Discussion

5. DISCUSSION

Free radical reactions, especially with participation of oxidative radicals, have been shown to be involved in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids, thus giving rise to a variety of diseases (Rahman, 2009). Many medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (Balsano and Alisi, 2009). It has been reported that there is an inverse relationship between the antioxidative status and occurrence of human diseases (Cemeli *et al.*, 2009). In addition, antioxidant compounds that are responsible for such antioxidants activity can be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders (Iannitti and Palmieri, 2009).

Naturally occurring dietary antioxidants found in medicinal plants can serve as alternatives to chemically designed anticancer agents (Ali *et al.*, 2008). This makes economic sense because the costs associated with the generation or isolation and development of natural compounds might well be lower than those associated with the discovery and development of new chemical entities. Therefore, it is reasonable for scientists to identify the bioactive natural compounds responsible and hope to find the magic bullet to prevent chronic diseases.

Thus, many research approaches globally are seeking to identify a good candidate plant and its phytochemicals for combating oxidative damage-associated diseases like cancer. Many plants are still in awesome darkness in this regard. One such plant is *Zea mays*. The present study is a systematic approach to analyse the antioxidant and anticancer properties of *Zea mays* leaves and to scientifically validate its candidature as a potent source of therapeutic components.

PHASE I

In the initial phase of the dissertation, the *Zea mays* leaves were analyzed for the levels / activities of non-enzymic and enzymic antioxidants at six different time points of

growth. The enzymic antioxidants (SOD, CAT, POD, GST and GR) and non-enzymic antioxidants (ascorbic acid, tocopherol, total carotenoids, lycopene, reduced glutathione, chlorophyll, total phenols and flavonoids) were analysed. The different time points selected were 5, 10, 15, 20, 25 and 30^{th} days after sowing (DAS), in order to find out whether any difference occurred in the antioxidant content at the different periods of growth. The results showed that the younger leaves possessed higher activities / levels of enzymic and non-enzymic antioxidants with a peak value on the 10^{th} day.

Phytochemicals serve numerous functions in plants and contribute to their colour, flavor, smell and texture (Heber, 2004). Many studies have been reported in the literature on the antioxidant status of leaves at different time periods of growth.

A steady increase in the GPX activity was observed in dark-germinated and light-germinated corn sprouts as the germination progressed (Randhir and Shetty, 2005). The levels of total phenolics and flavonoids in pomegranate leaves decreased significantly in the early stages of leaf growth, and then increased gradually (Zhang *et al.*, 2010a).

The POD, SOD and CAT activities and malondialdehyde content of different wheat genotypes showed changing trend at different stages of growth (HongBo *et al.*, 2005). Significant variation in the antioxidant properties was also observed between different maturity stages of daylily flowers (Fu *et al.*, 2009). These reports suggest that the antioxidant content varies with different time periods of growth, which support our findings.

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. The antioxidant activity of aqueous and ethanol extracts of wheat grass grown under different conditions over a period of 6, 7, 8, 10 and 15 days showed that the extracts of 10^{th} day grass supplemented with nutrients showed maximum antioxidant activity compared to the other time periods grown under other conditions (Kulkarni *et al.*, 2006).

Cano *et al.* (2006) have reported that five day old *Avena sativa* (oat) and *Triticum aestivum* (wheat) leaves exhibited higher hydrophilic antioxidant activity and ascorbic acid level than the 10 and 20 day old leaves under de-etiolation process and light stress.

The photosynthetic rates of 30 days old cotton leaves were reduced significantly when compared with 12 days old leaves upon UV-B exposure (Kakani *et al.*, 2004).

Sharma and Hall (1996) have reported that the 12 day 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. Among three leaf stages of growth (young, mature and senescent leaves) of *Zea mays* leaves subjected to stress, the accumulation of the major polyphenolic compounds was higher in young leaves (Hichem *et al.*, 2009).

The results of the present study are in agreement with the above reports. In our study, the leaves at their early stages of growth showed maximum antioxidant activity than the mature stages.

Our results revealed that the *Zea mays* leaves were found to be a rich source of both enzymic and non-enzymic antioxidants. The results also revealed that the 10^{th} day plant showed maximum antioxidant activity than the other time points. Therefore, further studies were conducted only on the ten day old leaves.

PHASE II

The capacity to scavenge free radicals is widely used as a parameter for *in vitro* evaluation of medicinal bioactive compounds. The radical scavengers are closely related to their biofunctionalities such as the reduction of chronic diseases like DNA damage, mutagenesis and carcinogenesis. They are also often associated with the termination of free radical propagation in biological systems (Zhu *et al.*, 2002).

In this phase, several cell free systems and *in vitro* assays were used to characterize the radical scavenging and antioxidant activities of the *Zea mays* leaves. The effect of the leaves was also tested on the molecular end points such as lipid and DNA damage. The influence of the leaf extracts was also tested on the antioxidant status of tissues (goat liver slices) exposed to oxidative stress.

The presence of different antioxidant components in the plant tissues makes it relatively hard to quantify each antioxidant component separately. Therefore, in many

studies, several intermediate extractions are used to ensure a maximum extraction of the available antioxidants (Djeridane *et al.*, 2006).

In the present study, *Zea mays* leaves were extracted into three different solvents with different polarity and were tested for their ability to scavenge free radicals in H_2O_2-induced oxidatively damaged systems. The solvents used were water, methanol and chloroform.

The antioxidant activity of plant extracts cannot be evaluated by only a single method due to the complex nature of phytochemicals. Numerous antioxidant methods have been developed to evaluate the antioxidant activity but to explain how antioxidants function, the different assays namely, DPPH scavenging, ABTS scavenging, deoxyribose degradation, reducing power, chelating power and lipid peroxidation are most commonly accepted ones (Chen *et al.*, 1999; Sanchez-Moreno *et al.*, 1999), which have been used in the present investigation.

DPPH RADICAL SCAVENGING EFFECTS

The DPPH system offers a convenient and accurate method for titrating the oxidizable groups of natural and synthetic antioxidants and has been accepted as a model compound for free radicals originating in lipids (Yasuda *et al.*, 2000).

In the present study, the effects of *Zea mays* leaves on DPPH were assessed as a measure of the radical scavenging ability of the leaf extracts. The aqueous, methanolic and chloroform extracts of the *Zea mays* leaves were tested for their DPPH-scavenging potential. The maximum DPPH quenching effect was mediated by the methanolic extract followed by the aqueous and chloroform extracts.

Several studies reported in the literature have used DPPH scavenging as an important parameter for evaluating the antioxidant effects of plant extracts. Among ten different extracts of the leaves of *Limoniastrum monopetalum,* the methanolic extract showed the highest DPPH scavenging activity (Trabelsi *et al.,* 2010). The methanolic extract of *Tanacetum densum* (Lab.) Schultz Bip. subsp. *Amani* exhibited maximum DPPH free radical scavenging among three different *Tanacetum* subspecies tested (Tepe and Sokmen, 2007).

The methanolic extracts of *Holoptelea integrifolia* (Roxb.) (Urticaceae) stem bark showed higher DPPH free radical scavenging effect than the leaves (Reddy *et al.*, 2008). The methanolic extracts obtained from leaves, root and flowers of *Stevia pilosa* and *Stevia eupatoria* possessed strong DPPH scavenging ability with an efficacy of more than 90% (Carino-Cortes *et al.*, 2007). The results reported by Tepe (2007) revealed that the methanolic extracts of *Salvia virgata*, *Salvia staminea* and *Salvia verbenaca* exhibited potent antioxidant activity.

Coban and Konuklugil (2005) have reported that the methanolic extract of *Linum arboreum* had a strong scavenging effect on DPPH radical. An aqueous extract of *Houttuynia cordata* Thunb showed high radical scavenging activity as determined by off-line DPPH assays (Nuengchamnong *et al.*, 2009).

Kalaivani and Mathew (2010) have reported that the ethanol extract of *Acacia nilotica* had potent DPPH scavenging ability. The ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn showed potent DPPH scavenging activity (Bouhlel *et al.*, 2007). The *Achillea santolina* extract demonstrated a concentration dependent scavenging activity by quenching DPPH radicals (Ardestani and Yazdanparast, 2007). A low DPPH activity was exhibited by the Japan sea-level type of *Chenopodium quinoa*, which in contrast possessed high phenolic content (Nsimba *et al.*, 2008).

The ethanolic extract of *Leucas aspera* root (Rahman *et al.*, 2007), the methanolic extract of *Port Oxford cedar* (Gao *et al.*, 2007a) and a methanolic extract of *Cherry blossom* (Lee *et al.*, 2007a) exhibited promising DPPH radical-scavenging activities. *Rubus ulmifolius* exhibited a high antioxidant activity by scavenging DPPH (Dall'Acqua *et al.*, 2008).

Gossypetin-8-O-b-D-xylopyranoside and 2, 6-di-O-galloylarbutin exhibited strong scavenging activitity against DPPH (Thuong *et al.*, 2007). The DPPH scavenging effect of the hydromethanolic extract of *Globularia alypum* phytochemicals was attributed to the presence of flavonoid and phenyl ethanoid constituents (Es-Safi *et al.*, 2007). Turnip (*Brassica rapa var. rapa* L.) flower buds exhibited a higher antioxidant capacity as reflected by its DPPH scavenging ability compared to leaves, stems and roots (Fernandes *et al.*, 2007).

These and several other studies provide evidence to the fact that DPPH-scavenging ability is a reliable index of antioxidant potential. Thus, our results show that *Zea mays* leaves possess strong antioxidant activity, which gets extracted maximally into methanol.

ABTS RADICAL SCAVENGING EFFECTS

The extracts of the *Zea mays* leaves were also analyzed for their scavenging effects against ABTS radical. The results revealed that the methanolic extract scavenged ABTS more effectively than the aqueous and chloroform extracts.

The chloroform extract of *Chromolaena odorata* leaves exhibited strong ABTS scavenging ability (Rao *et al.*, 2010). Dastmalchi *et al.* (2007) have reported that the extracts prepared from the aerial material of Moldavian balm (*Dracocephalum moldavica* L., Lamiaceae) using various solvents like petroleum ether, dichloromethane, acetonitrile, ethyl acetate, methanol, butan-1-ol and water were active in scavenging ABTS radical.

The ABTS scavenging effects of guava leaf extracts increased with increasing concentrations (Chen and Yen, 2007). Among three different species of *Annona, Annona muricuta* showed the maximum quenching of ABTS followed by *Annona reticulara* (Baskar *et al.*, 2007).

A methanolic extract of *Caesalpinia digyna* root exhibited strong scavenging effect on ABTS radical cation (Srinivasan *et al.*, 2007). The extracts of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf showed significant scavenging capacities against ABTS, DPPH, peroxyl and hydroxyl radicals (Su *et al.*, 2007). The methanolic extracts of cantaloupe showed a high total phenolic content accompanied with good antioxidant activity as determined by ABTS assay (Ismail *et al.*, 2010).

In the present study also, *Zea mays* leaf extracts possessed strong ABTS inhibitory activity, which substantiates their antioxidant action.

HYDROGEN PEROXIDE SCAVENGING EFFECTS

The measurement of hydrogen peroxide scavenging activity is one of the useful methods for determining the ability of antioxidants to decrease the level of prooxidants such as hydrogen peroxide (Pazdzioch-Czochra and Widenska, 2002).

The aqueous, methanolic and chloroform extracts of the leaves of *Zea mays* were tested for their H_2O_2-scavenging potential, among which, the methanolic extract exhibited the highest activity. Several studies have reported the H_2O_2 scavenging action of plant extracts.

Methanol extracts of five plants from the genus *Phyllanthus* showed strong hydrogen peroxide scavenging effect (Kumaran and Karunakaran, 2007). A similar effect was reported for the *Amygdalus communis* L. hulls and shells (Sfahlan *et al.*, 2009).

The scavenging effect of the medicinal tincture from Pedilanthus tithymaloids on H_2O_2 was suggested to be associated with its anti-inflammatory effect (Abreu *et al.*, 2006). The extracts of *Salvia miltiorrhiza* possessed weak and *Panax notoginseng* exhibited high scavenging activities against hydrogen peroxide (Zhao *et al.*, 2006).

Deerberry (*Vaccinium stamineum L.*) contained potent free radical scavenging activities for DPPH, ABTS, peroxyl radical, superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen radicals. These antioxidant capacities correlated to their antioxidant enzymes activities (Wang and Ballington, 2007).

Curcumin had an effective hydrogen peroxide scavenging activity (Ak and Gulcin, 2008). L-tyrosine and L-DOPA had an ability to scavenge hydrogen peroxide (Gulcin, 2007). Grape seed extracts showed strong antioxidant activity, as reflected by their capacity to scavenge hydrogen peroxide in a concentration dependent manner (Baydar *et al.*, 2007). In the context of these literature reports, the observation made in the present study of *Zea mays* leaf extracts exhibiting strong H_2O_2-scavenging activity gains significance in strengthening the antioxidant potential of the leaves.

HYDROXYL RADICAL SCAVENGING EFFECTS

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis, and cytotoxicity (Yasuda *et al.*, 2000).

In the present study, the *Zea mays* leaf extracts were capable of protecting 2'-deoxy-D-ribose from oxidative degradation by scavenging hydroxyl radicals. The methanolic extract exhibited the maximum scavenging effect, faring better than the aqueous and chloroform extracts.

Many studies on the hydroxyl radical scavenging properties of plants are reported in the literature. Among the methanolic and 70% acetone extracts of *Camellia sinensis* (L.) O. Kuntz (green tea), *Ficus bengalensis* L. (aerial root) and *Ficus recemosa* L. (stem bark), the methanolic extracts were found to have more hydroxyl radical scavenging activity (Manian *et al.*, 2008). The methanol extracts of aerial flowering parts of four endemic *Stachys* taxa, showed maximum scavenging of hydroxyl radical (Kukic *et al.*, 2006).

Achillea santolina extract exhibited a site-specific and non-site specific hydroxyl radical scavenging activity in a dose-dependent manner (Ardestani and Yazdanparast, 2007). Amtolmetin guacyl and tolmetin inhibited hydroxyl radical provoked deoxyribose degradation in a Fenton system, revealing a hydroxyl scavenging property (Kirkova *et al.*, 2007).

Whazosipmunja and Jasan (mulberry) exhibited antioxidant property, as reflected by its ability to scavenge hydroxyl radical (Bae and Suh, 2007). Siddhuraju (2007) has reported the antioxidative properties of methanol and aqueous acetone extracts of raw and dry heated seed coat of *Tamarindus indica* as reflected by its ability to scavenge hydroxyl radical. The chloroform extract of *Chromolaena odorata* leaves and roots showed powerful hydroxyl radical scavenging effect as reported by Rao *et al.* (2010).

It is evident from our results that the extracts of *Zea mays* leaves possess maximum hydroxyl radical scavenging activity. In the light of the available literature, these findings gain significance in establishing the antioxidant potential of the leaves.

SUPEROXIDE RADICAL SCAVENGING EFFECTS

Superoxide, the one-electron reduced form of molecular oxygen, is a precursor of other ROS such as hydrogen peroxide, hydroxyl radical and singlet oxygen that have the potential of reacting with biological macromolecules and thereby inducing tissue damages (Aruoma, 1998). It has also been implicated in initiating oxidation reactions associated

with aging (Wickens, 2001). Therefore, superoxide radical scavenging by antioxidants has physiological implications.

The leaf extracts of *Zea mays* were found to be effective in scavenging superoxide radicals, with the maximum effect being shown by the methanolic extract. This was followed closely by the water and chloroform extracts.

Many reports in the literature associate the $SO^{\bullet}$ scavenging activity of plants and their components with strong antioxidant activity. The leaves of *Stachytarpheta angustifolia* possessed antioxidant properties, which were expressed by their ability to scavenge superoxide radicals (Awah *et al.*, 2010).

Achillea santolina extract is a potent scavenger of superoxide radicals in a dose-dependent manner (Ardestani and Yazdanparast, 2007). Hseu *et al.* (2008) have reported that the aqueous extracts of *Toona sinensis* and gallic acid were active in inhibiting $SO^{\bullet}$ radical production.

Mansour *et al.* (2007) have demonstrated that the aqueous, petroleum ether, chloroform, ethylacetate and methanol extracts prepared from powdered *Acacia salicina* leaves were active in inhibiting $SO^{\bullet}$ 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 $SO^{\bullet}$ generating system. The superoxide scavenging property of *Andrographis paniculata* and *Swertia chirata* plant extracts were attributed to their high phenolic and flavonoid constituents (Tripati *et al.*, 2007).

In the backdrop of the above studies, the ability of *Zea mays* leaf extracts to effectively scavenge superoxide radicals reveals the strong radical scavenging potential of the leaves.

NITRIC OXIDE SCAVENGING EFFECTS

Nitric oxide (NO) and associated reactive nitrogen species (RNS) are involved in many physiological functions (Hofseth, 2008). It was found that all the three extracts of *Zea mays* leaves (aqueous, methanolic and chloroform) inhibited nitric oxide, with the methanolic extract performing better than the other two.

There are a lot of supportive reports for this observation in the literature. The ethyl acetate, petroleum ether and methanolic extracts of *Aporosa lindleyana* Baill root showed antioxidant activity by inhibiting nitric oxide radical (Badami *et al.*, 2005).

The ethyl acetate extract of the root of *Wikstroemia indica* was observed to inhibit nitric oxide production in a lipopolysaccharide and recombinant mouse interferon-g activated murine macrophage-like cell line (Wang *et al.*, 2005). *Amygdalus communis* L. hulls and shells possessed scavenging capacity for radical nitrite (Sfahlan *et al.*, 2009). Curcumin analogue 2,6-bis-4-(hydroxyl-3-methoxy-benzylidine)-cyclohexanone or BHMC showed a significant dose–response inhibitory action upon the synthesis of NO (Tham *et al.*, 2010).

In the present study, leaves of *Zea mays* showed significant inhibitory effect on NO generation, which reiterates their strong antioxidant activity.

METAL ION CHELATING AND REDUCING CAPACITIES

Metal ion chelating and reducing capacities play a significant role in antioxidant mechanism. It is reported that chelating agents, which form *s*-bonds with a metal, are effective as secondary antioxidants since they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon, 1990). The reducing capacity of various extracts might be due to their hydrogen-donating ability, as described by Shimada *et al.* (1992). Therefore, the extracts might contain reductones, which could react with free radicals to stabilize and terminate radical chain reactions.

The extracts of *Zea mays* were investigated for their reducing and chelating abilities. The results indicated that the methanolic extract was very effective in both reducing and chelating metal ions followed by the other two, with the aqueous extract faring better over the chloroform extract.

There are several reports, which support our findings. *Toona sinensis* extracts and gallic acid possessed antioxidant properties, which was expressed by their reducing power (Hseu *et al.*, 2008). The results reported by Kirkova *et al.* (2007) revealed that the celecoxib and amtolmetin guacyl possess antioxidant and metal-chelating abilities, which might contribute to their beneficial anti-inflammatory activity effects.

Mulberry (Whazosipmunja, Suwonnosang and Jasan) extracts possessed higher reducing power than BHT (Bae and Suh, 2007). Srivastava *et al.* (2006) have reported that the chelating and reducing power of *Decalepis hamiltonii* was concentration-dependent and that the methanolic extract was slightly more active than the aqueous extract.

Our results showed a trend comparable to that reported by Srivastava *et al.* (2006) that a methanolic extract of *Zea mays* leaves possessed strong reducing and chelating power when compared to the aqueous and chloroform extract.

Iron is known to generate free radicals through the Fenton and Haber-Weiss reaction (Halliwell and Gutteridge, 1990). Metal ion chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage. Metal ion chelating capacity plays a significant role in antioxidant mechanism since it reduces the concentration of the catalysing transition metal in LPO (Duh *et al.*, 1999).

Mau *et al.* (2004a) have reported that the methanolic extract of white mycelia of *Antrodia camphorata* (Zang and Su) exhibited maximum chelating and reducing properties compared to the red ones. Caffeic acid exhibited reducing power and metal chelating on ferrous ions (Gulcin, 2006).

The ethyl acetate extract/fractions of *Acacia auriculiformis* A. cunn possessed reducing as well as chelating power and also inhibited lipid peroxidation (Singh *et al.*, 2007). The monodesmosides and crude extract of *Leontice smirnowii* exhibited strong reducing power and metal chelating activities (Gulcin *et al.*, 2006).

The ethanolic, cold water and hot water extracts of *Hypsizigus marmoreus* (Peck) Bigelow (Tricholomataceae), an edible mushroom possessed chelating ability on ferrous ions as well as cupric ions (Lee *et al.*, 2007b). Methanolic extracts of three species of mushroom mycelia namely *Grifola frondosa* (maitake), *Morchella esculenta* (morel) and *Termitomyces albuminosus* (termite mushroom) showed good metal chelating and reducing powers (Mau *et al.*, 2004b).

The total anthocyanins from *Perilla pankinensis* samples were found to be very effective in total reducing power and metal chelating on ferrous ions (Gulcin *et al.*, 2005). The ethanolic, cold and hot water extracts of *Pleurotus citrinopileatus* fruit bodies,

mycelia possessed antioxidant properties as reflected by its reducing and chelating properties (Lee *et al.*, 2007c).

In the present study, it was observed that *Zea mays* leaf extracts also showed considerable metal chelating and reducing activities, adding weightage to the antioxidant capacity of the leaves.

Our results, thus, demonstrate that the *Zea mays* leaf extracts possess high activities/levels of both enzymic and non-enzymic antioxidants (as indicated in the results of the first phase). They also exhibited strong radical scavenging effects against a battery of radicals and oxidants. They were also effective in chelating and reducing metal ions. The maximum effect was mediated by the methanolic extract of *Zea mays* leaves.

EFFECT OF *Zea mays* LEAF EXTRACTS ON THE OXIDATIVE DAMAGE TO BIOMOLECULES

Oxidative assault to biological system manifests at the cellular level on important biomolecules. Of these, the lipid molecules form the primary targets and the DNA molecules, the ultimate targets. Therefore, as a next step, after ascertaining the radical scavenging properties of the extracts, their effects on membrane lipids and DNA were followed.

EFFECT OF *Zea mays* LEAF EXTRACTS ON LIPID PEROXIDATION

In aerobic organisms, one of the major targets of ROS are the cellular biomembranes, where they induce lipid peroxidation. Under this process, not only the membrane structure and its function are affected, but also some oxidation reaction products, for example, malondialdehyde (MDA), can react with biomolecules and exert cytotoxic and genotoxic effects (Pezzuto and Park, 2002).

Oxidants need to cross the membrane barriers to exert their damaging effects. This was given due to consideration in the present study, wherein three different sources of membrane lipids were challenged with an oxidant assault and the effect of the leaf extracts were studied on the damage inflicted to the lipid preparations. 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

Zea mays leaf extracts rendered strong protection to all the membrane model systems used.

The methanolic and aqueous extracts inhibited LPO more effectively than the chloroform extract. The protection was much better in the RBC ghosts followed by goat liver homogenate and goat liver slices. This is indicative of the fact that some components in the leaf extracts may not be readily membrane permeable, as the extent of LPO in the liver slices was lower.

Several plants have been shown to inhibit lipid peroxidation in various systems. Hsu (2008) has reported that the ethanol extract of *Pyrrosia petiolosa* exhibited strong antioxidant activity as reflected by their effectiveness in inhibiting lipid peroxidation. Phenyl propanoid glycosides have been shown to inhibit lipid peroxidation and LDL oxidation (Thuan *et al.*, 2008).

Gugliucci and Menni (2002) have reported that the *Achyrocline satureoides* extracts inhibit human LDL oxidation in three different systems (copper, peroxynitrite and lipoxygenase). The extract of *Adhatoda vasica*, *Amaranthus paniculatus*, *Brassica compestris*, *Mentha piperita* and *Spirulina fusiformis* inhibited lipid peroxidation in liver (Samarth *et al.*, 2008).

The leaves of perilla [*Perilla frutescens* (L.) Britt. var. *japonica* (Hassk.) Hara] reversed the *t*-BHP-induced lipid peroxidation in rat livers (Kim *et al.*, 2007a). A methanol extract of rhizomes of *Curculigo orchioides* Gaertn showed potent inhibition of lipid peroxidation induced by iron / ADP / ascorbate complex in rat liver homogenate (Bafna and Mishra, 2005).

Bhatia *et al.* (2006) have demonstrated that fenugreek (*Trigonella foenum-graecum* L.) extract inhibited LPO caused by cyclophosphamide and L-buthionine-SR-sulfoximine in the urinary bladder of mice. The alcoholic bark extract of *Butea monosperma* (*B. monosperma*) possessed antioxidant properties, as reflected by its ability to reduce lipid peroxidation (Sumitra *et al.*, 2005).

The sea weeds were potent in suppressing TBARS formation by H_2O_2 induced lipid peroxidation in RBC (Devi *et al.*, 2008). The essential oil from the crushed fruits of

Chaerophyllum libanoticum Boiss. *et* Kotschy inhibited LPO assayed using β-carotene bleaching and haemoglobin induced linoleic acid peroxidation (Demirci *et al.*, 2007).

Our results are in accordance with these reports. The methanolic extract of *Zea mays* leaves protected both plasma membrane and internal organelle membranes from lipid damage more effectively than the aqueous and chloroform extracts.

EFFECT OF *Zea mays* LEAF EXTRACTS ON THE OXIDATIVE DNA DAMAGE

DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions have been noted in various tumors, strongly implicating such damage in the etiology of cancer. It appears that the DNA damage is predominantly linked with the initiation process (Bao *et al.*, 2004; Evans *et al.*, 2004). Hence, the DNA protective effects of *Zea mays* leaf extracts were assayed in different *in vitro* systems. In order to understand whether they act by triggering any endogenous factors for the protection rendered, both intact cell DNA and also commercially available DNA were tested with the leaf extracts. Another major role of antioxidants is also to repair DNA damage caused during oxidative stress conditions. Hence the leaf extracts were also checked for their DNA-repairing capability in oxidant induced conditions.

In the present study, the protective abilities of the leaf extracts of *Zea mays* 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 were employed. The DNA samples used were λ DNA, haploid herring sperm DNA, diploid calf thymus DNA and intact cell DNA. H_2O_2 exposure caused extensive damage to DNA. However, *Zea mays* leaf extracts significantly reduced the extent of DNA damage in the different types of DNA. The extracts, by themselves, did not cause any DNA damage. The maximum protection was exerted by the methanolic extract in all the different kinds of DNA tested.

Many studies have reported the protection against oxidative DNA damage by herbal extracts and formulations. Irradiating DNA (λ phage, bovine spleen DNA, pUC 19 plasmid) with UV light in the presence of methyl resorcinol or hexyl resorcinol resulted in comparatively insignificant DNA destruction (Davydova *et al.*, 2005). Low molecular weight chitosan and chitooligosaccharides, obtained by persulfate-induced

depolymerization of chitosan offered protection against calf thymus DNA damage (Prasanth *et al.*, 2007).

Green tea polyphenols and/or trolox inhibited azo initiator, 2,2'-azobis(2-amidinopropane hydrochloride) (AAPH)-induced damage *in vitro*, by measuring the conversion of supercoiled pBR322 plasmid DNA to the open circular and linear forms (Wei *et al.*, 2006). Guarana (*Paullinia cupana* Mart. var. *Sorbilis*) showed a protective effect against DEN-induced DNA damage in mouse liver (Fukumasu *et al.*, 2006).

Hydroxylated 4-thiaflavan possessed effective protection against oxidation damage induced in herring sperm DNA by cumene hydroperoxide or by the glutathione / ferric ion system (Lodovici *et al.*, 2006). The rhizome extract of *Dioscorea alata* possessed radical scavenging activity and showed protective effect on calf thymus DNA and plasmid DNA as evaluated by EtBr (Wang *et al.*, 2004). The alcohol : water (1:1) extract of curry leaves (*Murraya koenigii* L.) showed the highest antioxidant as reflected by the inhibiton of ferrous sulfate : ascorbate-induced fragmentation and sugar oxidation of calf thymus DNA (Ningappa *et al.*, 2008).

Green tea consumption is associated with decreased DNA damage among GSTM1-positive smokers regardless of their hOGG1 genotype as determined by urinary 8-hydroxydeoxyguanosine (8-OHdG), a sensitive biomarker of the overall oxidative DNA damage and repair (Hakim *et al.*, 2008). The methanolic extracts of *Celastrus paniculatus* L. (Celastraceae), *Picrorhiza kurroa* L. (Scrophulariaceae) and *Withania somnifera* L. (Solanaceae) displayed a significant protective capability against H_2O_2-induced DNA damage in human non-immortalized fibroblasts (Russo *et al.*, 2001).

A methanolic extract of *Bacopa monniera* L. has been shown to be capable of scavenging free radicals and exerted a potent effect on DNA cleavage induced by H_2O_2 UV photolysis (Vanella *et al.*, 2003). Jurkat T-lymphocytes that were pre-incubated with relatively low concentrations of either EGCG or quercetin were less susceptible to DNA damage induced by either reactive oxygen species or reactive nitrogen species, as evaluated by the comet assay (Johnson and Loo, 2000).

H_2O_2-induced oxidative DNA damage was strongly protected by kahweol and cafestol as determined by the comet (single cell gel electrophoresis) assay (Lee and Jeong, 2007). Grape seed polyphenols and bearberry strongly protected U937 cells

against H_2O_2 and tert-butylhydroperoxide induced DNA damage in the alkaline single-cell gel electrophoresis assay (Carpenter *et al.*, 2006). Aqueous, methanol and ethyl acetate extracts of *Acacia salicina* leaves protected human lymphoblast cells K562 against H_2O_2 induced DNA damage as revealed by alkaline single-cell gel electrophoresis assay (Bouhel *et al.*, 2008).

The results of the present study showed that the leaves of *Zea mays* were able to protect DNA against oxidative damage in purified DNA and in intact cells.

ASSESSMENT OF DNA REPAIR

The *Zea mays* leaf extracts were very effective in reverting the DNA damage induced by H_2O_2. *Zea mays* leaf extract treatment effectively decreased the extent of DNA damage from baseline DNA damage. This reduction could be due to the prevention of DNA damage or due to the effective repair of the damaged DNA by the antioxidants present in the leaf extracts. In order to find out the exact mechanism operating behind the reduction observed in DNA damage, the extent of DNA repair was assessed using ^{3}HTdR.

The results obtained revealed that the exposure to hydrogen peroxide decreased incorporation of radioactive thymidine into the DNA. The cells exposed to the leaf extracts exhibited an increase in the incorporation of radioactive thymidine when co-administered with the hydrogen peroxide.

There is a lot of reported support for this observation in the literature. Glutathione depletion caused a pronounced retardation in DNA repair even under non-toxic irradiation conditions in fibroblasts and melanoma cells from three different patients (Eiberger *et al.*, 2008). Lunec *et al.* (2002) have proposed that those redox-active components of the diet, such as vitamin C, may promote DNA repair.

The prenylflavonoids xanthohumol, isoxanthohumol and 8-prenyl-naringenin decreased cell proliferation in breast cancer Sk-Br-3 cell line by inducing apoptosis shown by SRB, [3H]thymidine incorporation and DAPI staining (Monteiro *et al.*, 2007).

Rectification of DNA damage by inducing its repair is reflective of the anticancer activity of the plant components, as revealed by the above studies. Thus it is perceivable

that the DNA repair brought about by *Zea mays* leaf extracts could be reflective of its anticancer property.

EFFECT OF *Zea mays* LEAF EXTRACTS ON THE ANTIOXIDANT STATUS OF GOAT LIVER SLICES SUBJECTED TO OXIDATIVE STRESS

Our results demonstrated that the extracts of *Zea mays* leaves were very effective in protecting the cellular biomolecular targets from oxidative damage. Subsequently, the influence of the leaf extracts were 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.

Precision-cut tissue slices mimic specific organ toxicity because normal cellular heterogeneity and organ architecture are retained. Lerche-Langrand and Toutain (2000) have demonstrated the usefulness of precision-cut liver slices as an *in vitro* model system for investigating drug metabolism and toxicity. Precision-cut liver slices are described as a valuable tool for *in vitro* metabolism studies of potential drug candidates. Some papers have reported successful cryopreservation conditions for liver slices, facilitating a broader and more efficient use of the tissue (particularly of human origin) (Martignoni *et al.*, 2004).

In the current study, by employing the precision-cut liver slices generated from goat liver as a tool, the protective effects of the *Zea mays* leaves *in vitro* against hydrogen peroxide-induced oxidative stress was evaluated. Enzymic and non-enzymic antioxidants were assessed in the liver slices subjected to oxidative stress in the presence and the absence of the leaf extracts.

ENZYMIC ANTIOXIDANTS

The enzymic antioxidants analyzed were superoxide dismutase, catalase, peroxidase, glutathione S-transferase and glutathione reductase.

SUPEROXIDE DISMUTASE

The administration of H_2O_2 to goat liver slices significantly lowered the activity of SOD, whereas, the *Zea mays* leaf extracts significantly elevated the activity of SOD, with the methanolic extract showing the maximum effect.

The discovery of superoxide dismutases (SODs), which convert superoxide radicals to molecular oxygen and hydrogen peroxide, has been termed the most important discovery of modern biology never to win a Nobel Prize (Perry *et al.*, 2010).

The methanolic extract of *Berberis tinctoria Lesch* (Berberidaceae) leaves significantly increased CAT and SOD in a dose dependent manner. Their effects were comparable to that of the standard drug silymarin (Murugesh *et al.*, 2005). Four aqueous extracts from different parts of medicinal plants used in Ayurveda (an ancient Indian Medicine) viz., *Momardica charantia* Linn, *Glycyrrhiza glabra*, *Acacia catechu* and *Terminalia chebula* protected the SOD enzyme in rat liver mitochondria from the damage caused by irradiation (Naik *et al.*, 2003).

Chotimarkorn and Ushio (2008) have reported that the *trans*-ferulic acid and gamma-oryzanol-treated mice recovered from an ethanol-induced decrease in hepatic glutathione level together with enhancing superoxide dismutase activity. The leaf extract of *Passiflora alata* Dryander has been demonstrated to possess antioxidant activity *in vitro as* evidenced by increasing superoxide dismutase activitity in CCl_4 intoxicated rats (Rudnicki *et al.*, 2006). Liu *et al.* (2010) have reported that the administration of quercetin markedly restored Cu/Zn-SOD, Mn-SOD, CAT and GPx activities and upregulated mRNA expression levels of these proteins in the liver of lead-treated rats.

The green pods of *Acacia nilotica* caused a significant increase in the levels of SOD in the liver, lungs, kidneys and blood in CCl_4-intoxicated rats (Singh *et al.*, 2009). Curcumin restored the activity of SOD to normalcy in ethanol treated cells. The fact that the activity of SOD is kept low indicates that curcumin by its antioxidant activity reduced the oxidative stress induced by ethanol and protected the liver cells *in vitro* (Naik *et al.*, 2004).

Our results showed that *Zea mays* leaf extracts improved the activities of SOD in the oxidant stressed group, indicative of their antioxidant potential.

CATALASE

In the present study, during H_2O_2 intoxication, the catalase activities were found to be significantly reduced. Treatment with the leaf extracts, however, improved the status and the effect was more pronounced in the groups treated with the methanolic extract followed by the aqueous and chloroform extracts.

Catalase catalyses the decomposition of H_2O_2 to molecular oxygen and water thereby protecting cells from the toxic effects of H_2O_2 (Hua *et al.*, 2007). Yao *et al.* (2007) have reported that the *Ginkgo biloba* extract inhibited ethanol-derived inactivation of superoxide dismutase and catalase.

The study conducted by Mondal *et al.* (2005) showed a decline in catalase activity in CCl_4-administered rats, which were brought to near normal level by the administration of the defatted methanol extract of *Diospyros malabarica* bark. The ethanol extract of A*quilegia vulgaris* (Jodynis-Liebert *et al.*, 2009) imparted a protective effect on CCl_4 induced liver injury in male Wistar rats by significantly increasing the antioxidant enzyme activities.

Our results clearly demonstrate that the administration of *Zea mays* leaf extracts significantly increased the catalase activity in H_2O_2 exposed liver slices indicating their antioxidant potential. The effect elicited by the methanolic extract was effective in this regard.

SOD is the first line of defense in counteracting oxidant assault, the action of SOD results in the production of H_2O_2, which is also detrimental. Thus, effective detoxification can occur only if an increase in SOD is accompanied by a concomitant increase in catalase and / or peroxidase activities (Ramasarma, 1990). Our results are corroborative with these reports, emphasizing the antioxidant response evoked by *Zea mays* leaves.

PEROXIDASE

Treatment of liver slices with H_2O_2 caused a significant reduction in the activity of peroxidase. The adverse effects of H_2O_2 were reversed by the administration of *Zea mays*

leaf extracts. *Zea mays* leaf extracts, by themselves, significantly increased the peroxidase activity compared to control, except chloroform extract.

The exposure of hepatocytes to *S. cumini* peel extract rich in anthocyanins after CCl_4 treatment was found to elevate GSH and GPx activities by 2-folds, whereas the activities of catalase and superoxide dismutase were not significantly affected (Veigas *et al.*, 2008). Dehydrocavidine, a main active ingredient of *Corydalis saxicola* Bunting (*Yanhuanglian*) significantly prevented the depletion of glutathione peroxidase (GPx) in the liver of CCl_4-intoxicated male Sprague–Dawley rats (Wang *et al.*, 2008a). The hepatoprotective effect of ginsan, a polysaccharide extracted from *Panax ginseng* was attributed to the induction of anti-oxidant protein contents, such as SOD, CAT and GPx on carbon tetrachloride (CCl_4)-induced liver injury in BALB/c mice (Shim *et al.*, 2010).

Our results indicated that the *Zea mays* leaf extracts were able to protect the hydrogen peroxide-induced oxidative stress in liver slices by elevating the antioxidant enzyme activities.

GLUTATHIONE S-TRANSFERASE

Treatment of the goat liver slices with H_2O_2 caused a significant decrease in the activities of glutathione S-transferase. The toxic effects of H_2O_2 were effectively counteracted by the addition of *Zea mays* leaf extracts, which caused a significant increase in GST activities.

GSTs are active in the detoxification of numerous products, including reactive oxidant damage to DNA and lipids, such as organic epoxides, lipid hydroperoxides and unsaturated aldehydes (Hayes *et al.*, 2005). Yousef *et al.* (2009) have reported that grape seed proanthocyanidin extract significantly elevated the hepatic GST activity during cisplatin intoxication. Kundu *et al.* (2008) demonstrated that the methanol-aqueous fraction of *Cajanus cajan* leaf extract could prevent the chronically treated alcohol-induced rat liver damage by augmenting the antioxidant enzyme activities.

El-Demerdash *et al.* (2009) demonstrated that curcumin led to the recovery of the decreased GST, SOD and CAT activities against sodium arsenite-induced oxidative damage in rat. The decreased activites of key antioxidant enzymes such as SOD, CAT,

GPx and GST in diabetic rats were brought back to near normal range upon *Helicteres isora* L. (Sterculiaceae) bark treatment (Kumar *et al.*, 2008). *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_4 in rat (Young *et al.*, 2006).

Manna *et al.* (2006) have reported that the aqueous extract of the bark of *Terminalia arjuna* recovered the decline in the activity of CAT, SOD, GST and GSH in CCl_4 administered rats, thus revealing that oxidative stress elicited by CCl_4 intoxication had been nullified due to the antioxidant effect of the extract.

All these reports validate our results, wherein the treatment with the extracts significantly improved the activity of GST in the oxidatively stressed goat liver slices.

GLUTATHIONE REDUCTASE

The activities of GR significantly decreased upon H_2O_2 exposure and were restored to control levels by the co-administration of *Zea mays* leaf extracts.

Nevin and Vijayammal (2005) demonstrated that the partially purified petroleum ether extractable fraction of the whole plant *Aerva lanata* enhanced the antioxidant enzyme activities against liver damage induced by carbon tetra chloride (CCl_4) in Sprague Dawley rats. A methanolic extract of *Coscinium fenestratum* stem powder (Venukumar and Latha, 2004) retrieved the decreased activities of glutathione reductase towards near-normalcy in the plasma and liver of rats intoxicated with CCl_4.

The ethanol and ethyl acetate extracts of *Aquilegia vulgaris* (L.) (Ranunculaceae) herb increased the activities of CAT, GPx, GR and GST in rats treated with acetaminophen (*N*-acetyl-*p*-aminophenol, APAP) (Jodynis-Liebert *et al.*, 2005).

The administration of *Zea mays* leaf extracts in the present study, improved the GR activities from the effect of the oxidant assault. This observation shows that the leaf extracts are effective in ensuring GSH homeostasis in the cell, as GR replenishes GSH (reduced) from GSSG (oxidized). GSH, apart from being a strong antioxidant by itself also acts as a substrate for antioxidant enzymes like GPx and GST (Madrigal *et al.*, 2001)

The results obtained in the present study revealed that H_2O_2 exposure to the liver slices elicited an oxidative stress as evidenced by the decreased activities of the enzymic

antioxidants. The oxidative stress was mitigated by the *Zea mays* leaf extracts efficiently by increasing the activities of enzymic antioxidants.

NON-ENZYMIC ANTIOXIDANTS

The non-enzymic antioxidants analyzed in the present study were vitamin C, vitamin E, vitamin A and reduced glutathione.

VITAMIN C

H_2O_2 treatment caused a statistically significant decrease in vitamin C levels in the goat liver slices. The co-exposure of the liver slices to oxidant and the leaf extracts caused a rise in the levels of ascorbate. The maximum increase was observed with the methanolic extract of *Zea mays* leaves.

Withania somnifera extracts effectively counteracted the damage produced by H_2O_2 and replenished vitamin C levels in hydrogen peroxide treated goat liver slices (Sumathi and Padma, 2008). Vandana *et al.* (2006) observed that the addition of vitamin C inhibited chromium-induced cytotoxicity in murine macrophages.

The *Casearia esculenta* root extract improved the levels of vitamin C, vitamin E and GSH of the liver and kidney of streptozotocin diabetic rats (Prakasam *et al.*, 2005). *Ganoderma lucidum* polysaccharides treatment significantly and dose-dependently increased non-enzymic and enzymic antioxidants in STZ-induced diabetic rats (Jia *et al.*, 2009).

Thus, it is perceivable that the increase in vitamin C levels by the leaf extracts of *Zea mays* proves their ability to improve the antioxidant status in the tissues.

VITAMIN E

The goat liver slices, when treated with hydrogen peroxide, showed a slight decrease in the levels of vitamin E. This depleting effect was counteracted by the co-treatment with the leaf extracts.

Therapeutic treatment with the herbal drug *Aegle marmelos* in ethanol intoxicated rats significantly increased the levels of vitamin E and C through the influence of GSH regeneration (Singanan *et al.*, 2007). Bansal *et al.* (2005) reported that pretreatment with

vitamin E to NDEA induced rats provide protection against oxidative stress in liver caused by the carcinogen. El-Shenawy *et al.* (2010) demonstrated that the co-treatment of vitamin E with diazinon prevents the oxidative stress-induced liver tissue injury in mice.

Vitamin E treatment ameliorated the effects of atrazine suggesting it as a potential antioxidant against atrazine-induced oxidative stress in male Wistar rats (Singh *et al.*, 2010). Vitamin E effectively counteracted the bleomycin induced pulmonary fibrosis in the lungs of rats (Dede *et al.*, 2006).

In line with these reports, it is conceivable that the *Zea mays* leaf extracts can render protection to the membranes by increasing the levels of vitamin E, the major antioxidant present in the membrane.

VITAMIN A

H_2O_2 treatment caused a significant depletion in the levels of vitamin A compared to untreated controls. This depleting effect was restored by the co-treatment with the leaf extracts. The extent of increase was maximum with the methanolic extract followed by the aqueous and chloroform extracts.

Sampaio *et al.* (2007) demonstrated the chemopreventive potential of vitamin A and β-carotene during early hepatocarcinogenesis potentiated by 5-azacytidine in Wistar 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).

Prefeeding dehydrated amaranth leaves reversed the hexachlorocyclohexane-induced decrease in the levels of vitamin A and the actitivities of SOD, GPx, GST, GR and G6PD in rat liver (Anilakumar *et al.*, 2006). The oral administration of the ethanolic extract of *Terminalia arjuna* stem bark caused a significant improvement in the decreased levels of vitamin A in the liver and kidney of alloxan treated diabetic rats (Raghavan and Kumari, 2006).

In view of these reports, an increase in vitamin A by the leaf extracts of *Zea mays* in the present study presents very significant implications in the manifestation of the antioxidant defense by the leaves.

REDUCED GLUTATHIONE

GSH levels were significantly reduced when goat liver slices were exposed to H_2O_2. This may be due to its enhanced utilization by the hepatocytes to nullify the toxicity of H_2O_2. These levels were restored to normalcy by the *Zea mays* leaf extracts.

Reyes-Gordillo *et al.* (2007) have demonstrated the ability of curcumin to suppress acute carbon tetrachloride-induced liver damage. Wills and Asha (2006) observed that the treatment with *n*-hexane extract of *Lygodium flexuosum* (L.) significantly improved the levels of GSH in liver in CCl_4 intoxicated rats, indicating the hepatoprotection.

Tirkey *et al.* (2005) have reported that hesperidin, a potential citrus bioflavonoid improved the levels of GSH in the liver and kidney homogenates of the rats administered with CCl_4. Pretreatment with kahweol and cafestol prior to the administration of CCl_4 significantly prevented the depletion in glutathione content and lipid peroxidation, in the liver of mice in a dose-dependent manner (Lee *et al.*, 2007d).

Thus, it is perceivable that the increase in the GSH levels brought about by *Zea mays* leaf extracts could be reflective of the antioxidant effect.

EFFECT OF *Zea mays* LEAF EXTACTS ON OXIDATIVE STRESS-INDUCED APOPTOTIC DEATH IN VARIOUS TYPES OF CELLS

Oxidative stress can induce cell damage and eventual cell death (Yoshioka *et al.*, 2006). Several types of critical illnesses are associated with cell death, both necrotic and apoptotic. The significant contributions of apoptotic pathways in cell death and to the pathogenesis of variours disorders are well appreciated. Certainly, the ability to selectively induce or block apoptosis by pharmaceutical intervention is an area of active research (Thatte *et al.*, 2000).

As we felt the studies regarding the apoptotic modulation of medicinal plants would definitely pave a way for better understanding of its protective mechanism, we evaluated the influence of the *Zea mays* leaf extracts on apoptosis induced by H_2O_2 in different *in vitro* systems. *In vitro* data obtained with more complex systems, including isolated cells, cultured cells and precision-cut organ slices, can be used in drug

development to improve the drug development process (Lerche-Langrand and Toutain, 2000).

In tune with this background, in phase III of the study, the effect of *Zea mays* leaf extracts on oxidative stress-induced apoptosis was analysed in untransformed (chick embryo fibroblasts and *Saccharomyces cerevisiae*) and transformed (Hep2 laryngeal carcinoma) cells. H_2O_2 was used to induce oxidative stress.

EFFECT OF *Zea mays* LEAF EXTRACTS ON THE H_2O_2 INDUCED DEATH IN UNTRANSFORMED AND TRANSFORMED CELLS

Our results clearly indicated that H_2O_2 exposure caused a significant number of cells to commit to apoptosis, both in the case of transformed and untransformed cells. The extracts of *Zea mays* leaves, when administered alone, evoked a differential response depending on the cell type. In the normal (chick embryo fibroblasts and *Saccharomyces cerevisiae*) cells, the extract did not induce apoptosis. However, there was a marked increase in the number of apoptosing cancer (Hep2) cells when exposed to *Zea mays* leaf extracts.

Moreover, the oxidative stress-induced cellular death was effectively counteracted by the presence of *Zea mays* leaf extracts in the normal (untransformed) cells. But in the cancer cells, the leaf extract caused no change in the extent of cytotoxicity. The maximum cytotoxicity towards cancer cells was exhibited by the methanolic extract. Among the other two extracts, the aqueous extract pronounced a better effect than the chloroform extract.

These results suggest that *Zea mays* leaves possess anticancer activity and this action is mediated by selective cytotoxicity towards cancer cells. Agents that modulate apoptosis could be exploited as anticancer drugs. Thus, as the next step we studied the influence of *Zea mays* leaf extracts and etoposide (a standard chemotherapeutic drug) in both untransformed (chick embryo fibroblasts) and transformed (Hep2) cells.

EFFECT OF *Zea mays* LEAF EXTRACTS ON THE TRANSFORMED AND UNTRANSFORMED CELLS EXPOSED TO ETOPOSIDE

The results revealed that etoposide caused a significant number of cells to commit to apoptosis (both chick embryo fibroblasts and Hep2 cells). The *Zea mays* leaf extracts reduced the toxicity of etoposide in the normal cells and the leaf extracts, by themselves, did not cause any damage to normal cells when treated alone.

In contrast, the administration of the extracts alone increased the number of Hep2 cells showing apoptotic morphology. When the cells were exposed to both etoposide and the leaf extracts, the extent of cell death increased further, compared to that by etoposide alone. Thus, the leaf extracts helped to enhance the anticancer action of etoposide.

The results of various parameters employed for these studies such as cell viability (by SRB and MTT), membrane and nuclear changes (by PI, EtBr, DAPI and AO/ EtBr staining) are discussed below.

CYTOTOXICITY ASSAYS

MTT ASSAY

The cell viability was assessed by MTT assay, which is an ideal assay as reported by several studies. Using the MTT assay, many researchers found time- and dose-dependent inhibition in different cells with different agents.

The ethanolic extract from *Gynostemma pentaphyllum* inhibited cell proliferation in C6 glioma tumour cells and not in the proliferation of astrocytes of a primary cell culture (Schild *et al.*, 2010). This report supported our study that *Zea mays* induced apoptosis only in cancer cells and not in normal cells.

Ginkgo biloba extract kaempferol effectively inhibited pancreatic cancer cell proliferation and induced cancer cell apoptosis, which was suggested to sensitize pancreatic tumor cells to chemotherapy (Zhang *et al.*, 2008a). H_2O_2-induced damage in *Saccharomyces cerevisiae* cells was nullified by the treatment with *Ilex paraguariensis* infusion and α-tocopherol (Bracesco *et al.*, 2003). Kahweol and cafestol improved the cell viability in a dose-dependent manner in H_2O_2 treated NIH3T3 cells (Lee and

Jeong, 2007). Yi *et al.* (2005) have demonstrated that grape skin anthocyanin fractions showed more cancer inhibitory activity than phenolic acids.

Sebastian and Thampan (2007) have reported that the ethanol extract of fenugreek decreased the cell viability in MCF-7 cells, whereas soybean extract acted as a promoter as determined by MTT assay. Inhibition of cell proliferation by sesquiterpene lactones (Tomentosin and Inuviscolide) from *Inula viscosa* (*Compositae*) leaves against human melanoma cell lines was reported by Rozenblat *et al.* (2007).

The chemical drug 15,16-dihydrotanshinone I inhibited breast cancer cell proliferation and tumor growth, as shown by the MTT assay (Tsai *et al.*, 2007). Balasubramaniyan *et al.* (2007) have reported that leptin (antiobesity hormone) prevented ethanol elicited cytotoxicity as evidenced by MTT and trypan blue dye exclusion assays.

It was found that panaxydol markedly inhibited the proliferation of rat C6 glioma cells in a dose-dependent manner as assessed by the MTT reduction assay (Hai *et al.*, 2007). Vitamin A and its natural and synthetic analogs (retinoids) induced apoptosis in prostate cancer cells *in vitro* and in animal models, mainly through the induction of retinoic acid receptor-β (RARβ) (Zhang, 2002).

The viability of human osteoblast cells decreases in response to dose-dependent treatment of curcumin as shown by the MTT assay (Chan *et al.*, 2006). Peng *et al.* (2007) determined the cytotoxic activity of the root of *Spiranthes australis* on lung carcinoma, hepatoma cell line, gastric cancer, breast cancer, colon cancer, leukemia cancer and renal cancer by the MTT assay, where the cell viability was reduced by the plant extract.

Maslinic acid, a pentacyclic triterpene (olive pomace) decreased the viability of HT-29 and Caco-2 colon cancer cell lines as determined by the MTT assay (Reyes *et al.*, 2006). The ethanolic extract of *Celastrus orbiculatus* was studied for its cytotoxic effect on human melanoma A375-S2 and human cervical carcinoma HeLa cell lines (Xu *et al.*, 2008).

Etoposide suppressed the viability of gastric cancer cell lines SGC7901 and BGC823 cells in a time- and dose-dependent manner as shown by MTT assay (Liu *et al.*, 2006a). An ethanol extract of *Dunaliella salina* induced the reduction of A549 (human

non-small cell lung cancer) cells viability through apoptosis which was quantified using the MTT assay (Sheu *et al.*, 2008).

Human umbilical vein endothelial cells (HUVEL) subjected to oxidative stress were protected by the antagonist effect of telmisartan, an angiotensin II as revealed by the MTT assay (Cianchetti *et al.*, 2008). HepG2 cell proliferation was decreased by soya saponins in a dose-dependent manner as shown by MTT assay (Zhang and Poporich, 2008).

The determination of cell viability strengthens their anti-apoptotic and anticancer effects of *Zea mays* leaf extracts. Our results showed a trend comparable to that reported by Bracesco *et al.* (2003) that *Zea mays* leaf extracts protected *Saccharomyces cerevisiae* cells from H_2O_2-induced damage.

Gambogic acid chemosensitizes BGC-823 / DOC gastric cancer cells to docetaxol, an anticancer drug, which was assessed by the MTT assay (Wang *et al.*, 2008b). Our results also exhibit a similar trend in that the presence of *Zea mays* leaf extract increased the susceptibility of the Hep2 cells to etoposide-induced apoptosis. It can be inferred from these results that *Zea mays* leaves sensitize cancer cells to the cytotoxicity of chemotherapeutic agents, while protecting non-cancerous cells against their toxic effects.

SRB ASSAY

Several reports in the literature have validated the SRB assay as a relevant tool in quantifying the extent of survival. The cytotoxic effect of etoposide, vinblastine sulfate and dacarbazine against B16-F10 and HMEC-1 cells was determined by SRB assay, revealing their cytotoxic effects (Dandamudi and Campbell, 2007).

Mate tea (*Ilex paraguariensis*) inhibited the growth of human colorectal adenocarcinoma cells CaCo-2 and HT-29 cells (de Mejia *et al.*, 2010). 1-benzyl-3-(5-hydroxymethyl-2-furyl)indazole showed impressive selective toxicity against the NCI-H226 cell line as shown by SRB assay, where it inhibited NCI-H226 cell growth in a time- and a concentration-dependent manner (Chen *et al.*, 2007).

The results reported by Lin *et al.* (2007) revealed that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induced concentration-dependent increases in cytotoxic response in human breast cancer cells. The degree of cytotoxic response induced by TCDD was greater in MCF-7 cells than in MDA-MB-231 cells.

Chlorotriphenylphosphine-1,3-bis (diphenylphosphino) propanegold (I) markedly inhibits human melanoma cell growth (SRB assay) (Caruso *et al.*, 2007). Polyacetylenes are reported to have cytotoxic effect in various cancer cells, in a dose-dependent manner (Yang *et al.*, 2008).

An extract of the rhizomes of *Iris tectorum Maxim* exhibited very high cytotoxicity against MCF-7 (breast cancer) and C32 (melanoma) cell lines (Fang *et al.*, 2008). Carballo *et al.* (2008) investigated the cytotoxicity effect of mucronulatol from Caribbean propolis against cancer cells, using SRB.

The Buckwheat (*Fagopyrum esculentum Moench*) extracted with ethanol showed cytotoxicity against cancer cells lines MCF-7 (breast), Hep 3B (human hepatoma) and A549 (lung) cancer cells (Kim *et al.*, 2007b). Dichloromethane and n-butanol extracts of *Hippeastrum vittatum* showed antiproliferative activity against five human cell lines (HT-29 colon adenocarcinoma, H460 non small cell lung carcinoma, RXF393 renal cell carcinoma, MCF-7 breast cancer and OVCAR-3 epithelial ovarian cancer cells) in *in vitro* studies (Silva *et al.*, 2008a).

The combination of STI-571 with radiation or cisplatin had an additive antiproliferative effect in SKNMC cells and MCF-7 human breast cancer cells, which was determined by sulphorhodamine B cytotoxicity assay (Yerushalmi *et al.*, 2007). SRB assay also showed the antiproliferative effects of polysaccharides from fungus, *Phellinus gilvus* against B16F10 melanoma cell line in a dose-dependent manner (Bae *et al.*, 2005). The extracts of *Phyllanthus emblica* and *Terminalia bellerica* are reported to have high cytotoxic effects against the human hepatocellular carcinoma (HepG2) and lung carcinoma (A549) cells (Pinmai *et al.*, 2008).

From the cell viability assays in the present study, it can be inferred that oxidative stress caused significant death in *Saccharomyces cerevisiae* cells, primary fibroblast cells and Hep2 cells. The leaves of *Zea mays* offered significant protection against cell death in

the oxidatively stressed normal (untransformed) cells, while not hindering with the cytotoxic effects of oxidants in cancer cells.

MORPHOLOGICAL CHANGES ASSOCIATED WITH APOPTOSIS

Morphological changes including plasma membrane blebbing, changes to the cell membrane such as loss of membrane asymmetry and attachment and cell shrinkage, are the early stages of apoptosis that can be analyzed by giemsa staining. The observations made in this study clearly indicate that the *Zea mays* leaf extracts exerted anticancer effect towards cancer cells and also protected the normal cells from hydrogen peroxide and etoposide (oxidant) induced stress. Giemsa staining for apoptotic studies has been reported by many researchers.

EGCG (epigallocatechin gallate) effectively inhibited proliferation and induced apoptosis in rat ELT3 uterine leiomyoma cells *in vitro* as determined by morphological changes (Zhang *et al.*, 2010b). Morphological changes were observed in human multiple myeloma cells treated with parthenolide (sesquiterpene lactone) (Chen *et al.*, 2006).

Zhou *et al.* (2007) report that oridonin, a diterpenoid extracted from medicinal herbs, had potent antitumor activity with low adverse effects on U937 cells. Celecoxib treatment induced apoptosis associated morphological changes in K562 (erythroleukemia) cells (Subhashini *et al.,* 2005).

A crude ethanolic extract of the plant *Lycopodium clavatum* considerably inhibited the growth of HeLa cells through the induction of apoptosis via caspase-3 activation as determined by morphological changes (Mandal *et al.*, 2010). Ajith *et al.* (2006) confirmed that lovastatin induced apoptosis in ascites tumour cell lines from mice in a dose-dependent manner.

In vitro studies of parthenolide showed inhibition of the proliferation of human hepatocellular carcinoma cell line BEL-7402, as assessed by giemsa staining (Song and Zhang, 2006). The julibroside J8 isolated from *Albizia julibrissin* had high antiproliferative activity against BGC-823 (stomach cancer), Bel-7402 (hepatocellular carcinoma), HeLa (cervical cancer), PC-3MIE8 (prostate carcinoma) and MDA-MB-435 (breast cancer) cells (Zheng *et al.*, 2006).

Doxorubicin treatment induced apoptosis-associated morphological changes in MCF-7 breast cancer cells (Singh *et al.*, 2008). Custodio *et al.* (2002) have reported that etoposide caused an oxidative stress in cells that results in apoptosis. Our results are in agreement with this report.

Morphological changes were observed in human neuroblastoma cell line treated with thimerosal (Humphrey *et al.*, 2005). Compounds isolated from garlic were shown to induce apoptosis in SH-SY5Y cells, as reflected by the morphology visualized by Wright staining (Karmaker *et al.*, 2007).

Studies have demonstrated that wogonin, a monoflavonoid extracted from the root of *Scutellaria baicalensis*, could effectively inhibit the proliferation of several cancer cell lines as determined by giemsa staining (Zhang *et al.*, 2008b). Gao *et al.* (2007b) investigated the effect of lidamycin in human hepatoma BEL-7402 cells and showed cell multinucleation observed by giemsa staining. Apoptosis in ECV-304 cell line supplemented with sodium morrhuate and lipo-sodium morrhuate was demonstrated by giemsa staining (Tu *et al.*, 2006).

The results of the present study revealed that the methanolic extract of *Zea mays* leaves can exert a maximum activity followed by the aqueous and chloroform extracts. The leaf extracts exerts a differential response against the oxidative stress-induced apoptosis in different types of cells.

NUCLEAR CHANGES ASSOCIATED WITH APOPTOSIS

Apoptosis is a form of cell death that allows for the elimination of damaged or unwanted cells without damage to the organism. The most obvious characteristics of this form of cell death are cytoplasmic and nuclear condensation, followed by internucleosomal DNA cleavage, membrane blebbing, and finally cell fragmentation (Fritzer-Szekeres *et al.*, 2002).

The results revealed that, in all the cell types studied, oxidative stress (imposed by H_2O_2 or etoposide) caused an increased number of cells to commit to apoptosis. *Zea mays* leaf extract administration showed no cytotoxicity in the normal cells (chick embryo cells and yeast cells), but significant cytotoxicity towards Hep2 cells. Among the three extracts, the methanolic extract exhibited maximum protective effect in untransformed

cells and cytotoxic effect in cancer cells. The nuclear changes associated with apoptosis were quantified by PI, EtBr, DAPI and AO/EtBr staining.

PROPIDIUM IODIDE STAINING

The use of a fluorochrome, such as PI, that is capable of binding and labeling DNA makes it possible to obtain a rapid and precise evaluation of nuclear changes. Several studies have made use of this property.

Eupatilin, an extract from *Artemisia asiatica Nakai* dose-dependently inhibited H_2O_2-induced apoptosis as indicated by staining with annexin V and propidium iodide in human gastric (AGS) cells (Lee *et al.*, 2008). The ether extract of *Cremanthodium humile* induced apoptosis in HeLa cells as investigated by flow cytometry assays by PI (Li *et al.*, 2007a).

In HepG2 (hepatocellular carcinoma) cells, PI staining displayed the apoptotic cells when treated with *Trichosanthes kirilowii* tuber extract (Shin *et al.*, 2008). Pomegranate extract prepared from skin and arils minus seeds induces apoptosis in human prostate cancer cells by the modulation of the IGF–IGFBP axis (Koyama *et al.*, 2010).

Rhodiola imbricate aqueous extract decreased the cell viability of human erythroleukemic cell line K-562 by inducing apoptosis as determined by annexin V-FITC and propidium iodide staining (Mishra *et al.*, 2007). Wang *et al.*, (2007) demonstrated that emodin, a major constituent of rhubarb inhibited the proliferation of HK-2 cells. It exhibited its antiproliferation action in a dose- and time-dependent manner by the induction of apoptosis as studied by the annexin V/propidium iodide assay. Similarly, Cai *et al.* (2008) have examined the cytotoxic effect of emodin against human pancreatic adenocarcinoma cell lines : Mia Paca-2, BxPC-3, Panc-1, and L3. 6pl. WST-1.

Cytosine arabinoside (Ara-C) induced apoptosis in neuronal SH-SY5Y cells and it was best revealed by the propidium iodide–digitonin assay, which is based on the cell membrane integrity (Puttonen *et al.*, 2007). The methanolic and methane dichloride extract of the aerial parts of *Larrea divaricata* Lay (Jarilla) induced apoptosis in MCF-7 cell line as assessed by PI staining (Bongiovanni *et al.* 2008).

The effect of an ethanolic extract of *Tremella mesenterica* on the induction of apoptosis in human lung carcinoma A549 epithelial cells was also assessed by PI staining (Chen *et al.*, 2008). Increased apoptosis was seen in Hep G2 cells treated with genistein (Choden *et al.*, 2007). A similar effect was seen in human umbilical vein endothelial (HUVE-12) cells treated with genistein derivative, cytosine arabinoside Ara C, as determined by PI staining (Fu *et al.*, 2008).

Kapiszewska *et al.* (2007) have reported that the damaging activity of etoposide was reverted by quercetin in neutrophils as determined by FACScan flow cytometry using annexin/PI. Thabrew *et al.* (2005) have reported that the decoctions of traditional Sri Lankan medicines (*Nigella sativa* seeds, *Hemidesmus indicus* roots and *Smilax glabra* rhizomes) induced apoptosis in HepG2 (human liver cancer) cells, as measured by PI staining and annexin V binding. These reports supported our findings.

EtBr STAINING

EtBr has been used as a staining agent to quantify the number of cells showing nuclear changes in several studies. EtBr staining of Chinese hamster ovarian cells (CHO-K) showed that the extract of *Cochlospermum reguim* significantly reduced the cell proliferation and induced apoptosis (Ceschini and Campos, 2006).

Fluorescent staining of cultured peripheral blood mononuclear cells with EtBr showed the apoptotic morphology induced by cycloheximide (Baskic *et al.*, 2006). Hydrogen peroxide induced apoptosis or necrosis by ATP-dependent apoptosome formation in T-lymphoma Jurkat cells (Saito *et al.*, 2006). Two phytochemicals, resveratrol and citroflavon-3-ol, and four plant extracts (grape seed polyphenols, olive oil extract, bearberry and *Echinacea*) examined for their effect on the viability by the EtBr assay provided evidence for strong protection against oxidative stress in H_2O_2 stress-induced U937 cells (Carpenter *et al.*, 2006).

EtBr stained cells in the present study showed that the methanolic extract of *Zea mays* leaves inhibited apoptosis in primary chick embryo fibroblasts and *Saccharomyces cerevisiae* effectively than the aqueous and chloroform extracts, and in contrast, did not influence Hep2 cells, which reiterates its anti-apoptotic property against normal cells and its anticancer property.

DAPI STAINING

DAPI staining is used to observe the nuclear contents in shrunken cells. It has also been used as an index of apoptosis in viral toxin mediated apoptotic death in wild-type yeast cells (Reiter *et al.*, 2005). DAPI has been used to study pamidronate, anti-proliferative, apoptotic and anti-migratory effects in hepatocellular carcinoma cells (Wada *et al.*, 2006).

Chen *et al.*, (2007) have reported that valproic acid (drug) exposed to microglial cells showed typical apoptotic hallmarks including DNA fragmentation and chromatin condensation followed by TUNEL assays (red) and DAPI staining (blue). Ophiopogonin-D, one of the most bioactive components of *Radix Ophiopogon japonicus* prevented H_2O_2-induced injury in primary human umbilical vein endothelial cells (Qian *et al.*, 2010).

Rutin, an active flavonoid, rendered protective effects against apoptosis of human umbilical vein endothelial cells (HUVECs) induced by hydrogen peroxide (H_2O_2) as determined by DAPI staining (Gong *et al.*, 2010). These reports followed a similar trend of our study, where the *Zea mays* leaf extracts protected the primary chick embryo fibroblasts from H_2O_2-induced damage.

Human breast cancer cells treated with the extracts of *Astrodaucus persicus* also showed potential decrease in the cell proliferation by staining with DAPI (Abdolmohammadi *et al.*, 2008). Zoledronic acid, a third-generation nitrogen-containing bisphosphonate significantly slowed the line-1 tumor (in a murine lung adenocarcinoma cell line) growth with no signs of apoptosis as detected by annexin V/PI and DAPI staining (Li *et al.*, 2007b).

The cytotoxic effects of *Cochlospermum regium* aqueous root extract to immortal, non-tumorigenic mammalian cells *in vitro* were analyzed using DAPI staining (Ceschini and Campos, 2006). DAPI and propidium iodide permeability assays demonstrated that pigment epithelium-derived factor dose- and time-dependently induced apoptosis in THP-1 macrophages (Ho *et al.*, 2007).

The ethanolic extracts of *Euchresta formosana* radix showed apoptotic nuclear changes as determined by DAPI staining (Hsu *et al.*, 2007). Rajabalian (2008) observed

that anticancer drugs caused leukemia lines to undergo apoptosis using DAPI staining. SNU-668 (human gastric cancer) cells were exposed to *Coptis japonica* and the resulting apoptosis was assessed by staining with DAPI (Park *et al.*, 2005). These literature reports are in agreement with our results and support many aspects of our study.

AO/EtBr STAINING

AO is membrane permeable and marks the nuclei green, and EtBr, which binds to DNA, is mainly taken up by cells when membrane integrity is lost and stains the nuclei red. Since acridine orange intercalates in the DNA but only interacts with the RNA, and viable cells do not uptake ethidium bromide and these cells exhibit green nuclei. However, ethidium bromide is taken up by dying cells, which turn red (Giral *et al.*, 2007).

Treatment with a combination of acridine orange/ethidium bromide has been used as a reliable index of cellular degeneration (Campos-da-Paz *et al.*, 2008). Essential oil from aerial parts of *Ocimum viride* induced apoptosis in COLO 205 cells in dose- and time-dependent manner, as established by AO/EtBr staining (Sharma *et al.*, 2010).

Sanguinarine, a benzophenanthridine alkaloid derived from the root of *Sanguinaria canadensis*, induced apoptosis in human cancer cells, which was assessed by acridine orange/ethidium bromide staining (Han *et al.*, 2008). Rhodoxanthin, a carotenoid from *Potamogeton crispus* L., inhibited cell proliferation in HeLa cells and induced apoptosis in HeLa cells as shown by AO staining (Ren *et al.*, 2006). Analysis of the acridine orange/ethidium bromide staining revealed that the crude extract of *Solanum lyratum* induced apoptosis in a time- and dose-dependent manner in human colon cancer (Colo 205) cells (Hsu *et al.*, 2008). de Oliveira *et al.* (2007) have reported that laticifer proteins (LP) recovered from the latex of the medicinal plant *Calotropis procera* displayed considerable cytotoxicity to SF295 and MDA-MB-435 cells, but not to healthy peripheral blood mononuclear cells as determined by AO/ EtBr staining.

When HeLa cells were treated with diazene N-phenyl-2-(2-pyridinyl) diazenecarboxamide, apoptosis was established and the apoptotic morphology was viewed under microscope after AO/EtBr staining (Jakopec *et al.*, 2006). Analysis of the ethidium bromide and acridine orange staining revealed that *Lentinula edodes* reduced cell proliferation and induced apoptosis in a time- and dose-dependent manner in carcinoma cells (CH72) (Gu and Belury, 2005). Grifolin, a biologically active substance

from *Albatrellus confluens*, showed significant inhibition of tumor cell lines CNE1, HeLa, MCF-7, SW480, K562, Raji and B95-8 as detected by AO/EtBr (Ye *et al.*, 2005).

In the present study, AO/EtBr stained cells showed that the methanolic extract of *Zea mays* leaves induced apoptosis in Hep2 cells and not in primary chick embryo fibroblasts and *Saccharomyces cerevisiae* cells. Moreover, it protected the normal cells from oxidative stress, while no such protection was noted in the cancer cells.

EFFECT OF THE METHANOLIC EXTRACT OF *Zea mays* LEAVES ON THE OXIDATIVE STRESS INDUCED IN *Drosophila melanogaster*

The results obtained in the *in vitro* studies showed that *Zea mays* leaves possessed both antioxidant and anticancer activities. The plant extract may or may not render the same effect under *in vivo* conditions due to several factors. Hence, it was felt imperative to conduct *in vivo* studies, which was carried out using *Drosophila melanogaster.* Oxidative stress was induced in the flies by the administration of H_2O_2 (direct oxidant) and CCl_4 (which needs metabolic activation).

The activities of the enzymic antioxidants, superoxide dismutase, catalase and peroxidase were analyzed. These enzymic activities were found to decrease upon CCl_4 / H_2O_2 administration in both the male and female flies. Co-administration of the methanolic extract of *Zea mays* leaves significantly increased the activities of these enzymic antioxidants.

The level of non-enzymic antioxidants, vitamins C, E and reduced glutathione were also analyzed. There was a significant decrease in the level of these non-enzymic antioxidants in the flies subjected to oxidative stress induced by H_2O_2/CCl_4 at both the concentrations. The extent of decrease in the levels was related to the concentration of the oxidant used. When the methanolic extract of *Zea mays* was co-treated, there was a significant increase in the levels of non-enzymic antioxidants. There are several reports, which support these results.

Bacopa monnieri, Linn. (Brahmi) offered complete protection against rotenone induced oxidative stress and elevated the activities of enzymic antioxidants and GSH levels in Drosophila adult male flies (Hosamani and Muralidhara, 2009). This report is in

agreement with our results that the methanolic extract of *Zea mays* leaves increased the activities and levels of antioxidants and nullified the toxic effects of the oxidants.

Acai pulp has been reported to improve the survival of flies fed with a high fat diet through the activation of stress response pathways in *Drosophila melanogaster* (Sun *et al.*, 2010). Grape seed proanthocyanidins suppressed the DNA damage induced by doxorubicin in a dose-dependent manner in somatic cells of *Drosophila melanogaster* (de Rezende *et al.*, 2009).

Salvia officinalis (sage) showed antimutagenic effect against methyl methanesulphonate in Drosophila, which was attributed to its antioxidant activity and suppression of metabolic activation (Patenkovic *et al.*, 2009). Paraquat and H_2O_2 challenge tests demonstrated that black tea extract prolonged the survival time only for Oregon-R wild type flies but not for SOD^{n108} or Cat^{n1} mutants (Peng *et al.*, 2009).

The antioxidant vitamins (vitamins A, C, and E) lowered the aflatoxin-mediated gene mutation in Drosophila. This mitigation is based on the scavenging/trapping by antioxidant vitamins of DNA-reactive products (metabolites and radicals) emanating from aflatoxin metabolism (Khan and Sinha, 2008).

Cocoa exhibited a strong antioxidant activity and significantly increased the average life span in Drosophila under different oxidative stress conditions (Bahadorani and Hilliker, 2008). The SOD activity decreased markedly in the liver and kidney homogenates of the rats administered with CCl_4 (Shi *et al.*, 2006). The authors suggested that the mistletoe alkali could be a potential herbal medicine for improving SOD activity in liver and kidney, as their scavenging activity steadily increased with the increase of drug concentration.

A significant decrease in the activity of the enzymic antioxidants (SOD and CAT) was noted after a single administration of paracetamol. Upon administration of chloroform and methanolic extract of *Ichnocarpus frutescens* the activities of enzymic antioxidants were significantly reversed to near normal levels (Dash *et al.*, 2007).

The co-administration of ursolic acid increased the activity of SOD, CAT and GPx and enhanced the antioxidant capacity in the heart of rats chronically administered with ethanol (Saravanan and Pugalendi, 2006). Liu *et al.* (2006b) reported that the

methanolic extracts of *Ginkgo biloba* significantly increased the GPx activity upon CCl_4 intoxication in the liver and plasma of rats.

The oral administration of rutin improved the antioxidant status of streptozotocin-induced diabetic rats by increasing enzymic (SOD and CAT) and non-enzymic (GSH, vitamins C and E) antioxidants (Kamalakannan and Prince, 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_4 exposure (Raja *et al.*, 2007).

Adult *Drosophila melanogaster* of Oryon treated with different concentrations of $MgCl_2$ showed significant increase in the activities of SOD and CAT, as well as in the concentration of GSH (Matkovics *et al.*, 1997). 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.

Thus, the present study has confirmed the antioxidant potential of the *Zea mays* leaf extracts under conditions of oxidative stress in *Drosophila melanogaster*.

PHASE IV

The phytochemical screening revealed the presence of phenolics and flavonoids. To confirm the chemical nature of the active components present in *Zea mays* leaves, spectral analyses (UV, HPLC, HPTLC, IR and GC-MS) were carried out, which identified, polyphenolic compounds as the major components.

Polyphenolic compounds are widely distributed in plants and are known to be excellent antioxidants *in vitro*. They have the capacity to reduce free-radical formation by scavenging free radicals and enhancing antioxidant defences (Hashim *et al.*, 2005). Polyphenolic compounds also possess antimutagenic property (Teel and Castonguay, 1992).

The antitumor activity of polyphenolic compounds can be related to the immunomodulatory properties of the compounds, their cytotoxicity to tumor cells, and their capacity to induce apoptosis and necrosis (Orsolic *et al.*, 2004). Silva *et al.* (2008b)

suggested the importance of polyphenols as protectors of oxidative stress-induced DNA damage that commonly occurs in several pathological conditions.

In the present study also, polyphenols were identified as the major active components, which are probably responsible for the protective effects rendered by the *Zea mays* leaf extracts. Thus, the outcome of the present study highlights the protective effects rendered by *Zea mays* leaves under oxidatively stressed conditions. The present study also strengthens the candidature of the *Zea mays* 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.