Wicks and Garg, 2003). Noyan *et al.* (2006) have reported that administration of vitamin A effectively suppressed the hepatic injury caused by CCl<sub>4</sub> treatment.

Carotenoids, such as  $\alpha$ -carotene,  $\beta$ -carotene, lycopene, lutein and cryptoxanthine, have been shown to have antioxidant properties (Donaldson, 2004).  $\beta$ -carotene in *Dunaliella salina* marine alga has been reported to be effective against oxidative damage induced by  $\text{CCl}_4$  (Murthy *et al.*, 2005). Thus, an increase in vitamin A levels by *Triticum aestivum* leaf extracts is an indication of its antioxidant response.

### **REDUCED GLUTATHIONE (GSH)**

GSH levels were significantly reduced when goat liver slices were exposed to  $\text{H}_2\text{O}_2$ . This may be due to its enhanced utilization by the hepatocytes to nullify the toxicity of  $\text{H}_2\text{O}_2$ . These levels were restored to normalcy by the *Triticum aestivum* leaf extracts.

Glutathione is the most abundant intracellular thiol based antioxidant present in millimolar concentration and it serves as a component in the first line of defense against the oxidative stress (Das and Vasudevan, 2005).

GSH plays a vitally important role in cellular function, the maintenance of GSH homeostasis is essential for the organism to perform its many functions. GSH levels can be monitored as a non-specific indicator of cellular toxicity, because a decrease in GSH, and subsequently increase of its oxidized form (GSSG), is indicative of an increased potential for cellular injury (Fonnum and Lock, 2004).

The administration of *Indigofera oblongifolia* prevented the decrease in SOD, CAT, GPx and GSH caused by  $\text{CCl}_4$  and restored the levels towards normalcy in the liver of rats (Shahjahan *et al.*, 2005). The five benzophenones isolated from *Hypericum annulatum* in combination have been reported to alter the  $\text{CCl}_4$  induced damage in isolated rat hepatocytes *in vitro* (Mitcheva *et al.*, 2006). The crude extract and hydro-alcoholic fractions from leaves of *Ouratea parviflora*

enhanced the reduced levels of GSH caused by CCl<sub>4</sub> administration in rats (Carbonari *et al.*, 2006).

The results obtained from the phase II of this study showed the antioxidant potential of *Triticum aestivum* leaf extracts in all the *in vitro* systems studied.

### PHASE III

*In vivo* assessment of drug metabolism in experimental animals provides the most physiologically relevant test system and gives information that relates to the complexity of sequential and parallel routes of metabolic clearance not only by multiple enzymes but also by multiple organs. The results obtained in the *in vitro* studies showed that *Triticum aestivum* leaves possessed both antioxidant and hepatoprotective activity. Many factors such as signaling pathways involving hormones, regulatory substances and other molecules, physiological nature, multiple organ systems play an important role in influencing the *in vivo* environment. To confirm the results obtained *in vitro*, *in vivo* studies were carried out using male Wistar rats. Oxidative stress was induced in the animals by the combined administration of ethanol with CCl<sub>4</sub>.

Ethanol can increase the hepatic CYP2E1 upto ten folds. This induction is responsible for oxidative damage in hepatocytes (Lieber, 2004b). Induction of CYP2E1 by ethanol is one of the central pathways by which ethanol generates a state of oxidative stress in hepatocytes.

CCl<sub>4</sub>, a chemical with hepatotoxic and nephrotoxic effect, has been used as a model in many studies to induce liver injury (Chen *et al.*, 2005b) and kidney injury (Dogukan *et al.*, 2003). Metabolic activation of CCl<sub>4</sub> by CYP2E1 to the free radicals, namely trichloromethyl and trichloromethyl peroxy radicals is reported to enhance lipid peroxidation and protein oxidation in the liver, resulting in widespread membrane damage and liver injury (Sheweita *et al.*, 2001a).

Liver is the main target of CCl<sub>4</sub> and kidney is the main site of CCl<sub>4</sub> accumulation. Thus, in the present study, the oxidative status of both organs was studied in rats exposed to CCl<sub>4</sub>. Ethanol pretreatment sensitizes the liver to toxic challenge by CCl<sub>4</sub> so as to potentiate liver damage.

The extent of liver damage in different treatment groups were evaluated by the activities of the marker enzymes and the lipid profile in the serum. The results are discussed below.

### **SERUM MARKER ENZYMES**

One of the most sensitive and dramatic indicators of hepatocyte injury is the release of intracellular enzymes, such as transaminases, serum alkaline phosphatase and  $\gamma$ -glutamyl transpeptidase in the circulation after CCl<sub>4</sub> administration. The elevated activities of these enzymes are indicative of cellular leakage and loss of the functional integrity of the cell membranes in liver (Rajesh and Latha, 2004). Any damage in hepatic cells may result in an alteration in the serum levels (Tawta *et al.*, 2000).

The serum markers analyzed in the present study were aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP),  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GT) and bilirubin. A significant increase in the activities of the serum markers were observed in the groups treated with ethanol alone or along with CCl<sub>4</sub>.

An increase in the serum marker enzymes can be correlated to liver disease states. Hepatocellular necrosis leads to the elevation of the serum marker enzymes, which are released from the liver into blood (Shenoy *et al.*, 2002). The increased levels of AST, ALT, ALP and serum bilirubin are conventional indicators of liver injury (Achliya *et al.*, 2004).

The level of ALT is an indicator of the degree of cell membrane damage whereas that of AST is an indicator of mitochondrial damage, as mitochondria control 80% of the enzyme (Daba and Abdel-Rahman, 1998). Serum GGT has been proposed as a marker of oxidative stress (Lee *et al.*, 2004).

In our study a substantial increase in the activities of serum markers observed after the administration of ethanol and CCl<sub>4</sub> reflects the leakage of these enzymes into the blood stream, which can be attributed to the hepatic damage.

The co-treatment of *Triticum aestivum* leaf extracts elicited a significant protection against ethanol-CCl<sub>4</sub> induced liver damage by decreasing the serum marker enzymes. The leaf extracts of the 4<sup>th</sup> day plant was more effective in this respect followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by a hepatotoxin, is an index of its protective effects (Yadav and Dixit, 2003). The extracts of *Triticum aestivum* leaves possess strong hepatoprotective effects as revealed by our study.

In agreement with our results, Hung *et al.* (2006) have reported that the oral administration of the water extract of Du-Zhong (*Eucommia ulmoides* Oliv.) leaves for 28 consecutive days significantly decreased the serum AST, ALT and ALP levels in CCl<sub>4</sub> intoxicated rats. Recoupment in the activities of AST, ALT and ALP with the administration of different plant preparations have also been reported e.g. *Trianthema portulacastrum* L. (Kumar *et al.*, 2004), *Bupleurum kaoi* (Wang *et al.*, 2004), *Acanthopanax koreanum* (Nan *et al.*, 2004), and *Helminthostachys zeylanica* L. (Suja *et al.*, 2004).

Pretreatment of the ethanolic leaf extract of *Cassia fistula* Linn. followed by CCl<sub>4</sub> treatment reverted the elevated levels of the enzymes AST, ALT, ALP

and  $\gamma$ -GT in the serum of rats (Pradeep *et al.*, 2005). Rao *et al.* (2006b) had reported that the substantially elevated serum enzymic activities of AST, ALT, ALP and  $\gamma$ -GT due to CCl<sub>4</sub> treatment in rats were dose-dependently restored towards normalization upon the administration of rubiadin, a major constituent isolated from *Rubia cordifolia* Linn.

Asha *et al.* (2004) have reported that the administration of the water, alcohol and n-hexane extracts of *Phyllanthus maderaspatensis* remarkably prevented the acetaminophen-induced elevation of AST, ALT and ALP in rats with the maximum effect shown by n-hexane extract. The administration of the petroleum ether extractable fraction of the whole plant *Aerva lanata* was effective in restoring the elevated activities of liver marker enzymes in rats induced by CCl<sub>4</sub> (Nevin and Vijayammal, 2005).

Kim *et al.* (2005) have reported that the pretreatment of momordin Ic and oleanolic acid obtained from *Kochia fructus* fruit significantly lowered the increased AST, ALT,  $\gamma$ -GT activities in CCl<sub>4</sub> treated rats. The increase in the activity of serum AST and ALT induced by CCl<sub>4</sub> in rats were significantly inhibited by oral pretreatment with the chloroform extracts of *Terminalia catappa* L. leaves (Tang *et al.*, 2006).

Serum bilirubin is one of the most sensitive parameter employed in the diagnosis of hepatic diseases. It provides useful information on how well the liver is functioning (Harper, 1961). Determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and severe disturbance of hepatocellular function (Martin and Friedman, 1992).

The ethanol-CCl<sub>4</sub> treatment led to a significant increase in the serum bilirubin levels which were ameliorated by the *Triticum aestivum* leaf extracts treatment.

Many plant extracts have been shown to restore the oxidant induced hiked activities of serum markers and bilirubin levels under *in vivo* conditions. A significant increase in the activities of AST, ALT, ALP and bilirubin induced by CCl<sub>4</sub> and paracetamol in rats were restored to normalcy by the treatment with the *Abutilon indicum* leaf extracts in a dose dependent manner (Porchezian and Ansari, 2005). Similar results were reported for *Hemidesmus indicus* R. (Baheti *et al.*, 2006). Elevated activities of AST, ALT, ALP and bilirubin levels caused by CCl<sub>4</sub> toxicity were reverted back by the water extract of *Ballota glandlosissima* (Ozbek *et al.*, 2004).

Polyherbal formulations such as Amalkadi Ghrita (Achliya *et al.*, 2004), Haridradi Ghrita (Satturwar *et al.*, 2003) and Kasondi (Ahmad *et al.*, 2005) have been reported to alter the elevated serum enzymes AST, ALT, ALP and bilirubin levels against CCl<sub>4</sub> induced damage in rats. A hydroalcoholic extract of *Emblica officinalis* effectively reversed the CCl<sub>4</sub> and thioacetamide induced toxicity in rats by decreasing the elevated levels of serum marker enzymes and bilirubin (Tasaduq *et al.*, 2005).

In the present study, the administration of *Triticum aestivum* leaf extracts caused a significant decrease in the activities of serum marker enzymes (AST, ALT, ALP,  $\gamma$ -GT) and bilirubin levels against ethanol-CCl<sub>4</sub> induced toxicity which is indicative of their protective effect on both hepatocytes and their mitochondria. The protection rendered by the *Triticum aestivum* leaf extracts was comparable to that of a standard hepatoprotectant, silymarin. The maximum response was shown by the 4<sup>th</sup> day leaf extract, which was followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

## LIPID PROFILE

In the present study, there was a significant increase in the circulating levels of total cholesterol, triglycerides, phospholipids and free fatty acids in the rats treated with ethanol alone or with CCl<sub>4</sub>. Administration of *Triticum aestivum* leaf extracts effectively counteracted the toxicity by significantly decreasing the lipid levels in the serum of rats.

CCl<sub>4</sub> is an important model agent to study the pathogenesis of liver injury including fatty liver. This phenomenon is believed to be a result from an imbalance between hepatic fatty acid flow, triacylglycerol synthesis and excretion. One of the earliest manifestations for CCl<sub>4</sub> induced liver damage is the accumulation of fat by inhibition of triglyceride secretion (Boll *et al.*, 2001a). The synthesis of lipids was reported to be augmented while the oxidation of fatty acids was reduced upon CCl<sub>4</sub> treatment (Romero *et al.*, 1994; Boll *et al.*, 2001b).

CCl<sub>4</sub> intoxication elevated the levels of serum cholesterol (Venukumar and Latha, 2004), which were ameliorated by the methanolic extract of *Coscinium fenestratum* stem. The administration of *Piper betle* aqueous extract caused a significant reduction in the elevated cholesterol, phospholipids and free fatty acids levels against ethanol induced toxicity in the brain of rats (Saravanan *et al.*, 2003).

The aqueous extracts of *Boerhavia diffusa* leaves (Pari and Satheesh, 2004) and *Enicostemma littorale* extracts (Vasu *et al.*, 2005), significantly decreased the level of cholesterol and triglycerides in rats treated with alloxan and high fat diet.

The significant increase in the levels of cholesterol, triglycerides, phospholipids and free fatty acids caused by the administration of streptozotocin in rats were brought down to normalcy by the treatment with the ethanolic extracts of *Coccinia indica* leaves (Pari and Venkateswaran, 2003), *Aloe vera* leaf gel

(Rajasekaran *et al.*, 2006) and the aqueous extract of *Casearia esculenta* root (Prakasam *et al.*, 2003).

Herbal formulations such as Kalpaamruthaa (Veena *et al.*, 2006) and Diasulin (Saravanan and Pari, 2005) reverted back the plasma cholesterol, phospholipids, triglycerides and free fatty acids levels in rats against the 7,12-dimethyl benz(a)anthracene (DMBA) induced mammary carcinoma and alloxan induced hyperglycemia. The methanolic extracts of *Premna tomentosa* leaves (Devi *et al.*, 2004) and *Asteracantha longifolia* seeds (Shivashangari *et al.*, 2004) were effective in mitigating the acetaminophen induced elevated levels of lipid profile in rats.

*Triticum aestivum* leaf extracts have been suggested to act by decreasing the cholesterol biosynthesis especially by decreasing the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) activity (key enzyme of cholesterol biosynthesis) (Sharma *et al.*, 2003).

It is evident from our results that *Triticum aestivum* leaf extracts significantly reverted the elevated serum markers and lipid profile after ethanol-CCl<sub>4</sub> administration with the maximum effect shown by the 4<sup>th</sup> day leaf extract, thus, prove the hepatoprotective potential of the leaves. The response evoked were also comparable to that of the standard hepatoprotective agent, silymarin.

## **ANTIOXIDANT STATUS IN THE LIVER AND KIDNEY**

The enzymic (SOD, CAT, POD, GST, GR and G6PD) and non-enzymic antioxidants (ascorbate, tocopherol, vitamin A, reduced glutathione and protein thiols) were analysed in the liver (a metabolic organ) and kidney (an excretory organ) of the rats subjected to ethanol-CCl<sub>4</sub> induced oxidative stress in the presence and absence of pretreatment with *Triticum aestivum* leaf extracts. The results are discussed below.

## ENZYMIC ANTIOXIDANTS

A significant reduction in the activities of the enzymic antioxidants analysed (SOD, CAT, POD, GST, GR and G6PD) were observed in the liver and kidney of ethanol alone or with CCl<sub>4</sub> intoxicated rats, which was ameliorated by the treatment with *Triticum aestivum* leaf extracts. CCl<sub>4</sub> through its production of free radicals causes a decrease in the activities of enzymic antioxidants (Sheweita *et al.*, 2001a).

Alcohol-induced oxidative stress in the liver cells plays a major role in the development of alcoholic liver disease. This condition results from several processes related to alcohol metabolism, including decreased activity of antioxidant enzymes (Lieber, 2003).

The sensitivity of cells to oxidants is attenuated by antioxidant defense system such as GSH, GST, CAT, SOD, GPx, GR and G6PD. Among these enzymes, GPx, through reduction of both hydrogen peroxide and organic hydroperoxides, provides an efficient protection against oxidative damage and free radicals in the presence of GSH. Oxidized glutathione (GSSG) is regenerated by GR. SOD (metalloprotein) is the first enzyme involved in the antioxidant defense by lowering the steady state of O<sub>2</sub><sup>•-</sup>. CAT is a hemoprotein, localized in the peroxisomes and catalyses the decomposition of H<sub>2</sub>O<sub>2</sub> to water and oxygen (Venukumar and Latha, 2002). GST, a Phase II enzyme, confers protection against toxic chemicals by actively metabolizing it into less toxic compound (Wallig *et al.*, 1998).

In the presence of inadequate CAT or GPx activity to degrade H<sub>2</sub>O<sub>2</sub>, more H<sub>2</sub>O<sub>2</sub> could be converted to toxic hydroxyl radicals that may contribute to oxidative stress due to CCl<sub>4</sub> intoxication. A decline in the activities of these enzymes might be due to their inactivation caused by excess ROS production (Pigeolet *et al.*, 1990).

In the present study, the activities of the enzymic antioxidants increased significantly and reached near control values in the liver and kidney of animals treated with *Triticum aestivum* leaf extracts. The increase in the activities of the enzymic antioxidants is known to serve as protective responses to eliminate reactive free radicals (Cheung *et al.*, 2001). Several plant extracts have been reported to mitigate the toxic effects of CCl<sub>4</sub>.

The administration of the aqueous extract of the bark of *Terminalia arjuna* significantly elevated the reduced SOD, CAT and GST activities in the liver and kidney of CCl<sub>4</sub> challenged mice (Manna *et al.*, 2006). The administration of *Boerhavia diffusa* L. and *Gymnema montanum* leaf extracts enhanced the reduced activities of SOD, CAT, GPx, and GST in the liver and kidney tissues of alloxan induced diabetic rats (Ananthan *et al.*, 2004; Satheesh and Pari, 2004).

Treatment of rats with *Mistletoe alkali* significantly increased the activities of SOD, GPx, and GR in the liver and kidney after CCl<sub>4</sub> exposure (Shi *et al.*, 2006). Pretreatment of rats with *Cystisus scoparius* plant extract caused a significant increase in the SOD, CAT, GPx, GST and GR activities in the liver against CCl<sub>4</sub> exposure (Raja *et al.*, 2007).

Elevated levels of lipid peroxides with subsequent decrease in the activities of SOD, CAT, GPx, GR and G6PD were observed in the N-nitrosodiethylamine (NDEA) induced hepatocarcinogenesis in rats. In contrast, the administration of silymarin improved the activities of these enzymic antioxidants in both hemolysate and liver suggesting the chemopreventive action against NDEA administration during liver cancer progression (Ramakrishnan *et al.*, 2006).

The administration of black pepper (*Piper nigrum* L.) (Vijayakumar *et al.*, 2004) and the water extract of dried powder of the root and leaves of *Azadirachta indica* and *Abroma augusta* increased the reduced activities of SOD, CAT, GPx,

and GST caused by high fat diet and alloxan induced oxidative stress in the liver and kidney erythrocytes of rats (Halim, 2003).

A significant reduction in the activities of SOD, CAT and GPx caused by the streptozotocin and alloxan induced stress in the liver and kidney of rats were ameliorated by the administration of the aqueous leaf extract of *Annona squamosa* (Kaleem *et al.*, 2006), *Enicostemma littorale* (Srinivasan *et al.*, 2005). The (-) – epicatechin, a flavonoid was effective in reverting back the reduced activities of SOD, CAT and GPx in the liver and kidney of alloxan induced diabetic rats (Quine and Raghu, 2005).

In light of these reports, the reduced activities of the enzymic antioxidants observed by ethanol-CCl<sub>4</sub> administration being restored to near normal levels by the administration of *Triticum aestivum* leaf extracts gains significance in proving their antioxidant effects. The response elicited by the 4<sup>th</sup> day leaf extract was found to be maximum in this regard.

## **NON-ENZYMIC ANTIOXIDANTS**

The levels of vitamins C, E and A reduced glutathione and protein thiols were analyzed in the livers and kidneys of animals subjected to all treatment groups.

A single dose of CCl<sub>4</sub> administration induced oxidative stress in rats as indicated by the decreased levels of non-enzymic antioxidants (vitamins C and A, GSH and protein thiols) in the liver and kidney of rats compared to control. Pretreatment with the *Triticum aestivum* leaf extracts increased the levels of non-enzymic antioxidants and showed protective effects when CCl<sub>4</sub> was administered.

Vitamin C is a potent antioxidant, which widely acts on oxygen free radicals as well as by interaction with vitamin E (Garg and Bansal, 2000). Vitamin E, a major chain breaking lipid soluble antioxidant is the major lipophilic

antioxidant while all other antioxidants are present in much smaller quantity (Esterbauer *et al.*, 1992). It is well known that endogenous vitamin E and other antioxidant nutrients have a vital role in the maintenance of antioxidant defence in the mammalian system (Gutteridge and Halliwell, 2000).

GSH is a component of the first line of defense against free radicals and maintenance of protein thiols and as a substrate for GPx and GST (Edenharder *et al.*, 1999). The availability of sufficient amount of GSH can thus increase the detoxification of active metabolites through the involvement of GPx (Prakash *et al.*, 2001).

Vitamin E can transfer its phenolic hydrogen to a peroxy free radical of a peroxidized PUFA, thereby inhibiting ROS-induced generation of lipid peroxy radicals and protecting cells from peroxidation of PUFA in membrane phospholipids, from oxidative damage of plasma very low-density lipoprotein, cellular proteins, DNA and from membrane degeneration. Vitamin C reacts with vitamin E radical to yield a vitamin C radical while regenerating vitamin E. A vitamin C radical is converted back to vitamin C by GSH (Fang, 2002). Thus GSH reduction can explain the decreased concentrations of vitamin C.

The stressed rats showed a significant increase in the levels of  $\alpha$ -tocopherol, which suggests that vitamin E, a predominant antioxidant in the membrane (Bhandarkar and Khan, 2004) exhibited an increase in its level upon alcohol stress and decreased upon CCl<sub>4</sub> stress when compared to control levels. The levels of the antioxidant are known to be elevated in cells with response to free radical production (Quinn, 2004).

CCl<sub>4</sub> is metabolized by the cytochrome P450 enzyme system, specifically the CYP2E1 isoform of the enzyme. The induction of the microsomal enzymes in the liver to metabolise the administered CCl<sub>4</sub> results in the depletion of vitamin A

(Lall *et al.*, 1999). In our study also, the CCl<sub>4</sub> administration significantly decreased the vitamin A levels when compared to control rats.

One of the major consequences of oxidative stress is irreversible protein modification such as generation of carbonyls or loss of thiol residues (Bandyopadhyay, 1999). These are important in the pathophysiology of several degenerative diseases (Berlett and Stadman, 2001). Radical mediated modification of thiol groups, specifically cysteine residues, can be repaired by cellular antioxidants such as GSH or thioredoxin (Beal, 2002).

In the present study, the CCl<sub>4</sub> induced depletion of non-enzymic antioxidants were prevented by pretreatment with *Triticum aestivum* leaf extracts, which could be attributed to the antioxidant activity of the leaves.

Many reports are available in the literature that support our findings. The aqueous plant extract of *Scoparia dulcis* (Latha and Pari, 2003) and *Casearia esculenta* root extract (Prakasam *et al.*, 2005) improved the levels of vitamin C, vitamin E and GSH of the liver and kidney of streptozotocin diabetic rats. The oral administration of the ethanolic extract of *Terminalia arjuna* stem bark caused a significant improvement in the decreased levels of GSH, vitamins C, A and E, total sulfhydryl and non protein sulfhydryl in the liver and kidney of alloxan diabetic rats (Raghavan and Kumari, 2006).

Murugan and Pari (2006) have reported that the administration of tetrahydrocurcumin significantly elevated the reduced levels of GSH, vitamin C, vitamin E to normalcy in the liver and kidney of streptozotocin-nicotinamide induced diabetic rats. Pretreatment with green tea (*Camellia sinensis*) significantly improved the levels of vitamins E and A in the liver and kidney of ammonium metavanadate induced toxicity in rats (Soussi *et al.*, 2006).

The aqueous and the alcoholic extracts of the herbal supplement Amrit nectar tablets reversed the effects of cisplatin in the liver and kidney of rats by increasing the decreased levels of GSH (Dwivedi *et al.*, 2005). Prefeeding dehydrated amaranth leaves at 20% level reversed the hexachlorocyclohexane induced decrease in the levels of vitamin A and glutathione and the activities of SOD, GPx, GST, GR and G6PD in rat liver (Anilakumar *et al.*, 2006).

Rajagopal *et al.* (2003) have reported that the administration of *Cassia auriculata* leaf extract significantly elevated the decreased levels of vitamins E and C to normalcy in the serum of rats supplemented with alcohol. Depletion in the levels of GSH, total thiols, non-protein thiols, vitamin E, vitamin C and cytochrome P450 in aflatoxin B1 induced hepatocellular carcinoma in rats were restored to normal by the administration of *Semecarpus anacardium* nut extract in the liver and kidney tissues (Premalatha and Sachdanandam, 1999).

Thus, in the present study, the activities / levels of both enzymic and non-enzymic antioxidants which declined significantly by alcohol-CCl<sub>4</sub> treatment were restored to normalcy by the administration of *Triticum aestivum* leaf extracts in both the liver and kidney of rats. This suggests the strong protective action rendered by the *Triticum aestivum* leaf extracts against oxidative damage in CCl<sub>4</sub> induced hepatorenal toxicity in rats. The maximum effect was elicited by the 4<sup>th</sup> day extract followed by the 8<sup>th</sup> and 12<sup>th</sup> day leaf extracts.

## **EXTENT OF LIPID PEROXIDATION**

The level of lipid peroxide is a measure of membrane damage and alterations in structure and function of cellular membranes. In the present study, LPO levels were found to be significantly elevated in the liver of CCl<sub>4</sub> treated rats. The increase in the MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant mechanisms to prevent the formation of excessive free radicals (Shenoy *et al.*, 2001).

CCl<sub>4</sub> is a toxic substance known to induce lipid peroxidation, liver damage and steatosis (Jeon *et al.*, 2003). The hepatotoxicity of CCl<sub>4</sub> has been reported to be due to the formation of the highly reactive trichlorofreeradical, which attacks polyunsaturated fatty acids. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals (Shenoy *et al.*, 2001).

Lipid peroxidation has been implicated in the pathogenesis of hepatic injury by ethanol and which leads to membrane dysfunction (Bandyopadhyay *et al.*, 1999). In the present study, increased malondialdehyde (MDA), a product of lipid peroxidation, observed in the liver of ethanol administered rats indicated excessive formation of free radicals resulting in hepatic damage.

Potent antioxidant extracts and compounds are known to increase the levels of catalase and SOD and decrease the level of TBARS in blood and tissues when compared with CCl<sub>4</sub> treatment (Badami *et al.*, 2005). In the present study, the administration of *Triticum aestivum* leaf extracts caused a significant decrease in the level of TBARS when compared to CCl<sub>4</sub> treated rats in the liver.

Several plant extracts have been reported for their antioxidant activity as reflected by a reduction in LPO. Yadav and Dixit (2003) reported that the leaves of *Kalanchoe pinnata* Pers. when administered intraperitoneally, exhibited hepatoprotective activity in rats with CCl<sub>4</sub>-induced hepatotoxicity. Ustundag *et al.* (2005) have reported that soy isoflavones are effective in liver injury caused by experimentally induced CCl<sub>4</sub>, reducing the lipid peroxidation products and stimulating paraoxonase enzyme, which has an antioxidant characteristic.

Pretreatment of rats with ascorbic acid, vitamin E or garlic as repeated doses prior to the administration of CCl<sub>4</sub> was found to reduce the induced level of TBARS caused by CCl<sub>4</sub> below the normal levels (Sheweita *et al.*, 2001b). Administration of *Melissa officinalis* L. plant extract reduced the lipid peroxidation levels in the liver of hyperlipidemic rats (Bolkent *et al.*, 2005). The

ethanol extract of *Puerariae radix* reverted back the increased extent of lipid peroxidation to near normal levels in diabetic rats (Kang *et al.*, 2005).

The elevated levels of hepatic lipid peroxidation of CCl<sub>4</sub> treated rats were reversed by the administration of *Terminalia belerica* Roxb extract and gallic acid (Jadon *et al.*, 2007), *Cassia siamea* flower extracts (Kaur *et al.*, 2006), *Echinacoside*, one of the phenylethanoids isolated from *Cistanches salsa* (Wu *et al.*, 2006) and flavonol glycosides-rich fraction from *Vicia calcerata* Desf (Singab *et al.*, 2005).

In tune with these reports, the present study proves that the treatment with the *Triticum aestivum* leaf extracts protected the liver against ethanol alone or ethanol-CCl<sub>4</sub> induced LPO in rats.

#### **RADICAL SCAVENGING ACTIVITY**

The overall antioxidant status of the tissues of rats subjected to oxidative stress by alcohol and CCl<sub>4</sub> was assessed by the ability of liver homogenate to scavenge the stable free radical DPPH. From the results obtained, the DPPH scavenging effect was not significantly affected in the alcohol and CCl<sub>4</sub> treated groups. But in the *Triticum aestivum* leaf extracts treated groups, a significant increase in DPPH scavenging activity over the controls was observed with the 4<sup>th</sup> day leaf extract showing the maximum effect, which was comparable to silymarin treatment.

Many plants have been reported for their DPPH radical scavenging effects. The free radical scavenging effect of methanolic extracts of fresh leaves of selected Chinese medicinal plants (Fenglin *et al.*, 2004), *Ginkgo biloba* leaves (Ellnain-Wojtaszek *et al.*, 2003) and *Cecropia pachystachya*, *Eugenia uniflora*, *Schinus Weinmannifolia* and *Schinus terebinthifolia* (Velazquez *et al.*, 2003) has been reported in terms of DPPH scavenging capacity.

The DPPH scavenging ability of *Dorstenia mannii* (Dufall *et al.*, 2003) and *Lycium chinense* (Qian *et al.*, 2004) has been attributed to the presence of flavonoids. Yu *et al.* (2004) have reported that the flour extracts from three winter wheat varieties exhibited significant DPPH scavenging activities. Extracts prepared from the fruits of *Cycium* species (Kosar *et al.*, 2003) and *Annona squamosa* leaves (Shirwaikar *et al.*, 2004) scavenged DPPH in a dose dependent manner.

The present study proves that the treatment with *Triticum aestivum* leaf extracts protected the liver of rats against alcohol-CCl<sub>4</sub> induced toxicity which can be attributed to the presence of antioxidants present in the leaf extracts, as well as to their modulation of the antioxidant components in the tissues.

Thus the results obtained clearly demonstrate the strong antioxidant potential of the *Triticum aestivum* leaf extracts in cell free systems, *in vitro* and *in vivo* models tested under oxidatively stressed conditions. These observations proved the candidature of the *Triticum aestivum* leaves as a rich source of antioxidants.

## HISTOPATHOLOGICAL CHANGES

Histopathological studies revealed that ethanol with CCl<sub>4</sub> treatment showed centrilobular fatty infiltration with focal necrosis in hepatic cells and the renal tubules revealed suffused glomeruli tufts in glomeruli. In accordance with our results Chung *et al.* (2005), Ha *et al.* (2005) Shyamal *et al.* (2006), Sureshkumar and Mishra (2006) and Tang *et al.* (2006), have reported that the administration of CCl<sub>4</sub> to rats induced degeneration of hepatic cords and hepatocytes, infiltration of lymphocytes and necrosis.

Ogeturk *et al.* (2005) have reported that CCl<sub>4</sub> induced nephrotic changes which included glomerular and tubular lesions, interstitial inflammatory cell

infiltration and interstitial fibrosis in the kidney. Ozturk *et al.* (2003) have observed that CCl<sub>4</sub> exposure resulted in glomerular necrosis and tubular necrosis in rat kidney.

In the groups treated with *Triticum aestivum* leaf extracts changes induced by CCl<sub>4</sub> were reverted. A better protection was shown by the 4<sup>th</sup> day leaf extract of *Triticum aestivum*, wherein the hepatocytes showed normal cord pattern with central vein and portal tracts, with no signs of necrosis. A similar effect was observed by the administration of Du-Zhong leaf extract (Hung *et al.*, 2006) and *Amalkadi Ghrita*, a polyherbal formulation (Achliya *et al.*, 2004).

In the groups treated with the 8<sup>th</sup> day extract of *Triticum aestivum* perivenular single cell necrosis was seen around central veins and 12<sup>th</sup> day extract treated groups showed fatty change in the perivenular area in the liver of rats. Rudnicki *et al.* (2007) have reported that *Passiflora alata* leaf extract pretreatment demonstrated mild hepatocellular necrosis and moderate inflammatory cell infiltration in the liver of CCl<sub>4</sub> exposed rats.

In the present study, the 4<sup>th</sup> day leaf extract of *Triticum aestivum* treatment exhibited significant protection against ethanol-CCl<sub>4</sub> induced damage in the liver and kidney of rats, as evidenced by the hepatorenal histological picture. The histopathological response elicited by the 4<sup>th</sup> day leaf extract was comparable to that observed with silymarin.

Thus, it can be inferred from the results obtained from the histological architecture that the extracts of *Triticum aestivum* protected the liver and kidney from the hepatorenal histological damage by ethanol and CCl<sub>4</sub>.

From the results of the first three phases of the study, protective and antioxidant properties of the leaf extracts become evident. This effect may be due to the presence of various phytochemical principles present in the leaves.

In order to throw light on the major phytochemical causing such an effect, the identification of the major phytochemical component of the leaf extract was attempted in the next phase of the dissertation.

#### PHASE IV

The preliminary phytochemical screening revealed the presence of alkaloids, phenols and flavonoids. To confirm the chemical nature of the active component present in *Triticum aestivum* leaves, spectral analyses (FT-IR, <sup>1</sup>H-NMR and GC-MS) were carried out, which identified, phytosterols and steroidal alkaloids as the major components.

Phytosterols are chemical homologs of cholesterol that are found exclusively in most plant foods. They interfere with the micellar solubilization of cholesterol in the intestine and reduce the efficiency of cholesterol absorption (Ostlund and Lin, 2006). They lower blood cholesterol (Piironen *et al.*, 2000) and provide protection against certain types of cancer (Awad and Fink, 2000).

Phytosterols affect host systems potentially enabling the more robust antitumor responses, including the boosting of immune recognition of cancer, influencing hormonal dependent growth of endocrine tumors, and altering steroid biosynthesis. Phytosterols have effects that directly inhibit tumor growth, including the slowing of cell cycle progression, the induction of apoptosis and the inhibition of tumor metastasis (Bradford and Awad, 2007).

In our study, the spectral patterns of the *Triticum aestivum* leaves were suggestive of the presence of phytosterols like  $\beta$  sitosterol and campesterol. The IR, <sup>1</sup>H NMR and mass spectra showed the presence of beta-sitosterol-D-glucoside in the leaves of *Ficus lyrata* (Basudan *et al.*, 2005). Similarly, the presence of beta-sitosterol was identified in the leaves and branches of *Opuraka seriserrate* as evidenced by the spectral data (Velandia *et al.*, 2002).

The methanolic extract from the herb *Erigeron acris* showed the presence of campesterol (Nazaruk, 2006). The hexane extract of the plant *Eryngium foetidum* L. possessed alpha-cholesterol, campesterol, beta-sitosterol and stigmasterol (Garcia *et al.*, 1999).

The gamoderma total sterol significantly reduced malondialdehyde content and reactive oxygen species production and increased manganese superoxide dismutase (Mn-SOD) activity in the rat cortical neuronal cultures exposed to hypoxial reoxygenation *in vitro* (Znao *et al.*, 2005).

Beta sitosterol offers protection from breast cancer metastasis by inhibiting cell invasion of the basement membrane components that are implicated in signaling tumor cell invasion of MDA-MB cells, which may be mediated by its ability to limit the adhesive interaction of the tumor cell and the basement membrane (Awad *et al.*, 2001a). Another study also showed that both beta-sitosterol and campesterol (*in vivo* as a dietary supplement) and directly inhibited the growth and metastasis of PC-3 cells (human prostate cancer cells) (Awad *et al.*, 2001b). Total phytosterols intake is associated with a strong inverse relationship with stomach cancer and gastric cancer risk (De Stefani *et al.*, 2000).

Plant sterols reduce biomarkers of oxidative stress and inflammation. They exert their hypocholesterolemic effects by interfering with the uptake of both dietary and biliary cholesterol from the intestinal tract (Devaraj and Jialal, 2006). The consumption of phytosterol-enriched snack bar effectively reduced plasma total and LDL cholesterol levels in a population with cholesterolemia (Polagruto *et al.*, 2006).

The phytosterols such as steryl glycosides and free sterols such as ergosterol, cholesterol stigmasterol, campesterol,  $\beta$ -sitosterol, esterified sterols and acylated steryl glycosides have been identified in the wheat bran (*Triticum*

*aestivum*) as identified by LC/APCI-MS analysis (Rozenberg *et al.*, 2003). These results are in agreement with our findings.

Thus, the outcome of the present study highlights the protective effects rendered by *Triticum aestivum* leaves under oxidatively stressed conditions.

Oxidative stress is the major causative factor underlying in the pathogenesis of several disease conditions. The present study strengthens the candidature of the *Triticum aestivum* leaves for use in medicinal preparations to combat the diseases arising due to oxidative stress.

The findings of the present study are summarized and the conclusions drawn therein are elaborated in the next chapter.