DISCUSSION

5. DISCUSSION

The generation of free radicals has been implicated in the causation of several diseases of known and unknown etiologies such as rheumatoid arthritis, cancer, chronic degenerative conditions, some forms of anemia, autoimmune diseases and the entire comorbidity of uremia and diabetes (Galli *et al.*, 2005; Lee and Lee, 2006). Compounds that can scavenge free radicals have great potential in ameliorating these disease processes (Narendrakannan *et al.*, 2005).

An increase in the intracellular concentration of ROS is tightly regulated by multiple defense mechanisms involving ROS scavenging enzymes and small antioxidant molecules (Zatorska *et al.*, 2003). One approach to control the level of ROS is to use dietary antioxidants that can scavenge ROS (Haenold *et al.*, 2005). Among the most important constituents of the edible plant products, low molecular weight antioxidants are emerging the most predominant ones (Khopde *et al.*, 2001). The additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their potent antioxidant and anticancer activities (Liu, 2003). Thus, there is a global effort to search for widely available and rich sources of antioxidants and to validate their use.

Therefore, the present investigation aimed to evaluate the antioxidant and anticancer effects of two varieties of *Solanum nigrum* (L) leaves, BBL and RBL, which are used as leafy vegetables. The results obtained are discussed in this chapter with reference to the relevant published literature.

PHASE I

Naturally occurring antioxidants in leafy vegetables and seeds such as ascorbic acid, α-tocopherol and phenolic compounds possess the ability to reduce the oxidative damage associated with many diseases (Lee *et al.*, 2000; Middleton *et al.*, 2000). The antioxidative protection also includes β-carotene as well as selenium and zinc (Halliwell and Gutteridge, 1999).

The formation of ROS is prevented by an antioxidant system comprising of low molecular weight antioxidants and enzymes, which include those regenerating the reduced forms of antioxidants and ROS interacting enzymes such as SOD, peroxidases and catalase (Blokhina *et al.*, 2003; Zacchini and Agazio, 2004).

This research work was initially started by analyzing the antioxidant contents of the *Solanum nigrum* leaves, BBL (leaves of plants bearing black barriers) and RBL (leaves of the plants bearing red berries). The antioxidant status was assessed by evaluating enzymic, non-enzymic, and mineral antioxidants. The results indicated that BBL and RBL extracts exhibited differential antioxidant profile.

BBL showed significantly greater activities of SOD, CAT and PPO. It was also found to be a rich source of total tocopherols, total carotenoids, total phenols, chlorophylls and copper. RBL showed higher activities of glutathione dependent enzymes and glucose 6-phosphate dehydrogenase. The GSH content was also more in RBL. There was no significant change in AAO activity, and the levels of ascorbate and the minerals Zn, Mn and Se.

Plant extracts and plant derived antioxidants can elicit a number of *in vivo* effects such as promotion of increased synthesis of endogenous antioxidant defenses or themselves acting directly as antioxidants (Halliwell, 1990; Aruoma, 1999). It is also reported that the composition of antioxidants varies widely with several factors like the stage of maturity, variety, climatic conditions, part of the plant analysed, post-harvest handling, processing, storage etc, which influence the composition of antioxidants in plants (Redriguez-Amaya, 2003).

Wang *et al.* (2003a) have shown that there were significant differences in the plant polyphenolics among varieties of *Cynara scolymus* (Artichoke). Both enzymic and non-enzymic antioxidants exhibited wide variations in different species of Piper (Karthikeyan and Rani, 2003). In another study, five Sicilian varieties of tomato fruits were compared, which showed significant differences in

the activity of PPO (Spagna *et al.*, 2005). Neill *et al.*, (2002) showed a differential activity in SOD and CAT between red and green leaves of *Elastostema rusoum*.

These studies are in agreement with our results, wherein the two varieties of *Solanum nigrum* leaves (BBL and RBL) showed different levels of antioxidants. It has been demonstrated that plants over-expressing Fe-SOD exhibit enhanced tolerance to oxidative stress (Van Camp *et al.*, 1996). Our results show that both BBL and RBL are rich sources of not only SOD, but also the other enzymic as well as non-enzymic antioxidants. This perceivably will result in rendering a high protection against oxidative damage.

Among the enzymic antioxidants studied, higher activities of SOD, CAT and PPO were observed in BBL than RBL, while Px, GPx, GR and G6PD were higher in RBL ($p<0.05$). Analysis of these results suggests that RBL exhibits higher activities of the enzymes that are involved with glutathione metabolism in the cell.

This suggestion is further strengthened by the results obtained in the non-enzymic antioxidants, wherein higher levels of reduced glutathione were observed in RBL than BBL. Thus, the results of the phase I of this study threw open the exciting possibility that the two varieties of *Solanum nigrum* leaves acted via different biochemical mechanisms. They also iterated the antioxidant potential of the leaves, which possessed considerably high levels of both enzymic and non-enzymic antioxidants.

PHASE II

Free radicals arising from either the normal metabolism or induced by environmental sources interact continuously in the biological systems (Packer, *et al.*, 1997). Reactive oxygen species (ROS) such as superoxide ($SO^{\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$) are formed in the course of cellular metabolism (Fridovich, 1995). These moieties are also called as oxidants. These ROS together with unstable intermediates lead to the peroxidation of lipids, which is a well known inducer of cellular and tissue pathogenesis (Witzturm, 1993). Thus, the ultimate targets of the oxidants are lipids, proteins and DNA molecules.

Any strong antioxidant is thus, expected to react with oxidants and neutralize or scavenge them, making them harmless. The pharmacological effects of medicinal plants are reported to be related to their free radical scavenging properties.

Therefore, in this phase of the study, the effect of the crude aqueous extracts of *Solanum nigrum* leaves on various radical and non-radical oxidants, as well as oxidant generating systems were studied *in vitro.*

RADICAL SCAVENGING ACTIVITY OF *Solanum nigrum* LEAVES

The radical scavenging activity of *Solanum nigrum* leaf extracts was analaysed against a battery of oxidants namely DPPH, $SO^{\bullet}$, H_2O_2, $^{\bullet}OH$ and NO. Crude aqueous extracts of *Solanum nigrum* leaves (BBL and RBL) elicited an effective scavenging activity on DPPH radicals and H_2O_2. Both the extracts also significantly inhibited superoxide radical and hydroxyl radical generation. However, both the leaves did not influence the extent of generation of nitric oxide. Comparatively, BBL exerted a better inhibitory activity on free radicals and oxidants than the RBL. These observations prove that both BBL and RBL are good sources of antioxidants.

The reported scientific literature is rich in studies that have shown the scavenging activity of the aqueous and other extracts of plants and plant products against several radical and non-radical oxidants. The aqueous extract of the whole plant *Coronopus didymus* Linn effectively scavenged DPPH (Mantena *et al.*, 2005). A hot water extract of Japanese rice bran (Okai and Higashi-Okai, 2006) showed scavenging activity against DPPH. In another study, among the different fractions of germinated fenugreek seeds, the aqueous fraction exhibited the highest activity against DPPH radicals (Dixit *et al.*, 2005).

An aqueous acetone extract of *Gynura formosana* kiamnna compositai exhibited an effective scavenging of DPPH (Hou *et al.*, 2004). Methanolic and aqueous extracts of the leaves and fruits of *Phyllanthus niruri* (Harish and

Shivanandappa, 2006) and *Cassia fistula* (Ilavarasan *et al.*, 2005) exhibited significant antioxidant activity on DPPH radical.

Banskota *et al.* (2003) reported that water, water/methanol and methanolic extracts of the wood of *Taxus yunnanensis* exhibited DPPH radical scavenging activity. The water, methanol and n-butanol fractions of *Eriobotrya japonica* seeds exerted high levels of scavenging activity on DPPH radical (Yokota *et al.*, 2006). In another study, the methanol, methanol/water extracts, infusions and decoction of *Cymbopogon citrates* exerted considerable scavenging activity on DPPH radical (Cheel *et al.*, 2005).

In vitro experiments showed that the extract of both leaves and roots of the celery *Apium graveolens* were good scavengers of DPPH (Popovic *et al.*, 2006). The seed of *Trichopus zeylanicus* effectively scavenged DPPH radical (Tharakan *et al.*, 2005).

Scavenging of DPPH radical has been used as a routine test for establishing the antioxidant properties of plant extracts (Atawodi, 2005) and their components (Summa *et al.*, 2005). However DPPH is not a natural intermediate in the biological system. In the biological system, $SO^{\bullet}$, H_2O_2, $^{\bullet}OH$ and NO gain more importance as oxidants, as they are products formed during normal metabolism.

In the present study, the effect of BBL and RBL have been analysed on all these oxidants apart from DPPH. In tune with the analyses, several reports are available in the literature. BBL and RBL exhibited an effective scavenging activity on superoxide generation. BBL was found to have a better scavenging activity than RBL. The generation of superoxide ($SO^{\bullet}$) radical has been shown to be inhibited by the crude water extracts of *Piper nigrum* (Gulcin, 2005) and *Tabernaemontana caronaria* flowers (Priya *et al.*, 2006).

Pilarski *et al.* (2005) observed that among the aqueous and ethanolic extracts of *Uncaria tomentosa* bark, the ethanolic extracts had a higher antioxidant activity against $SO^{\bullet}$ radical, which was attributed to their higher total phenolic content.

However, in another study, among various solvent extracts of *Morus bombycis* koidzumi, the aqueous extract exhibited higher superoxide scavenging effect (Jin *et al.*, 2005a).

A crude hydroalcoholic extract, and butanolic and ethyl acetate fractions of *Cuphea carthagenensis* rendered effective scavenging of $SO^{\bullet}$ radical (Schuldt *et al.*, 2004). Potent $SO^{\bullet}$ inhibitory activity was also observed in the water as well as polar extracts of Korean herbal medicines (Kang *et al.*, 2003). Herbal medicinal preparations, like Triphala (an ayurvedic Rasayana drug) have also been reported to possess $SO^{\bullet}$ scavenging effect (Jagetia *et al.*, 2004).

In the light of the above reports, the ability of BBL and RBL extracts to scavenge $SO^{\bullet}$ radical gains more importance. These extracts also scavenged H_2O_2 to a significant extent. Adequate support is available in the scientific literature that H_2O_2 scavenging property is associated with strong antioxidant and medicinal properties.

Hydrogen peroxide (H_2O_2) is an ROS that can react with various cellular targets, thereby causing cellular damage or cell death (Bienert *et al.*, 2006). BBL and RBL extracts exhibited a marked scavenging effect of H_2O_2. Among the two extracts, BBL was found to exhibit higher scavenging activity on H_2O_2.

The water and ethanol extracts of jumper (*Juniperus communis* L) fruits showed effective H_2O_2 scavenging activity (Elmastas *et al.*, 2006). Crude water and ethanolic extracts of black pepper (*Piper nigrum)* exhibited a strong scavenging activity on H_2O_2 (Gulcin, 2005). Ethanolic extract of *Punica granatum* flowers showed an effective scavenging activity on H_2O_2 molecule (Kaur *et al.*, 2006). A methanolic exract of *Mucuna pruriens* seeds significantly scavenged H_2O_2 in a dose dependent manner (Rajeswar *et al.*, 2005a).

The extent of H_2O_2 scavenging was reported to depend on the position of the hydroxyl groups of phenolic acids (Dinis *et al.*, 1994). The effective scavenging of H_2O_2 by the BBL and RBL extracts, might be due to the action of enzymic

antioxidants like catalase and peroxidases and other non-enzymic antioxidants. Comparatively BBL showed a better scavenging activity than RBL.

Another major free radical in the aerobic cells is the hydroxyl radical.It is an extremely reactive species that readily oxidises all cellular macromolecules including proteins, sugars, lipids and DNA (Bhupinder *et al.*, 2004).

When BBL and RBL crude aqueous extracts were tested on an *in vitro* $^{\bullet}OH$ radical generating system, it was found that both the leaves significantly scavenged the radical, with BBL showing a better effect than RBL.

The ability of plant extracts and components to scavenge $^{\bullet}OH$ radicals is an important reflection of their antioxidant potential as evidenced by several studies. The water extract of some Korean herbal medicines showed potent inhibitory effect on hydroxyl radical (Kang *et al.*, 2003). The crude hydroalcoholic extract of *Cuphea carthagenesis* leaves showed an effective scavenging of $^{\bullet}OH$ radical (Schuldt *et al.*, 2004).

The aqueous extract and crude polymeric fraction of fermented *Aspalthus linearis* rooibas displayed $^{\bullet}OH$ radical scavenging activity (Jourbert *et al.*, 2005). Popovic *et al.* (2006) observed that the extracts of both celery of *Apium graveolens* leaves and roots in ether, chloroform, ethyl acetate, n-butanol and water exhibited good hydroxyl scavenging activity. The aqueous extracts of *Tabernaemontana coronaria* flower possessed significant hydroxyl radical scavenging activity (Priya *et al.*, 2006).

Hsieh and Yen (2000) reported that the water extract of leaves of *Du zhong* markedly inhibited the oxidation of deoxyribose induced by Fe^{+3}-EDTA / H_2O_2 / ascorbic acid. Similarly the water extracts of *Cassia tora* (Yen and Chung, 1999) and *Tinospora cordifolia* (Goel *et al.*, 2002) were found to inhibit the oxidation of deoxyribose by the same reaction.

Not only the plant extracts but herbal medicinal preparations also exert scavenging effect on $^{\bullet}OH$ radicals, as demonstrated for Triphala (Jagetia *et al.*,

2004), Dianex (Mutalik *et al.*, 2005) and a drug mixture containing *Equiseti herba, Myrtilli folium,Phaseoli fructus sine seminibus* and *Urticae-folium* (Then *et al.*, 2005).

The ability of BBL and RBL extracts to scavenge hydroxyl radicals, thus, gains significance in strengthening their antioxidant potential.

Nitric oxide (NO), a simple free radical, elicits a surprisingly wide range of physiological and pathophysiological effects. NO interacts with soluble guanylate cyclase to evoke many of these effects, however, NO can also interact with molecular oxygen and superoxide radicals to produce reactive nitrogen species, that can modify a number of macromolecules including proteins, lipid and nucleic acids. NO can also interact directly with transition metals (Davis *et al.*, 2001). BBL and RBL extracts did not elicit a profound effect on the *in vitro* generation of NO at the dose level tested.

Solanum nigrum has been reported to increase the production of NO by γIFN-(gamma) primed mouse peritoneal macrophages, and NF – (Kappa) plays a critical role in mediating these effects (An *et al.*, 2005). However, in the present study, the leaves of *Solanum nigrum* failed to evoke an inhibitory response against the *in vitro* generation of NO this discrepancy can be attributed to two reasons. On the one hand, it is possible that an influence on NO metabolism can be observed only under *in vivo* conditions, which fails to show up in the *in vitro* system. On the other hand, it is also possible that higher doses of the leaf extracts are needed to elicit a response on the *in vitro* NO generation system.

The literature cited above indicates that the extracts of various medicinal plants and plant products were found to exhibit differential effect against the various free- and non-radical oxidants. The results obtained for the cell-free systems in the present study demonstrated that BBL and RBL extracts were effective scavengers and inhibitors of a battery of oxidants falling into both radical and non-radical categories. These effects reflect the strong antioxidant potential lying unexplored and under exploited in these leaves.When these scavenging

effects of the leaf extracts are considered in light of the results obtained in the first phase of the study, wherein the leaves were shown to be rich sources of both enzymic and non-enzymic antioxidants, it can be perceived that the radical-scavenging effects of the leaf extracts are due to the presence of the antioxidants.

EFFECTS OF *Solanum nigrum* LEAVES ON OXIDATIVE DAMAGE INDUCED IN BIOMOLECULES

The primary targets of oxidants are the membrane lipids, as the membrane is the macromolecular assembly that immediately comes in contact with the generated oxidants. The ultimate targets however, are the DNA molecules, the damage of which results in mutations, which can manifest themselves into various diseases, including cancer.

It was, therefore, felt imperative to study the effect of crude aqueous extracts of BBL and RBL on oxidative damage caused to lipids and DNA. Oxidative damage to lipids results in peroxidation reactions and damage to DNA results in strand breaks and mutations (Imlay and Lin, 1988). The extents of the damage were measured following oxidative stress in the presence and absence of leaf extracts.

EFFECTS OF *Solanum nigrum* LEAVES ON LIPID PEROXIDATION

Both the leaves, BBL and RBL, inhibited the process of lipid peroxidation in goat liver homogenate. Comparatively, BBL exhibited better inhibitory activity on LPO. These results suggest the protective effect of the leaf extracts on lipid molecules. Several studies are supportive of our results.

A water extract of *Selaginella involvens* showed inhibition of lipid peroxidation in rat liver homogenate (Gayathri *et al.*, 2005). An aqueous extract of *Scoparia dulcis* showed a marked antioxidant activity *in vitro,* which was assayed by measuring the TBARS in fowl egg yolk (Ratnasooriya *et al.*, 2005).

Among the different fractions of germinated fenugreek seeds, the aqueous fraction exhibited the highest inhibitory effect on lipid peroxidation in mitochondrial preparation from the rat liver (Dixit *et al.*, 2005). The aqueous and methanolic extracts of leaves and fruits of *Phyllanthus niruri* exhibited inhibition of membrane lipid peroxidation (Harish and Shivanandappa, 2006).

Tilak *et al.* (2004) reported that the aqueous and ethanol extracts of two major preparations of turmeric effectively inhibited membrane lipid peroxidation. The aqueous and ethanol extracts of *Tabernaemonatana coronaria* flowers possessed significant inhibitory activity on lipid peroxidation (Priya *et al.*, 2006). An effective inhibitory activity was exerted by the methanol extract of *Secamone afzelii* stems on lipid peroxidation in liposomes (Mensah *et al.*, 2004). The methanolic and alcoholic extracts of *Smilax china* root exhibited a marked inhibitory activity on lipid peroxication (Lee *et al.*, 2001; Tripathi *et al.*, 2001). Alcoholic fraction of the rhizomes of *Podophyllum hexandrum* (Chawla *et al.*, 2005) significantly inhibited iron-ascorbate and $FeSO_4$ induced lipid peroxidation in mouse and rat liver homogenate.

Liposem (a polyherbal formulation) inhibited Fe^{+2}-ascorbate induced lipid peroxidation in rat liver homogenate (Mary *et al.*, 2002). The roasted barley yielded strong antioxidant activity against lipid peroxidation induced by CCl_4 in rat liver hepatocyte microsomes (Papetti *et al.*, 2006).

The bioflavonoids (rutin, catechin and naringin) showed a dose dependent inhibitory effect on lipid peroxidation (Russo *et al.*, 2000). A 43 KD protein from the leaves of the herb, *Cajanus indicus* effectively reduced the chloroform induced lipid peroxidation both *in vivo* and *in vitro* (Ghosh *et al.*, 2006).

The inhibitory activity of the crude aqueous extracts of *Solanum nigrum* on LPO of the liver homogenate suggests the direct antioxidant action of the BBL and RBL. These results pave the way for using these extracts to treat the ulcers where these extracts could be applied directly.

EFFECTS OF *Solanum nigrum* LEAVES ON OXIDATIVE DNA DAMAGE

Cells are constantly under threat from cytotoxic and mutagenic effects of DNA damaging agents. These agents can either be exogenous or formed within the cells (Norbury and Hickson, 2001). ROS induced DNA damage can be described both chemically and structurally and shows a characteristic pattern of modifications (Valko *et al.*, 2004).

Reactive oxygen species lead to oxidative damage of nucleobases and sugar components of nucleotides in double stranded DNA, which can result in DNA strand scission, mutagenesis and covalent cross-linking to DNA. If these lesions are not repaired, they can initiate a cascade of biological sequences and can also promote cancer development via several mechanisms (Bagchi *et al.*, 2000).

The forms of DNA damage produced by ROS experimentally include modifications of all bases, production of base-free sites, deletions, frame shifts, strand breaks, DNA-protein crosslinks and chromosomal rearrangements. An important reaction involved in DNA damage is the generation of hydroxyl radical through Fenton chemistry (Brezova *et al.*, 2003).

In the present investigation the effect of crude aqueous extracts of *Solanum nigrum* leaves on H_2O_2 - induced oxidative DNA damage was evaluated using purified DNA preparations as well as DNA within live cells. The purified DNA samples used were commercially purchased λ phage DNA, pUC 18 plasmid DNA and herring sperm DNA. The DNA were selected from different hierarchies of evolutionary development (viral, bacterial and animal) in order to check if the extent of DNA damage and /or its protection by the plant extract was influenced by the source of the DNA. Finally, the DNA damage was measured by single cell gel electrophoresis in KB oral carcinoma cell line.

In all these DNA sources, the oxidative damage induced by H_2O_2 was significantly decreased by the extracts of BBL and RBL. H_2O_2 treated phage DNA and pUC 18 plasmid DNA were fragmented completely and the treatment with the crude aqueous extracts of BBL and RBL exhibited a significant protective effect on

these DNAs. The TBARS level of oxidative stress induced herring sperm DNA was inhibited significantly. Additionally, a reduction in the number of comet bearing cells was observed in oxidative stress (H_2O_2) induced live cells (KB oral carcinoma cells) treated with BBL and RBL extracts. DNA damage is known to be one of the most sensitive biological markers for evaluating oxidative stress, representing the imbalance between free radical generation and the efficiencies of the antioxidant system (Donelly *et al.,* 1999).

Many studies have reported the protection against oxidative DNA damage by herbal extracts, formulations and their products. Crude aqueous extracts from *Cistus incanus* and *Cistus monspeliensis* showed a protective effect on DNA cleavage (Attaguile *et al.*, 2000). Some plant derived drugs also inhibited the damage induced in pUC 18 DNA (Okubo *et al.*, 2000).

Essiac tea (a tea prepared from a mixture of herbs) exhibited DNA protective effects by inhibiting hydroxyl radical induced DNA damage (Leonard *et al.*, 2006). Field inversion gel electrophoresis studies showed the protection offered by curcumin (turmeric extracts) against X-ray induced DNA damage in *E.coli* WPZS (lambda cells) (Pal and Pal, 2005).

A methanolic extract of *Nelumbo nucifera* inhibited H_2O_2 induced damage to pUC 18 DNA (Wang *et al.*, 2003b). An ethanolic extract of *Piper betel* prevented radiation–induced DNA strand breaks in pBR 322 plasmid DNA in a concentration–dependent manner (Bhattacharya *et al.*, 2005). The chloroform fraction of the rhizome of *Podophyllum hexandrum* exhibited maximum protection to plasmid (pBR 322) DNA in the plasmid relaxation assay (Chawla *et al.*, 2005).

Phenolic components of *Calcareous formosana* completely inhibited the UV-induced strand cleavage in ∅X-174 plasmid DNA (Wang *et al.*, 2004). A phenolic compound in ginger was able to suppress the oxidative damage in supercoiled PTZ 18U plasmid DNA (Ippoushi *et al.*, 2003). Hydroxychavicol, a major phenolic compound in *Piper betel,* inhibited pUC 18 plasmid DNA damage by hydroxyl radicals (Chang *et al.*, 2002).

The phenolics, genistein and resveratrol, were also effective in inhibiting the damage induced by H_2O_2 in ∅X-174 plasmid DNA (Win *et al.*, 2002). Pycnogenol significantly minimized the iron-ascorbate induced damage in pBR 322 plasmid DNA (Nelson *et al.*, 1998).

The plant extracts of *Solanum nigrum* and *Cichorium intybus* prevented the free radical mediated DNA sugar damage dose dependently in calf thymus DNA (Sultana *et al.*, 1995). The green shell cover extract of almond completely arrested peroxyl radical induced DNA scission in comparison with brown skin and whole seed extracts (Wijaeratne *et al.*, 2006). Among the enzymatic extracts (produced by the action of commercially available carbohydrate splitting enzymes and proteases) from seven species of brown seaweeds, two enzymatic extracts strongly inhibited DNA damage (Heo *et al.*, 2005).

An antioxidant, hydrooxylated-4-thiaflavans, showed protection of oxidative DNA damage in herring sperm DNA induced by cumene hydroperoxide (Lodovici, 2006). Methanolic extracts of rice hulls showed a protective effect against oxidative DNA damage induced by H_2O_2 (Jeon *et al.*, 2003). Schaefer *et al.* (2005) explained that the polyphenolic extracts of apples of different origins (Cidar and table apples) modulated DNA damage. The bioflavonoids like rutin, catechin and naringin showed a protective effect on DNA cleavage (Russo *et al.*, 2000).

Complex polyphenols and tannin extracts from red wine, with or without small molecular weight phenols prevented oxidative DNA damage (Lodovici *et al.*, 2001). Ascorbic acid rendered protection against DNA double strand breaks in a dose-dependent manner (Yoshikawa *et al.*, 2006).

In our study, the crude aqueous extracts of *Solanum nigrum* leaves offered a significant protection against oxidative DNA damage in purified DNA samples treated under physiological conditions. The leaves of *Solanum nigrum* are also rich sources of both enzymic and non-enzymic antioxidants, as demonstrated in phase I of this study. Thus, the DNA protective effect observed can be attributed to the

antioxidants present in the leaves. Following this, the oxidative DNA damage was observed in intact cells using comet assay.

The comet assay or single cell gel (SCG) test is a microgel electrophoresis technique that measures DNA damage at the level of single cells (Speit and Hartmann, 2005). This assay detects single-strand breaks and alkali labile sites in DNA, and DNA degradation due to necrosis and apoptosis (Frenzilli *et al.*, 2006). It is increasingly used in genotoxicity testing of substances such as industrial chemicals, biocides, agrochemicals, food additives and pharmaceuticals (Brendler-Schwaab *et al.*, 2005).

In this dissertation, an increased number of comet-bearing KB cells were observed in the oxidant (H_2O_2) treated group, which was significantly reduced when co-treated with BBL and RBL extracts. Many studies have been reported in the literature, wherein plants, their parts and chemical components in them have been shown to protect against DNA damage as revealed by the comet assay. The water extracts of *Cassia tora* L. reduced the benzo (a) pyrene induced DNA damage in human cell line HepG2 in a dose-dependent manner in the comet assay (Wu *et al.*, 2001). Consumption of Kiwi fruits led to an increased resistance of DNA to oxidative damage induced *ex vivo* by H_2O_2 in isolated lymphocytes as measured by comet assay (Collins *et al.*, 2001). The extracts of red algae (*Grateloupia filicina*) inhibited H_2O_2 induced DNA damage in rat lymphocytes (Athukorala *et al.*, 2005).

The supplementation of green tea extract significantly decreased the DNA damage induced by Fe^{+2} treatment in Jurkat T-cell lines (Erba *et al.*, 1999). A North American ginseng extract effectively inhibited the non site-specific DNA strand breakage caused by Fenton agents (Kitts *et al.*, 2000).

Russo *et al.* (2001) showed that the methanolic extracts of *Celastrus paniculatus, Picrorhiza kurroa* and *Withania somnifera* protected the DNA cleavage induced by H_2O_2 in non-immortalized human fibroblasts. Basal levels of oxidative DNA damage upon treatment with quercetin and caffeic acid documented

protection in the comet assay (Szeto and Benzie, 2002). Baicolin, a flavonoid isolated from the root of *Scutellaria baicalensis,* decreased H_2O_2 induced comets in NIH3TC mouse fibroblasts (Chen *et al.*, 2003a).

Resveratrol was found to inhibit the oxidative DNA damage in mammalian cells challenged with H_2O_2 (De Salvia *et al.*, 2002). Vitamin C supplementation at high dose decreased the steady-state level of oxidative DNA damage in mononuclear blood cells of smokers (Moller *et al.*, 2004).

The results of the present investigation suggest the antioxidant and the protective effects of BBL and RBL extracts on Fe^{+2} and H_2O_2 induced oxidative lipid and DNA damage respectively. This activity could be due to the presence of the antioxidant molecules like phenolics, vitamin C, vitamin E, carotenoids and GSH in the leaf extracts. Thus, the results suggest that these extracts may be useful inhibitors of oxidative injury to biomolecules and in turn the cells.

INFLUENCE OF *Solanum nigrum* LEAVES ON THE ANTIOXIDANT STATUS OF GOAT LIVER SLICES SUBJECTED TO OXIDATIVE STRESS

The studies conducted on oxidant moieties and biomolecular preparations showed BBL and RBL of *Solanum nigrum* to be significantly protective against oxidative damage. All the said studies were conducted, however, on either purified compounds or on cell-free systems, with the exception of the assay of LPO and comet assay, which were conducted in liver homogenate and intact cells respectively.

The data derived from cell-free systems should be handled with caution when being extrapolated to living cells because, in the intact cellular environment, there are several factors that can influence the effects of plant extracts, both effecting and affecting their biological actions. Thus, it becomes imperative that the extracts be studied for their antioxidant effects in the environment of an organ and an organism to make clear the interplay of their components in the living system.

The ultimate aim of evaluating the medicinal properties of any preparation, including plant extracts, is to validate its use on human diseases and disorders, or for general health and wellbeing. Experimental animals are highly reflective of the reactions of medicinal preparations in humans, and are therefore used widely in life science research. However, the recent awakening to the ethical aspects of the use of animals has necessiated the search for viable, reliable and reproducible alternative experimental systems (Hartung and Goldberg, 2006).

In tune with this, in the present dissertation, the antioxidative responses evoked by the leaf extracts of *Solanum nigrum* werestudied *in vitro* using goat liver slices, which were maintained in conditions that simulated the liver *in vivo.* The slices were treated with leaf extracts in the presence and absence of the oxidative stress, and the enzymic and non-enzymic antioxidants were determined. LPO was also measured as a reflection of the extent of oxidative stress.

INFLUENCE OF *Solanum nigrum* LEAVES ON ENZYMIC ANTIOXIDANTS IN GOAT LIVER SLICES EXPOSED TO CCl_4 INDUCED OXIDATIVE STRESS

The enzymic antioxidants, SOD, CAT, GPx, GST, GR and G6PD were assayed in liver slices exposed to CCl_4 and/or *Solanum nigrum* leaf extracts.

SUPEROXIDE DISMUTASE (SOD)

Superoxide radicals are generated as by-products of metabolic oxidation and SOD has evolved to inactivate superoxide (Itoh *et al.*, 2005). A reduced activity of this enzyme may indicate a reduction in the cellular efficacy to detoxify the potentially toxic oxy-radicals, which will lead to an increase in the levels of lipid peroxidation (Fridovich, 1983). The activity of SOD dropped significantly in oxidatively stressed liver slices. This reduction was efficiently counteracted by the leaf extracts, with BBL showing a better effect than RBL.

In the present investigation, the reduction in SOD activity in the liver slices, is suggestive of the increased oxidative stress. Additionally, the significant increase

in the activity by the leaf extracts implies that they can effectively alleviate the oxidative stress.

Bhattacharyya *et al.* (2003) reported that Himoliv, a polyherbal formulation, reversed the increase in SOD activity due to CCl_4 or paracetamol reaction in rats. Satturwar *et al.* (2003) reported that the oral administration of *Haridradi ghrita* (a ghee based polyherbal formulation) enhanced the decreased activity of SOD during CCl_4 exposure in rats.

The rice flavonone imparts a protective effect on hepatic injury due to the increased activity of SOD and GPx and scavenging the free radicals produced in CCl_4 intoxication (Xu *et al.*, 2005). Administration of ferulic acid (a naturally occurring phenolic compound) improved the depletion of SOD activity during CCl_4 induced toxicity (Srinivasan *et al.*, 2005).

Increased activity of SOD was observed in S-8300 (from shark liver) treated mice intoxicated with CCl_4 (Huang *et al.*, 2005). The aqueous suspension of bark extract of *Lawsonia alba* Lam enhanced the decreased activity of SOD during CCl_4 exposure in rats (Bhandarkar and Khan, 2003). Loki and Rajmohan (2003) have reported that tender coconut water retained almost normal level of SOD in the liver of CCl_4 treated rats.

Our results are also in good corroboration with the above cited reports indicating the effective antioxidant activity of BBL and RBL extracts.

CATALASE (CAT)

CAT is a heme protein, localized in the peroxisomes or microperoxisomes. This enzyme catalyzes the decomposition of H_2O_2 to water and oxygen, thus protecting the cell from oxidative damage by H_2O_2 and $^{\bullet}OH$ (Venukumar and Latha, 2002). CAT appears to be the most effective defense agent against high concentration of H_2O_2 (Panda and Kar, 1997).

A significant decrease in the activity of catalase was observed in CCl_4 treated liver slices. These toxic effects were reduced by the addition of *Solanum nigrum* leaves, i.e. the extracts of BBL and RBL; BBL extract exhibited a greater effect when compared to RBL extract.

Several studies reported in the literature support our finding that the leaf extracts increase CAT activity. Lee *et al.* (2001) observed that the V79-4 cells treated with methanolic extract of *Similax china* root induced the of superoxide dismutase, catalase and glutathione peroxidase activities in a dose-dependent manner.

Dibenzylbutyrolactone lignans of *Torreya nucifera* significantly preserved the activities of SOD, CAT, GPx and GR in the CCl_4 injured rat hepatocytes (Kim *et al.*, 2003a). Meera and Rana (2006) reported that the pretreatment with hydro alcoholic extracts of *Taraxacum officinale* roots improved the levels of SOD, CAT and peroxidases in rats intoxicated with CCl_4.

Maheswari and Rao (2005) showed that the co-administration of grapeseed oil improved the activity of SOD and CAT in CCl_4 treated rats. Ethanolic extracts of dried flowers of *Hibiscus sabdariffa* L. showed a significant increase in hepatic SOD and CAT in sodium - arsenite - induced oxidative stress (Usoh *et al.*, 2005). The catalese activity in rat liver decreased with CCl_4 treatment and upon treatment with *Strychnos potatorum* extracts, the CAT activity improved significantly (Sanmugapriya and Venkataraman, 2006).

Similar to these experimental evidences, BBL and RBL extracts also improved the activity of catalase in CCl_4 exposed liver slices. This observation, thus, implies that BBL and RBL can evoke a strong antioxidant response.

GLUTATIONE PEROXIDASE (GPx)

GPx activity was elevated in the liver, which may be due to the adaptive response to remove hydrogen peroxide (Anuradha and Balakrishnan, 1998). A significant reduction in the activity of GPx was observed during CCl_4 intoxication

in goat liver slices. This toxic effect was counteracted by the addition of extracts of BBL and RBL. Unlike the trend in SOD and CAT, in GPx RBL was found to be more effective than BBL.

The activity of SOD, though very vital in detoxifying superoxide, releases hydrogen peroxide, which is also a strong oxidative species. Thus, improvement in the activity of SOD, unaccompanied by an increase in H_2O_2 detoxifying enzymes like CAT and GPx will be meaningless (Ramasarma, 1990). Thus, the observation that BBL and RBL are effective in increasing the CAT and GPx activities, gaining significance, implying their ability to scavenge both $SO^{\bullet}$ and H_2O_2.

Another striking observation that can be made from our results is that while BBL is more effective on CAT activity, RBL showed a better effect on GPx activity. This differential response indicates that the biochemical mechanism of antioxidant response of the two leaf varieties is different.

The literature is rich with studies reporting an increase in GPx activity by plant extracts and products. *Solanum trilobatum* extract treatment caused a recovery of reduced levels of SOD, CAT and GPx in CCl_4 treated rats (Shahjahan *et al.*, 2004). Administration of *Lawsonia alba* extract significantly elevated the hepatic GPx activity during CCl_4 stress in rats (Bhandarkar and Khan, 2003).

The activities of SOD, CAT and GPx were significantly preserved in CCl_4 intoxicated hepatocytes when treated with the lignans of *Machillus thunberagii* (Yu *et al.*, 2000). Methanolic extracts of *Phyllanthus* significantly elevated the activity of hepatic glutathione peroxidase activity in CCl_4 intoxicated rats (Lee *et al.*, 2006).

Kim *et al.* (2003b) and Shahjahan *et al.* (2005) reported that *Artemisia apiacea* and *Indigofera oblongifolia* respectively elevated the activities of SOD, CAT and GPx during CCl_4 intoxication. Melatonin treatment reversed the decreased levels of hepatic SOD and GPx levels in CCl_4 intoxicated animals (Wang *et al.*, 2005).

All these reports add strength to the observations made in the present study, wherein the extracts of *Solanum nigrum* leaves exhibited an effective antioxidant activity, including the improvement of GPx activity.

GLUTATHIONE S-TRANSFERASE (GST)

Induction of Phase II enzymes is an effective and sufficient strategy for achieving protection against the toxic and neoplastic effects of many carcinogenesis (Talalay, 2000). The GSTs are believed to detoxify the endogenous substances that are formed as a consequence of oxidative stress including lipid hydroperoxides and hydroalkenols (Vandam and Vandewater, 2005).

A significant reduction in GST activity was observed in the liver slices when treated with CCl_4. The co-exposure of crude aqueous extracts of *Solanum nigrum* leaves caused an elevation in GST activity. RBL treated groups were found to exhibit a better GST activity than BBL.

Many herbs and herbal products, and medicinal plants were found to induce GST activity to counteract the oxidative stress. Sheweita *et al.* (2001) showed that among L-ascorbate, vitamin E and garlic, vitamin E is more effective in restoring the inhibition of hepatic GST activity caused by CCl_4.

Farombi (2000) showed that simultaneous administration of kolaviron, a biflavonoid fraction of an extract from *Garcinia kola* seeds with CCl_4, modulated the effect of CCl_4 on GST activity in rats. Koul *et al.* (1994) investigated the diterperes from *Andrographis paniculata* treatment and reported that they increased the activities of SOD, GPx and GST in CCl_4 intoxicated mice.

Singh *et al.* (1999) reported that the administration of ellagic acid recovered the activities of SOD, GPx and GST in CCl_4 treated rats. The activity of GST has been found to be decreased in cancerous conditions in experimental rats (Balasenthil and Nagini, 2000). *Spirulina fusiformis,* a blue green microalga, significantly enhanced the activities of SOD, CAT, GPx and GST in the liver of

experimental mice in retaliation to the genotoxicity induced by cisplatin and urethane (Premkumar *et al.*, 2004).

The results of the present study follow the same trend as reported by all these studies, reiterating the antioxidant protective effect of the *Solanum nigrum* leaf extracts.

GLUTATHIONE REDUCTASE (GR)

GR maintains the cell glutathione homeostasis and is responsible for keeping the TBARS levels under control (Kiranjit, 2003). Within the cell, GR ensures that a high ratio of GSH/GSSG is maintained. It replaces GSH from GSSG in an NADPH dependent reaction in the cell (Kanzok *et al.*, 2001; Bhuvaneswari *et al.*, 2002; Zaka *et al.*, 2002).

In the present part of the research work, the CCl_4 treatment significantly reduced the levels of GR and treatment with extracts of BBL and RBL counteracted the effect of CCl_4. RBL exhibited a better GR activity when compared to BBL.

Venukumar and Latha (2004) reported that the co-administration of methanol extract of *Coscinium fenestratum* stem powder significantly elevated the levels of SOD, CAT, GPx and GR in CCl_4 intoxicated rats. Oral administration of brahma rasayana significantly increased the liver antioxidant enzymes such as SOD, CAT, GPx, GST and GR against radiation induced oxidative stress in rats (Rekha *et al.*, 2001).

Rastogi *et al.* (2001) reported that the picroliv treatment significantly increased the activities of SOD, CAT, GST, GPx and GR in aflatoxin B, intoxicated rats. Ellagic acid, a naturally occurring polyphenol, promoted the reactivation of hepatic GR enzyme in CCl_4 treated rats (Singh, *et al.*, 1999).

Ilavarasan *et al.* (2003) reported that *Thespesia populnea* bark induced an increase in GR activity in CCl_4 intoxicated liver injury in rats. Rubiadin, a major constituent isolated from *Rubia cordifolia* Linn, significantly prevented the hepatic

injury by elevating the activities of GST and GR and scavenging the free radicals produced by CCl_4 exposure in animals (Rao *et al.*, 2006).

The results observed in the present study also show that *Solanum nigrum* leaves improve the GR activities, which in turn will maintain the GSH levels.

GLUCOSE 6-PHOSPHATE DEHYDROGENASE (G6PD)

G6PD, the first enzyme in HMP shunt, generates NADPH, which generates reduced glutathione from oxidized glutathione. The inhibition of G6PD causes decreased supply of reducing equivalents like NADPH for conversion of GSSG to GSH by glutathione reductase (Mayes, 1993).

The activity of G6PD was depleted significantly in CCl_4 treated goat liver slices. Co-administration of BBL and RBL extracts elevated the level of G6PD. Among the two extracts, RBL was found to evoke a higher G6PD activity than BBL.

Khan and Sultana (2005) reported that the oral treatment of rats with *Nymphaea alba* caused a significant recovery of GPx, GR, CAT, G6PD and GST in oxidative stress induced by ferric nitrilo-triacetate in renal system. Khan *et al.* (1997) have observed that curry leaf and mustard seeds enhanced the G6PD activity in rats fed with high fat diet.

In our investigation also we found that the supplementation of *Solanum nigrum* leaf extracts caused an improvement in G6PD activity which parallels with GR activity, indicating its role in antioxidant response of the leaf extracts.

INFLUENCE OF *Solanum nigrum* LEAVES ON NON-ENZYMIC ANTIOXIDANTS IN GOAT LIVER SLICES EXPOSED TO CCl_4 INDUCED OXIDATIVE STRESS

The non-enzymic antioxidants analysed were vitamin C, E, A and GSH, in liver slices exposed to CCl_4 and/or *Solanum nigrum* leaf extracts.

VITAMIN C (ASCORBIC ACID)

Vitamin C is regarded as a major natural antioxidant defense and a powerful inhibitor of lipid peroxidation. It also regenerates another major antioxidant, tocopherol (vitamin E) in lipoproteins and cell membranes. Intracellular mechanisms exist, which can regenerate vitamin C from its inactive metabolite dehydroascorbate using reduced glutathione (Maxewell, 1995), showing the interplay of these components. Ascorbic acid is a water soluble antioxidant that protects lipids against peroxidation (Maneesh *et al.*, 2005).

The vitamin C levels were significantly reduced in liver slices exposed to CCl_4. The BBL and RBL extracts brought back the levels to near normal. Though there was no significant difference between BBL and RBL treatments, RBL resulted in a high level of vitamin C than BBL.

Jain *et al.* (2004) reported that vitamin C is capable of quenching the oxyradicals generated by UV radiation. Vitamin C has also been suggested to play an important role in reducing the mutagenic and carcinogenic activity of the carcinogen MNNG (Biasiak *et al.*, 2002).

CCl_4 treatment to rats caused a significant depletion in plasma and tissue antioxidants, including vitamin C, and this effect was counteracted by the oral administration of curcumin (Kamalakkannan *et al.*, 2005). An ethanolic extract of *Striga orobanchioides* significantly elevated the ascorbate levels in the liver and kidney of rats (Badami *et al.*, 2003a).

Vitamin C and E, alone or in combination, can be given as therapeutic supplement (Zaidi *et al.*, 2005). Polidori *et al.* (2004) showed that the short term and long term vitamin C supplementation in humans significantly increased plasma ascorbate and improved the resistance of plasma lipids to LPO.

The change observed in the levels of vitamin C in our study, thus, indicates that BBL and RBL influence this non-enzymic antioxidant, resulting in the favourable modulation of the disturbance caused by oxidative stress.

VITAMIN E

Vitamin E is nature's most potent lipid soluble antioxidant (Schneider, 2005) and it acts as a stabilizer of membrane (Quinn, 2004). It is the major chain breaking antioxidant, protecting the cell membranes against lipid peroxidation at an early stage of free radical attack through its free radical quenching activity (Niki and Noguchi, 2000).

The vitamin E levels significantly reduced in liver slices when challenged with CCl_4. Supplementation with BBL and RBL extracts elevated the levels of vitamin E. BBL extract was more effective in elevating the vitamin E content than RBL.

Vitamin E and other radical–scavenging antioxidants can inhibit the free radical mediated-oxidation of LDL (Niki, 2004).Vitamin E treatment decreased the elevated TBARS levels in plasma and RBCs, and increased the reduced vitamin E levels, thus preventing the RBC membrane destruction and hemolysis in acetone induced oxidative stress in rats (Armutcu *et al.*, 2005). In humans, vitamin E supplementation decreases the susceptibility to LDL oxidation (Reaven *et al.*, 1993). Antioxidant therapy to oxidant stressed rabbits with vitamin E provides protection against death due to free radical stress (Singh *et al.*, 1997).

Vitamin E inhibits cell proliferation, platelet adhesion and formation of N-nitroso compounds (Azzi *et al.*, 2004), all of which are associated with cancer. MacDonald-Wick and Garg (2003) have reported that dl-alpha-tocopherol acetate supplementation was protective of lipid peroxidation when oxidative stress is induced by the prooxidant, CCl_4 in rats.

Experimental and epidemiologic investigations suggest that α-tocopherol and β-carotene might reduce the risk of cancer (Albanes *et al.*, 1996). Piperine, the

major plant alkaloid present in *Piper nigrum* and *Piper longum* significantly elevated the vitamin E level in lung and liver of cancer bearing mice (Selvendiran *et al.*, 2003).

Thus, it is perceivable that the increase in the vitamin E levels brought about by *Solanum nigrum* leaf extracts could be reflective of their antioxidant effects.

VITAMIN A

Carotenoids and other antioxidant pigments are involved in several physiological processes and signaling in animals, which cannot synthesize them and therefore, must acquire them from food (Biard *et al.*, 2005). Vitamin A, a fat soluble vitamin, plays a role in trapping peroxy radicals in tissues. The ability of β-carotene as an antioxidant is due to the stabilization of organic peroxide free radicals within its conjugated alkyl structure (Murray *et al.*, 1998).

Vitamin A level in the liver slices decreased significantly when assaulted with CCl_4. BBL and RBL leaf extracts were very effective in increasing the vitamin A levels nearer to the control values. BBL extract was more effective in this aspect than RBL.

Administration of β-carotene during CCl_4 treatment reduced several signs of fibrosis (Knook *et al.*, 1995). Beta-carotene attenuated liver cirrhosis induced by thioacetamide, which was suggested to be due to the scavenging of free radicals by β-carotene (Wardi *et al.*, 2001). Seifert *et al.* (1995) reported that β-carotene administration could prevent the long term loss of retenoids from the CCl_4 injured liver in rats.

Murthy *et al.* (2005) reported that the carotenoids obtained from the algal sources (*Spirulina platensis* and *Dunaliella salina)* exerted higher antihepatotoxic effect with synthetic β-carotene and with β-carotene alone from a natural source. Vitamin A administration has been reported to prevent hepatic injury caused by CCl_4 treatment (Noyan *et al.*, 2006).

Thus, an increase in vitamin A level, evoked by *Solanum nigrum* leaf extracts *in vitro* gains significance in the *in vivo* conditions, and strengthens their antioxidant role.

REDUCED GLUTATHIONE (GSH)

Glutathione protects the hepatocytes by combining with the reactive metabolites and thereby prevent their covelent binding to liver protein (Jaya *et al.*, 1993). In the present investigation, CCl_4 caused a significant reduction in GSH and this level was elevated upon in BBL and RBL extracts treatment. GSH content was found to be more in RBL treated group than BBL treated groups.

Meera and Rana (2006) reported that the pretreatment with hydroalcoholic extract of *Taraxacum officinale* roots improved the GSH content in CCl_4 treated group. Lee *et al.* (2003) reported that long term administration of *Salvia miltiorrhiza* increased the level of hepatic glutathione level in CCl_4 induced liver injury.

Treatment with diterpenes of *Andrographis paniculata* elevated the level of reduced glutathione in CCl_4 treated mice (Koul *et al.*, 1994). Ellagic acid administration caused a significant elevation in GSH content in CCl_4 treated rats (Singh *et al.*, 1999). Hung *et al.* (2006) have reported that the administration of water extracts of Du-zhong leaves reduced the CCl_4 toxicity and resulted in hepatic GSH depletion.

An ethanolic extract of propolis significantly elevated the reduced glutathione level in the liver of rats intoxicated with CCl_4 (Shukla *et al.*, 2004b). Significantly reduced GSH level was elevated in the rat hepatocytes challenged with CCl_4, when treated with phenyl propanoids from *Scrophularia buergeriana* roots (Lee *et al.*, 2002a). Sanchinone, a lignan from *Saururus chinensis*, attenuated the CCl_4 induced toxicity including GSH, SOD and GPx in rat hepatocytes (Sung *et al.*, 2000).

In our study, an increase in GSH by *Solanum nigrum* leaves presents very significant implications in the manifestation of antioxidant defense by the leaves.

The analysis of the overall pattern of the enzymic and non-enzymic antioxidants and their modulation during CCl_4 induced stress, in the presence and the absence of *Solanum nigrum* leaf extracts (BBL and RBL) revealed an interesting pattern in the responses evoked. BBL caused a better elevation than RBL in the activities of SOD and CAT, and the levels of vitamin E and A. On the other hand, a better increase of the activities of GPx, GST, GR and G6PD and the levels of reduced glutathione was evoked by RBL extract than BBL extract. Vitamin C level was elevated in both the treatments but there was no significant difference between them.

These observations suggest an interesting possibility in the mechanism of action of the two extracts. The differential picture in the responses shows that the leaves exert their antioxidant effects by different biochemical mechanisms. It can be suggested, from our results, that RBL extract acts via a glutathione dependent mechanism, while BBL follows a different route. This suggestion is validated by the observation that all the glutathione-dependent components (GPx, GST, GR, G6PD and GSH) respond more significantly to RBL administration than to BBL. This suggestion needs to be confirmed by further molecular level studies.

Following these analyses, which showed that BBL and RBL influenced the antioxidant status positively, thus counteracting the oxidative stress, the extent of oxidative stress on the organ (liver) slices was quantified as lipid peroxidation.

INFLUENCE OF *Solanum nigrum* LEAVES ON LIPID PEROXIDATION IN GOAT LIVER SLICES EXPOSED TO CCl_4 INDUCED OXIDATIVE STRESS

Lipid peroxidation is believed to be an important underlying cause of initiation of oxidative stress, its related tissue injury, cell death and further progression of many acute and chronic diseases (Halliwell and Gutteridge, 1999).

Significantly elevated levels of lipid peroxidation were observed in CCl_4 assaulted goat liver slices. Supplementation of BBL and RBL extracts significantly reduced the LPO product, the malondichdehyde. BBL exhibited better inhibitory activity on lipid peroxidation than RBL.

Jin *et al.* (2005a) reported that the treatment with an aqueous extract of *Morus bombycis* koidzumi recovered the CCl_4 caused liver injury and showed antioxidant effects against $FeCl_2$-ascorbate induced lipid peroxidation. Treatment with extracts of Du-zhong leaves (Hung *et al.,* 2006) and *Nelumbo nucifera* seeds (Rai *et al.,* 2006) decreased the levels of TBARS in CCl_4 induced liver injury in rats.

Ilavarasan *et al.* (2001) observed that the *Cassia angustifolia* reduced the level of lipid peroxides in CCl_4 intoxicated rats. Decreased levels of TBARS was observed in *Rhus verniciflua* stokes treated CCl_4-injured mice (Lee *et al.,* 2002b).

The level of lipid peroxidation was reduced by brahma rasayana in radiation induced oxidative stress in rats (Rekha *et al.,* 2001). The administration of kolaviran decreased the level of MDA in CCl_4 intoxicated rats (Farombi, 2000).

Oral administration Zinc (Camps, 1992) and taurine (Miyazaki *et al.,* 2005) caused a significant reduction in LPO level in CCl_4 intoxidated rats. S-8300 (from shark liver) treated animals exhibited a reduction in MDA level in CCl_4 intoxicated rats (Huang *et al.,* 2005).

Higher levels of lipid peroxidation of CCl_4 treated hepatocytes were significantly reduced upon treatment with the drug ursolic acid (Martin-Aragon *et al.,* 2001). In CCl_4 injured rat hepatocytes, the increased formation of malondialdehyde was reduced by the treatment with phenylpropanoids from *Scrophularia buergeriena* roots (Lee *et al.,* 2002a). Sung *et al.* (2000) also reported a similar effect with Sauchinone, a lignan from *Saururus chinensis*. Rodrigo and Bosco (2005) reported that the elevated levels of lipid peroxides were decreased by the administration of wine polyphenols in the liver of ethanol treated rats.

In the present investigation, potent inhibitory activity was found to occur on lipid peroxidation, which might be due to the action of all enzymic and non-enzymic antioxidants of *Solanum nigrum* leaf extracts, thus confirming their antioxidant potential on antiperoxidative activity.

In vitro CYTOTOXIC ACTIVITY OF *Solanum nigrum* LEAVES ON KB CELLS (ORAL CARCINOMA CELLS)

Cancer is essentially a problem of abnormal cell death; under the influence of chemicals, viruses, and free radicals, the normal cells are converted to tumor masses that divide in an uncontrollable manner (Nwafor *et al.*, 2001). Since higher plants have proven to be an important source of anticancer compounds, discovery of new drugs through screening efforts using cytotoxicity is currently of major interest (Farnsworth and Kass, 1981; Suffness and Douros, 1982).

The crude aqueous extracts of *Solanum nigrum* leaves caused a remarkable cytotoxic effect on KB oral carcinoma cells. RBL extract was found to exhibit a better cytotoxic activity than BBL. However, when KB cells were exposed to H_2O_2 in addition to the leaf extracts, BBL elicited a better effect than RBL. H_2O_2 by itself, was highly cytotoxic. It is inferable from our results that BBL and RBL possess components that, may have anticancer properties, as KB cells are derived from oral carcinoma.

The 50% ethanol extract of the whole plant of *Solanum nigrum* significantly inhibited the gentamycin-induced toxicity on Vero cells (Kumar *et al.*, 2001). The hydroalcoholic extract of branchlets of female *Taxus baccata* L showed inhibitory activity against KB cells (Emami *et al.*, 2005).

Lee *et al.* (2002c) showed that the water extract of *Paeoniae radix* showed an inhibitory effect on both HepG2 and Hep 3B cell lines. Glei *et al.* (2003) reported that the water extracts of green tea and black carrots caused a reduction in cell viability and cell growth, and DNA damage in human colon cancer cells (HT-29 Clons 19A).

Mongelli *et al.* (1997) reported that dichloromethane extract of *Baccharis cordifolia* inhibited the growth of the KB cells. Ethyl acetate extract of *Marchantia convoluta* had a significant cytoxicity against lung (H1299) and liver (HepG2) carcinoma cells (Xiao *et al.,* 2006). Wong and Tan (1996) reported that ether fraction of *Rhaphidophora korthalsii* caused an effective (50%) killing of KB carcinoma cells.

Methanolic extract of the leaves of *Combretum fragrans* and a fruit extract of *Combretum zeyheri* gave a very strong antiproliferative and cytotoxic effects against Hela, T24 and MCF carcinoma cell lines (Fyhrquist *et al.,* 2006). A significant cytotoxic effect was exerted by the crude methanolic extract of *Centella asiatica* on EAC and DLA cells under *in vitro* conditions (Babu *et al.,* 1995).

Moongkarndi *et al.* (2004) showed that the ethanolic extract of *Garcinia mangostana* exhibited a potent antiproliferative activity against SKBR3 human breast carcinoma cell line. Sivalokanathan *et al.* (2006) reported that the ethanolic extract of *Terminalia arjuna* bark induced cytotoxity in HepG2 cells *in vitro.*

The flavonoid mixture from *Gomphrena martiana* exhibited an *in vitro* cytotoxicity against cultured KB cells (Pomilio *et al.,* 1994). The terpenoids isolated from *Asteraceae* species showed significant toxic action towards KB cells (Villarreal *et al.* 1994). Tian *et al.* 2006) reported that among cycloartane triterpenoids isolated from the aerial parts of *Cimicifuga foetida* showed moderate cytotoxic activity on R-HepG2 cells.

Indap and Barkume (2003) reported that curcumin showed potent antiproliferative effect and is superior to quercetin and ferulic acid in inhibiting the growth of the K-562 and S180 tumour cells. The substances, gallic acid, ethyl gallate and luteolin isolated from *Terminalia arjuna* exhibited moderate inhibitory activity against several cancer cell lines (Pettit *et al.*, 1996). The cell growth of KB cells in culture was inhibited by the active principle from *Nigella saliva* seeds (Salomi *et al.,* 1992)

Among the compounds, khellin, berberin, lupeol, scopolin and rapanone obtained from Colombian plants, barberine and raponone exhibited cytotoxic

effects against HT-29 (colorectal cancer), MCF-7 (breast cancer), Hep2 (larynx cancer) and MKN-45 (gastric cancer) cells lines (Cordero *et al.,* 2004). Guieranone A, a naphthyl butanone, purified from the leaves and roots of *Guiera senegalensis,* presented a strong cytotoxicity against cancer cell lines (Fiot *et al.,* 2006).

Among the alkaloids isolated from the seeds of *Strychnos nux-vomica,* brucine, strychnine and isostrochmine exhibited inhibitory effects against HepG2 cell proliferation (Lamchouri *et al.,* 2000). Pretreatment with the alkaloid fraction of *Alstonia scholaris* caused a significant elevation in the death of KB and Hela cells, followed by HL 60, MCF-7 and HepG2 cells during exposure to gamma-radiation (Jagetia and Baliga, 2003). The oxoaporphine alkaloids, iiriodenine and oxostephanine were strongly cytotoxic to the both epidermoid carcinoma (KB) and breast cancer (BC) cell lines (Wirasathien *et al.*, 2006).

The total alkaloid fraction of the methanolic extract of unripe fruits of *Solanum pseudocapsicum* showed strong cytotoxic activity against Hep-2, RD and vero cell lines (Vijayan *et al.,* 2002). The same authors (2004) also showed that the total alkaloid fraction of *Solanum pseudocapsicum* strongly inhibited the DLA cells (short term) and Hep2 cell (long term).

Lee and Lim (2006) reported that the glycoprotein isolated form *Solanum nigrum* (L) has an apparant cytotoxic effect on HCT-116 cells. Heo and Lim, (2005) reported that a glycoprotein isolated from *Solanum nigrum* (L), is a potential natural anticancer agent because of its ability to induce apoptosis in MCF-7 cells. Lim (2005) suggested that glycoprotein isolated from *Solanum nigrum* (L) kills HT-29 cells through apoptosis. From these reports it can be seen that the glycorprotein and solamargine (Hu *et al.,* 1999) present in *Solanum nigrum* exhibited cytotoxic effect on various cancer cell lines.

There is reported evidence that these compounds and other glycoalkaloids are present in the leaves, stems and roots of *Solanum nigrum* (Ivanchenko and Tukalo, 1975; El-Ashall *et al.,* 1999). Therefore, it is likely that these compounds, or other similar compounds, present in the leaves (BBL and RBL), may be

responsible for the cytotoxicity observed in KB cells. In our own study, in the last phase, the phytochemical analysis revealed the presence of alkaloids and phenolics.

Thus, it is possible that these components cause cytotoxictity to the cancer (KB) cells. The additional action of the antioxidants present in the leaves may enhance this effect. In order to probe further into this possibility, the effect of the leaf extracts of *Solanum nigrum* were tested in an *in vivo* tumour system, namely, in mice bearing the Dalton's Lymphoma Ascitic (DLA) tumour, the results of which are elaborated in phase III of this study.

PHASE III

The results obtained for the various *in vitro* models proved the antioxidant potential and anticancer activity of the leaves of *Solanum nigrum.* Eventhough the data from *in vitro* system gives an insight into the mechanism of action of plant extracts, and are likely to be the same as evoked *in vivo*, there remains a chance that the results *in vivo* differ from the ones *in vitro* due to interfering factors like transport to target organs, hormonal regulation, cellular homeostasis etc. Therefore, in order to confirm the results of the experiments conducted in Phase II, *in vivo* studies were conducted using experimental animals in Phase III.

Female albino rats belonging to the Wistar strain were used as experimental animals to determine the antioxidant effect of *Solanum nigrum* leaves. Oxidative stress was induced with ethanol-CCl_4 as described in the methodology.

Carbon tetrachloride (CCl_4) is a classic hepatotoxin, which has been developed by researchers into an important experimental hepatotoxin over 10 years (Plass, 2000). Studies have also reported the potentiation of CCl_4-induced liver injury upon the pre-administration of a non-lethel dose of ethanol to mice (Strubelt *et al.*, 1978).

CCl_4 is known to be metabolized into trichloromethyl ($CCl_3^{\bullet}$) radicals by a specified isoenzyme of the cytochrome P450 monooxygenase system, namely CYP 2E1 (Kim *et al.,* 1996). This isoenzyme is known to be induced by alcohol and

stress conditions (Kessova and Cederbaum, 2003). This is the reason that alcohol pretreatment potentiates the toxicity of CCl_4.

Exploiting this knowledge, in the present dissertation, alcohol pretreatment was given to induce CYP2E1, followed by a single subcutaneous dose of CCl_4 at sub-acute level (0.5ml/kg body weight). In many other studies, higher or repeated doses of CCl_4 have been used to induce oxidative stress (Augusti *et al.*, 2005; Dakshayani *et al.*, 2005; Kamalakkannan *et al.*, 2005).

The regime was followed to minimize the necrotic toxicity of CCl_4, at the same time maximizing the oxidative stress. In order to ascertain whether this regime did cause oxidative stress to the liver, as the first step, the circulating levels of liver function marker enzymes and the circulating lipid profile were analysed in the experimental rats.

To compare the effects of BBL and RBL administration to oxidatively stressed rats and to validate the observations made, a standard hepato protective antioxidant silymarin was used. All the parameters were analysed in the liver of the experimental rats.

LIVER FUNCTION MARKER ENZYMES IN SERUM

Carbon tetrachloride is a widely used chemical to induce liver damage in the experimental studies, and its toxicity has been studied extensively. The resulting hepatic injury is characterized by the leakage of cellular enzymes into the blood stream (Muriel *et al.*, 2001). Their estimation in the serum is a useful quantitative marker of the extent of hepatocellular stress (Mitra *et al.*, 1998).

The various liver function marker enzymes analysed were aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Significantly elevated levels of these enzymes were observed in the serum of animals treated with ethanol alone or alongwith CCl_4. Treatment with BBL and RBL aqueous extracts or silymarin caused a reduction in the levels of liver function marker enzymes.

An aqueous extract of *Anoectochilus formosanus* showed reducing actions on the levels of ALT and AST caused by CCl_4 in rats (Shih *et al*, 2005). Pre- and post-treatment with the aqueous extracts of the whole plant of *Asteracantha longifolia* Linn. reduced the ALT level against CCl_4 treated mice (Hewawasam *et al.*, 2003).

Components extracted from the roots of *Bupleurum kaoi* water extracts, polysaccharide-enriched fractions markedly reduced serum ALT and AST levels in dimethyl nitrosamine-induced hepatic fibrosis in rats (Yen *et al.*, 2005). Manjunatha (2006) reported that the crude aqueous and ethanol stem bark extracts of *Pterocarpus santalinus* administration resulted in a decrease in serum levels of the AST, ALT and ALP in CCl_4 intoxicated rats.

Treatment with Handqooqa (*Boerhaavia diffusa)* produced significant reduction in serum AST, ALT and ALP compared to CCl_4 intoxicated albino rats (Fakhr-E-Alam *et al.*, 2003). Lin and Lin (2006) reported that CCl_4 caused an increase in plasma transaminases and this level was markedly decreased by the extract of *Ganoderma lucidum* in mice. Treatment with carrot extracts afforded a significant protection against CCl_4 induced increase in serum AST, ALT, LDH and alkaline phosphatase levels in mice (Bishayee *et al.*, 1995).

In methanol extract of *Pterocarpus marsupium* stem bark treated animals, the toxic effect of CCl_4 was controlled significantly by the restoration of AST, ALT and ALP activities when compared to normal and silymarin treated rats (Mankani *et al.*, 2005). Shyamal *et al.* (2006) have reported that the methanolic extract of the stem bark of *Pittosporum neilgherrense* decreased the levels of AST and ALT in CCl_4, d-galactosamine (d-Gal N)- and acetaminophen induced acute hepatotoxicity in Wistar rats.

Administration of acetone fraction of *Rosa damascena* reduced the elevated levels of ALP, ALT and AST in serum of rats (Achuthan *et al.*, 2003). The treatment with n-hexane extract of *Lygodium flexuosum* prevented the elevation of serum AST, ALT and LDH levels in CCl_4 treated rats (Wills and Asha, 2006).

Picroliv, the active constituent isolated from the plant *Picrorhiza kurroa*, restored the altered levels of AST, ALT and ALP in alcohol intoxicated rats (Saraswat *et al.*, 1999).

Achliya *et al.* (2003) found that the administration of *Panchagavya ghrita* markedly prevented CCl_4 induced elevation of ALT, AST and ALP which was comparable with silymarin in rats. Treatment with silymarin, CH100 and CH101 (Chinese herbal preparations) reduced the ALT elevation in mice exposed to CCl_4 (Li *et al.*, 2003). Pretreatment with water extracts of *Limonium wrightii* significantly reduced the elevated serum AST and ALT in rats (Aniya *et al.*, 2002).

In the present investigation, a significant reduction was observed in the liver function marker enzyme levels in the serum of the CCl_4 exposed rats treated with BBL and RBL extracts, which might be due to their hepatoprotective and antioxidant properties, including the stabilization of the membrane.

LIPID PROFILE IN SERUM

Significantly elevated levels of cholesterol and triglycerides occurred in the serum of the ethanol alone or along with CCl_4 treated groups. Treatment with both BBL and RBL extracts exerted a significant reduction in the cholesterol and triglyceride levels.

The circulating lipid profile has a direct bearing that reflects the status of the liver. The alterations in the lipid profile in response to the administration of hepatotoxins like CCl_4, and their modulation by herbal extracts and products have been the focus of many studies worldwide.

Lin *et al* (2002) have reported that the treatment with ethanol and CCl_4 elevated the levels of cholesterol and triglycerides, which were altered by the administration of *Arctium lappa* Linne in experimental rats. The elevated serum cholesterol level was reduced in CCl_4 intoxicated rats when treated with the whole plant slurry of *Asteracantha longifolia* Nees (Shailajan *et al.*, 2005).

Both hot water extract and methanol extract of *Artemisia iwayomogi* lowered serum cholesterol levels in fibrosis induced by CCl_4 in rats (Park *et al.*, 2000). Methanolic extract of *Asteracantha longifolia* seeds significantly reduced the elevated levels of triglycerides and cholesterol in the serum and liver of rats intoxicated with acetaminophen (Shivashangari *et al.*, 2004). An ethanolic extract of *Beta vulgaris* roots exhibited a significant dose dependent hepatoprotective activity against carbontetrachloride induced hepatotoxicity in rats as reflected by the cholesterol and triglyceride levels in serum (Agarwal *et al.*, 2006).

The oral administration of Livex, a compound herbal formulation, caused a significant reduction in serum cholesterol and triglycerides in erythromycin estolate-induced hepatotoxicity in rats (Venkateswaran *et al.*, 1997). Liv 52, an indigenous herbal preparation in which *Solanum nigrum* is a major constituent, used in the treatment of various hepatic dysfunction and disorders, significantly decreased the serum lipids, in the rats treated with CCl_4 (Subbarao and Gupta, 1978a, 1978b) and alcohol (Subbarao, 1976). Treatment with *Elephantopus scaber* Linn roots caused a significant reduction in cholesterol and triglyceride levels in serum of CCl_4 intoxicated rats (Rajesh and Latha, 2001).

Thus, the treatment regime in the present study to induce oxidative stress to the liver with a single subacute dose of CCl_4 following ethanol pretreatment was very effective as reflected by the increase in the stress elevated components (AST, ALT, ALP, cholesterol and triglycerides). The observation that the extracts of BBL and RBL decreased these stress effects is indicative of the protective effects of the leaves.

HEPATIC CYTOCHROME b_5 AND CYTOCHROME P450

Liver is the major organ for the metabolism of xenobiotics and its capacity to carryout the several oxidative metablism is associated with its high cellular contents of cytochromes P450 (Coon *et al.*, 1992). Nearly fifteen isoenzymes of CYPs are involved in the metabolism of drugs and xenobiotic chemicals and have received the most attention from pharmacologists (Guengerich, 2003).

The initial event in the rats given CCl_4 has been believed to be lipid peroxidation of the endoplasmic reticulum of liver cell initiated by trichloromethyl radical generated by the action of CYP on CCl_4 (Recknagel *et al.,* 1967). Ethanol-CCl_4 intoxication caused an elevation in the levels of cytochrome P450 and cytochrome b_5. In the animals pretreated with BBL and RBL extracts, there was a significant reduction in both CYP and cytochrome b_5 during oxidative stress.

Treatment with ethanol caused an increase in hepatic CYP2E1 activity (Song *et al.*, 2003). Synthetic zinc formate and plant-derived zinc formate from the aqueous extracts of *Cochlospermum planchonii* Hook.E. rhizomes were effective inhibitors of cytochrome P450 enzymes and acted as hepatoprotective agents in carbontetrachloride treated rats (Aliyu *et al.*, 1995). Pretreatment with *Quercus aliena* acorn in rats caused a significant reduction on the impression of cytochrome P450 2EI (CYP2EI) mRNA, in liver and these results are related to the antioxidative activity and expression of CYP2E1 (Jin *et al.*, 2005b).

Oral administration of Liv-100 modulated the alterations in xenobiotic metabolizing system and microsomal lipid peroxidation in experimental rats (Saraswathy and Shyamala Devi, 2001). A hydroalchoholic (50%) extract of *Emblica officinalis* (fruit) (EO-50) effectively reversed the CYP2E1 level in hepatic fibrosis induced by carbontetrachloride and thioacetamide in rats (Tasduq *et al.*, 2005).

Ha *et al.* (2005) reported that the expression level of CYP2E1 mRNA and protein was significantly decreased in the liver of *Lycium chinese* fruit pretreated rats when compared with that in the liver of control group. The administration 2,3,7,8-tetrachlorodibenzo-p-dioxin resulted in an increased activity of CYT reductace activity in the guinea pig liver, and this was significantly inhibited by *Panax ginseng* extract (Lee *et al.*, 2002d).

The activity of CYT P450 and CYT b_5 induced by enthanol-CCl_4 administration was effectively counteracted by the components of the *Solanum nigrum* leaves which might be due to the inhibitory property of some components

of the leaves. This suggests the antioxidative of hepatoprotective activity of BBL and RBL extracts, since inhibition of CYT P450 results in the decreased levels of formation of free radicals and other oxidative products.

Following the assertation that oxidative stress was induced, the antioxidant status in the metabolic organ, namely the liver, was assessed by analysing the enzymic and nonenzymic antioxidants as well as the extent of lipid peroxidation. The results obtained are discussed below.

ENZYMIC ANTIOXIDANTS

The rats treated with ethanol, alone or along with CCl_4, exhibited a significant reduction in SOD, CAT, GPx, and GSTs when compared to control. In animals treated with *Solanum nigrum* leaves, there was a significant elevation in the activities of these enzymes. The activities of SOD and CAT were significantly higher in BBL, while those of GPx and GST were greater in RBL.

Free radical induced oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient natural antioxidant defences. Potential antioxidant therapy should therefore include either natural free radical scavenging antioxidant enzymes or agents that are capable of augmenting the activity of these enzymes, which include SOD, CAT and GPx (Bast *et al.,* 1991).

Superoxide is inactivated by SOD, the only enzyme known to use a free radical as a substrate. However, the free radical scavenging activity of SOD is effective only when it is followed up by increase in the activity of CAT and/or GPx (Ramasarma, 1990). Since SOD generates hydrogen peroxide as a metabolite, which is more tissue toxic than oxygen radicals and has to be scavenged by CAT or GPx. Thus, a concomitant increase in CAT and/or GPx activity is essential if a beneficial effect from increase in SOD activity is to be expected (Harman, 1991).

Among the treatments with different medicinal plants (*Piper cubeba* (fruit), *Physalis angulata* (flower), *Rosa hybida* (flower)), SOD and CAT activities by

Piper cubeba and CAT activity by *Rosa hybrida* were significantly increased while SOD and CAT activities by *Physalis angulata* were not significantly changed in the plasma of rats (Choi and Hwang, 2005).

Latha and Pari (2003) have reported that the aquous extracts of the flowers of the *Cassia auriculata* caused a significant increase in the activities of SOD, CAT, GPx and GST in the brain of streptozotocin induced diabetic rats. The administration of *Andrographis paniculata* increased the activities of SOD, CAT, GPx and GR in BHC treated mice (Trivedi and Rawal, 2001).

The activities of SOD, CAT, GPx and GST in the gastric mucosal tissues of rats preteated with methanolic extract of *Solanum nigrum* berries were found to be increased in aspirin induced ulcerative rats (Jainu and Shyamala Devi, 2004). Pretreatment with an alcoholic extract of *Indigofera tinctoria* L. significantly elevated the activities of SOD, CAT, GPx and GST in D-galactosamine endotoxin induced liver injury in rats (Sreepriya and Devaki, 2001).

HD-03, a herbal formulation, significantly restored the changes in cellular antioxidant system of the CCl_4 intoxicated rats (Mitra *et al.*, 1998). Pepticare, a herbomineral formulation, increased the levels of SOD and CAT in gastic ulcers in ethanol induced mucosal injury in rats (Bafna and Balaraman, 2005).

Protandium, a preparation consisting of the extracts of five medicinal platns, increased the levels of SOD and CAT in the erythrocytes of healthy human beings (Nelson *et al.*, 2006). Garlic oil dose-dependently increased the hepatic SOD, GR and GST activities but decreased the GPx activities in rats (Chen *et al.*, 2003b).

Oral administration of dimethyl dimethoxy biphenyl dicarboxylate alleviated the oxidative status of tamoxifen-intoxicated liver injury in rats as observed by significant increments in the antioxidant enzymes, CAT, GPx and GST (El-Beshbishy, 2005). The active tannoids of *Emblica officinalis* (amla) induced an increase in SOD, CAT and GPx activities in the brain tissues of rats (Bhattacharya *et al.*, 1999).

Gupta *et al.*, (2004a) reported that methanolic extracts of *Bauhinia racemosa* Lam significantly elevated the reduced SOD activity in CCl_4 induced liver damage. Treatment with bioflavanoid hesperidin significantly elevated the reduced SOD activity in CCl_4 induced liver damage in rats (Tirkey *et al.*, 2005).

The co-treatment with betaine and taurine significantly elevated the reduced activity of catalase in ethanol and CCl_4 induced liver damage in rats (Erman *et al.*, 2004). Young *et al.* (2007) showed that the extract of *Piper betel* leaves enhanced the hepatic SOD and CAT activities in CCl_4 treated rats.

NON-ENZYMIC ANTIOXIDANTS

The non-enzymic antioxidants analysed were ascorbate, tocopherol, vitamin A and reduced glutathione. All the components tested decreased significantly in the livers of the animals subjected to oxidative stress. This decrease was efficiently counteracted by the pre-treatment with the extracts of BBL and RBL. The levels of reduced glutathione increased to a better extent in RBL treatment than in BBL treatments, while all the other components responded better to BBL exposure.

Defence against oxidative stress is primarily dependent upon an orchestrated synergism between several endogenous and exogenous antioxidants. For example, vitamin E is a major lipid phase antioxidant that protects against oxidative lipid damage and it is continuously recycled as it acts as an antioxidant. Exogenous nutrients such as vitamins C and E are not produced in the human body. Thiols like GSH and dihydrolipoate support vitamins C and E recycling (Gul *et al.*, 2000). Thus, vitamins C and E, and GSH react co-operatively *in vivo*, resulting in greater protection of the organism against radical damage which could not be provided by any single antioxidant.

Administration of green tea to ethanol treated rats of different ages partly normalized the levels of vitamins C, E, A and β-carotene (Augustyniak *et al.*, 2005). Saravanan *et al.*, (2002) reported that *Piper betle* extract improved the tissue antioxidant status by increasing the levels of GSH, vitamin C and vitamin E in ethanol treated rats. Silymarin prevents CCl_4-induced lipid peroxidation and

hepatotoxicity in mice, firstly by decreasing the metabolic activation of CCl_4 and secondly by acting as a chain breaking antioxidant (Letteron *et al.*, 1990).

A decrease in the plasma vitamins E, C and A levels, which was observed in rats after irradiation, was maintained at near normal levels with N-acetylcysteine pretreatment (Sridharan and Shyamaladevi, 2002). Jung *et al.*, (2005) observed that the cardiovascular protective properties of kiwi fruits are considered to be due to antioxidants like vitamin C, carotenoids and flavonoids. It was found that the protective effect of the juice of *Opuntia ficus-indica* fruit in CCl_4-induced hepatic injury is related to the flavonoid fraction as well as vitamin C in rats (Galati *et al.*, 2005).

The extracts of *Nigella sativa* L and the oil extracts of *Urtica dioica* elevated the levels of vitamin C and vitamin E in CCl_4 treated rats (Kanter *et al.*, 2005). Augusti *et al.*, (2005) reported that supplementation with vitamin E prevented the liver damage in rats. Campo *et al.* (2001) analysed that IRFI 042, a novel vitamin E like compound possessing a marked sulphydryl group in the aliphatic side chain, restored the hepatic concentrations of vitamin E and endogenous antioxidants like GSH in CCl_4 induced liver toxicity in rats.

Low hepatic vitamin A levels, which can be the result not only of dietary intake but also of interference with vitamin A metabolism by agents such as ethanol and CCl_4 have been implicated to be a risk factor for the development of liver fibrosis in rats (Seifert *et al.*, 1994). Seifert *et al.* (1995) also reported that β-carotene (provitamin A) could prevent long term loss of retinoids from the CCl_4-injured rat liver.

Jainu and Shyamala Devi (2004) reported that the GSH content in gastric mucosa was reverted to near normal in the aspirin induced ulcerative rats pretreated with a methanolic extract of *Solanum nigrum* berries. Trivedi and Rawal (2001) reported that *Andrographis paniculata* caused a significant elevation in the GSH content in BHL treated mice. Treatment with Propolis brought back the GSH level in rat liver intoxicated with alcohol and CCl_4 (Sharma *et al.*, 1997). Administration

of water extract from adzuki bean (*Vigna angularis)* hulls, reversed the decreased level of GSH in acetaminophen induced liver damage in rats (Han *et al.*, 2004).

In our study, the levels of ascorbate, vitamin E, vitamin A and GSH were reduced significantly in CCl_4 induced oxidative stress and these levels were found to be elevated in the animals pretreated with *Solanum nigrum* leaves. This could be because, in CCl_4 intoxicated animals, there was increased level of free radicals and formation of oxidative products, resulting in excessive utilization of antioxidant molecules to neutralise the damaging effects. Administration of *Solanum nigrum* leaf extracts, a rich source of antioxidant molecules including polyphenols and carotenoids (Phase I) raised these levels in oxidative stress induced animals.

When the effects of the extracts of BBL and RBL on the enzymic antioxidants were compared with each other, it was observed that BBL caused a higher level of increase in SOD and CAT activities than RBL, while RBL caused a better increase in GPx and GST activities than BBL. In the context of the results observed in Phase II, in the liver slices, the results observed in phase III of the study reiterated the possibility that RBL exerts its action via glutathione-dependent mechanism. BBL seems to follow a different biochemical mechanism that may involve glutathione or related components to a lesser extent. The involvement of these components is definitely indicated by our results, because BBL also causes significant increase in GPx, although to a lower extent that RBL. The trend observed in the levels of GSH, in *vitro* and *in vivo*, also followed a similar trend, lending more support is credibility to this suggestion. More in-depth molecular studies are needed in this direction to dissect the metabolic pathways involved.

LIPID PEROXIDATION

Lipid peroxidation occurs mainly in membranes, where the content of unsaturated fatty acids is relatively high. Peroxidation of membrane lipid arising out of oxidative damage in intact cells results in decreased fluidity, inactivation of membrane bound enzymes and receptors and changes in non-specific permeability (Bast *et al.,* 1991). The greater the unsaturation, the greater is the lipid peroxidation

and cumulative effects of lipid peroxidation have been implicated as the underlying mechanism in various pathological conditions like atherosclerosis, hemolytic anemia and ischemia (Halliwell, 1991).

In the present investigation, significantly elevated levels of LPO products (MDA, CD and HP) in CCl_4 administrated animals indicated the excessive formation of free radicals and activation of LPO system, resulting in the hepatic damage. Pretreatment with *Solanum nigrum* leaves, BBL and RBL, lowered the extent of LPO in the liver tissue of CCl_4 intoxicated animals.

The fruits of *Aronia melanocarpa* rich in anthocyanins prevented the CCl_4-induced elevation of MDA formation in rats (Valcheva-Kuzmanova *et al.*, 2006). The extract of *Antrodia camphorata* dose-dependently inhibited the formation of lipid peroxidative products in mice during CCl_4 treatment (Hsiao *et al.*, 2003).

Hsiao *et al.* (2001) reported that the 2,2,5,7,8-pentamethyl-6-hydroxychromane, a derivative of α-tocopherol, dose-dependently inhibited the lipid peroxidation in mice. Kim *et al.* (2003b) observed that n-butanol extract of *Artemisia apiacea* significantly decreased the MDA production in rats. Camps (1992) reported that oral zinc supplementation was associated with a decrease in lipid peroxidation in CCl_4 intoxicated rats. Lipid peroxidation was also significantly decreased in the group that received ethanol with green tea when compared to control group in rats (Skrzydlewska *et al.*, 2002).

The elevated lipid peroxidative products (CD, HP and MDA) were attenuated by treating fructose-fed diet rats by taurine, an antoxidant aminoacid (Nandhini *et al.*, 2002). Treatment with the root extract of *Withania somnifera* dose dependently and significantly reduced the lipid peroxidation induced by reserpine in rat brain homogenate (Naidu *et al.*, 2006). Mandal and Das (2005) reported that the two fold increase in conjugated diene by the induction of CCl_4 was decreased to normal level by galactosylated liposomal quercetin pre-treatment. An extract of *Ganoderma lucidum* decreased the level of hepatic MDA level in CCl_4 intoxicated rats (Lin and Lin, 2006).

Wang *et al.* (2005) reported that the treatment with melatonin reversed the hepatic MDA level in CCl_4 intoxicated rats. Campo *et al.* (2004) showed that the level of conjugated dienes in the liver was reduced in CCl_4 treated rats treated with glycosaminoglycans. The lipid peroxide and CD formation was significantly inhibited by *Glycyrrhiza glabra in* rats treated with CCl_4 (Rajesh and Latha, 2004). Treatment with vitamins E, C or Spirulina caused a significant reduction of the elevated peroxidation products such as CD, HP and MDA in rat liver (Upasani and Balaraman, 2001). Liv-52, a polyherbal formulation prevented the lipid peroxidation in CCl_4 induced liver damage in rats (Pandey *et al.*, 1994; Kataria and Singh, 1997).

In tune with these reports, the significant decrease in lipid peroxidation products like CD, HP and MDA upon treatment with BBL and RBL extracts and silymarin in animals treated with CCl_4 is indicative of the antilipid peroxidative effect of *Solanum nigrum* leaves and silymarin.

There are reports that the activated $CCl_3^{\bullet}$ radical formed by mixed function oxidases binds covalently to macromolecules and induces peroxidative degradation of membrane lipids of the endoplasmic reticulum rich in polyunsaturated fatty acids (Kornbrust and Mavis, 1980). The *in vitro* experiments performed also indicated a reduction in LPO after the addition of BBL and RBL extracts to CCl_4 treated goat liver slices, investigated in Phase II. Both the *in vitro* and *in vivo* experimental systems confirm the antilipidperoxidative activity of *Solanum nigrum*. BBL was found to be more effective than RBL in both the systems.

Histopathological results showed that the ethanol-CCl_4 induced centrilobular necrosis and fatty changes of hepatocytes, sparing of hepatocytes around the portal tract. The researchers, Ulicna *et al.*(2003), Jiang *et al.*(2004) and Yang *et al.* (2006), have reported a similar type of results, which might be due to the development of highly reactive oxidant metabolites formed during CCl_4 metabolism.

These adverse changes were found to be reduced tremendously in BBL and RBL treated oxidatively stressed, experimental animals. Similar results were observed when *Ginkgo bilbo* (Lou *et al.*, 2004), *Arctium lappa* (Lin *et al.*, 2005) and *Cassia fistula* Linn (Pradeep *et al.*, 2005) were administered to CCl_4-induced hepatotoxic rats, suggesting their hepatoprotective activity by the action of the antioxidant molecules supplemented through BBL and RBL treatments.

The liver of animals treated only with BBL or RBL showed normal histological picture indicating their non-toxicity property. The antioxidant potential and hepatoprotective effect could have been brought about by various phytochemical components of the leaves.

EFFECT OF *Solanum nigrum* LEAVES ON DLA TUMOUR INDUCED IN SWISS ALBINO MICE

In the antitumour studies, Ascites Dalton's Lymphoma has been commonly used as an important murine experimental tumour model (Nicol and Prasad, 2002). Many of the classes of phytochemicals in herbal medicine are finding therapeutic use. In particular cancer patients are reported to benefit from treatment with herbal medicine and survivability in many cases is significantly increased (Ho *et al.*, 2002).

Non-Hodgkin's lymphoma is etiologically related to suppressed immune status, and certain nutrients found in fruits and vegetables have been associated with increased immune reponses (Zheng and Sheppard, 2004). The reliable criteria for judging the value of any anticancer drug are prolongation of lifespan and disappearance of leukemic cells from blood (Lin *et al.*, 1988). Anemia encountered in tumour bearing mice is mainly due to the reduction in RBC or hemoglobin percentage and this either may occur due to iron deficiency or due to hemolytic or myelopathic conditions (Fenninger and Mider, 1954).

In the present study, the DLA tumour induced animals showed a high mortality rate compared to the other groups and the mice administered with the BBL and RBL extracts showed greatly enhanced the lifespan of the DLA tumour

animals. The leaf extracts were capable of controlling the tumour bearing development, which was indicated by the change in the body weight. Treatment with BBL and RBL extract exhibited a tremendous change in the hemoglobin level, RBC, total and differential WBC counts in DLA tumour induced animals.

Aqueous extract of *Phyllanthus amarus* was found to prolong the lifespan of Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) bearing mice and reduced the volume of transplanted solid tomours (Rajesh Kumar *et al.*, 2002). Rosangkima and Prasad (2004) observed that the aqueous extract of the root of *Ageratum conyzoides* and *Potentilla fulgens* and the methanolic extract of stem bark of *Dillenia pentagyna* significantly prolonged the survival time of mice bearing DLA.

The total alkaloid fraction of the methanolic extract of *Solanum pseudocapsicum* leaves exhibited an increase in the mean survival time and percent increase in lifespan of DLA bearing mice (Badami *et al.*, 2003b). Oral administration of the methanolic extract of *Emilia sonchifolia* to mice reduced the development of both solid and ascites tumours and increased the lifespan of these tumour bearing mice (Shylesh and Padikkala, 2000).

Babu *et al.* (1995) observed that the administration of crude methanolic extract and the acetone fraction of *Centella asiatica* significantly reduced the development of murine solid tumour and increased the lifespan of tumour-bearing mice. DLA tumour bearing mice treated with the methanolic extract of *Enicostemma littorale* caused an increase in the mean survival time, decrease in tumour growth and the reversal of changes in the hematological parameters (Kavimani and Manisenthlkumar, 2000).

Gupta *et al.* (2004b) reported that the methanol extract of *Bauhinia recemosa* stem bark showed a decrease in tumour volume and increased the mean survival time in EAC tumour bearing mice. The study also showed that the hematological profile reverted to more or less normal levels in mice treated with the extract. Treatment with an ethanolic extract of *Indigofera aspalathoides* in

Ehrlich ascites carnicoma induced mice caused alterations in hematological parameters, and increased the survival time of the experimental animals (Rajkapoor *et al.*, 2004).

Thirumurugan *et al.* (2000) observed that when the Rhinacanthone treated mice underwent i.p. inoculation with DLA cells, tumour cell growth was found to be inhibited and there was also a reversal of changes in hematological parameters consequent to tumour inoculation. Nair *et al.* (1991) showed that the lifespan of S-180, EAL and DLA tumour bearing mice was found to be increased when treated with the saffron (*Crocus sativus*) extract, wherein the hematological and biochemical parameters were also found to be within the normal range.

Tinospora cordifolia whole plant influenced the proliferation and myeloid differentiation of bone marrow hematopoetic precursor cells in mice bearing DLA (Singh *et al.,* 2006). Ajit and Janardhanan (2002) reported that pretreatment with the ethyl acetate, methanol and aqueous extracts of a wood inhabitating polypore macrofungus, *Phellinus rimosus* (Berk) pilat, were highly effective in inhibiting the growth of solid tumour induced by DLA cells in mice.

In another investigation, Nevin and Vijyammal (2005) found that a petroleum ether extract of the whole plant of *Aerva lanata* significantly reduced the development of solid tumour induced by DLA cells in mice. Inoculation of 5,6,7-trisubstituted flavones of *Gomphrena martiana* on murine tumour cell lines, sarcoma-180 and Ehrlich's carcinoma, decreased the tumour growth in mice (Pomilio *et al.*, 1994). Treatment with quercetin arrested the growth of S180 ascites tumour in mice (Indap and Barkume, 2003).

The results of the present investigation indicate the anticancer activity of the BBL and RBL extracts. The anticancer activity might be attributed to the phenols, flavanoids, alkaloids and steroidal glycosides present in the plant extracts which were analysed during phytochemical screening. Several plants belonging to *Solanaceae* showed strong cytotoxic and antitumour properties like Solamargine from *Solanum nigrum* (Cham *et al.*, 1987), incanumine from *Solanum* incamim

(Lin *et al.,* 1990) and several other steroidal alkaloidal glycosides (Nakamura *et al.,* 1996; Mohanan and Devi, 1997). Spectral studies indicate the possibility of the presence of flavonoidal and also terpenoidal or steroidal glycosides which might be responsible for all these above changes and for controlling the development of the tumour, indicated by the prolonged lifespan and change in the body weight, and modification of all the hematological parameters in a favourable manner.

MDA, the end product of lipid peroxidation was reported to be higher in cancer tissues (Yagi, 1987). In the present study, MDA level was higher in the liver of DLA tumour bearing mice than the BBL and RBL extracts treated groups. Lipid peroxidation level was increased in DLA tumour induced animals whereas these levels were markedly reduced when treated with BBL and RBL extracts indicating their antiperoxidative activity

Lipid peroxidation was increased in DLA tumour induced animals whereas the levels of LPO indicators markedly reduced when treated with BBL and RBL extracts indicating their antiperoxidative activity. This observation is supported by several reports in the literature. Senthilnathan *et al.* (2002) reported that there was an increase in LPO level and decreased activities of antioxidant enzymes in lungs, liver and kidney of animals having lung cancer. These levels were reversed when treated with paclitaxel and *Withania somnifera.* Pretreatment of caffeine and continuation of its treatment during the course of development of EAC cells restored the EAC-cell induced changes in liver CAT, SOD and LPO (Mukhopadhyay *et al.*, 2003).

Rohini *et al.* (2004) reported that the treatment with *Bacopa monniera* significantly increased the antioxidant enzyme status and inhibited the lipid peroxidation in fibrosarcoma bearing rats. Rajeswar *et al.* (2005b) reported that *Mucuna pruriens* (Fabaceae) seeds decreased the levels of lipid peroxidation in Ehrlich ascites carcinoma in swiss albino mice.

The co-treatment with BBL and RBL extracts reduced the MDA level in tumour induced animals. This might be due to the supplementation of the

antioxidant molecules, including the phenolic compounds, which are responsible for reducing the oxidation of biolgocial molecules like lipids, DNA and proteins. They act either by preventing the formation or neutralising the free radicals and the non-radical oxidant molecules.

The above findings suggest the potential antioxidant and antitumour activities of BBL and RBL.

Thus, the outcome of the first three phases of the present study clearly demonstrates the strong antioxidant potential of the leaves of *Solanum nigrum*. Scrutiny of the results revealed an interesting insight into the differential biochemical mechanisms involved in exerting the antioxidant activities between the plants bearing black and red berries. The leaves of the black berried plants (BBL) improved the antioxidant components of all the categories, in both enzymic and non-enzymic antioxidants. The leaves of red berried plants (RBL) also improved the enzymic and non-enzymic antioxidants, but to a lower extent than BBL in most components.

The interesting trend that was noted was that the glutathione-related components (GSH, GPx, GST, GR and G6PD) responded better to RBL exposure than BBL, suggesting that RBL exerts its action via a glutathione dependent mechanism, while BBL adopts different, as yet unidentified, mechanisms. This trend was observed both *in vitro* and *in vivo*, lending strength to the above suggestion.

However, the overall response of the leaf extracts, on biomolecules (lipids and DNA), intact cells (viability), tissue slices (antioxidant status and LPO) and in animals (antioxidant status, LPO and tumour burden), shows that BBL is a better protector than RBL. Thus it is perceivable that the glutathione dependent biochemical mechanisms adopted by BBL.

With these inferences, in the next phase, an attempt was made to analyse the phytochemicals present in the leaves (BBL and RBL) of *Solanum nigrum*.

PHASE IV

A wide variety of plant-derived active principles representing numerous classes of chemical compounds has shown a potential for the use in the treatment of various diseases. Among the classes of chemical compounds isolated from plants with documented biological activity are alkaloids, glycosides, glycopeptides, aminoacids, inorganic ions etc. (Shukla *et al.*, 2000). The major classes of phytochemicals with potential for antioxidant activity include carotenoids, bioflavonoids, phytosterols, tannis, chlorophylls, terpenoids, and indoles (Cooper *et al.*, 1997).

The preliminary phytochemical and TLC analyses of BBL and RBL showed the presence of the secondary metabolites such as phenols, flavonoids and alkaloids. Some phytochemicals affect the antioxidant status directly, others indirectly and some may have both direct and indirect effects. For example, phytates, tannins and other phenolic compounds, may have a direct antioxidant effect by scavenging free radicals (Tuntawiroon *et al.*, 1991).

The relative concentration of alkaloids and phenolics in dry leaves of *Remus bodlers* and their activity suggested that free radical scavenging effect was mainly due to phenolics (Schemeda-Hirschmann *et al.*, 2003). Alkaloids are also reported to possess antilipidperoxidative effect and direct radical scavenging activity (Kongure *et al.*, 2003).

A positive, significant linear relationship between antioxidant activity and total phenolic content showed that phenolic compounds were the dominant antioxidant components in many medicinal herbs (Cai *et al.*, 2004). The radical scavenging activity of root extract has been reported to reflect their phenolic composition (Pellati *et al.*, 2004).

Flavonoids are a group of polyphenolic substances, very widespread in nature, which are found in plants predominantly in the form of glycosides. Aglycons are especially pharmacologically effective. Many of them show hepatoprotective, diuretic, vasodilatory, anti-inflammatory, antidiabetic, antiallergic, antibacterial and chemoprotective effects, (Read 1995; Calomme *et al.*, 1996).

The FT-IR and NMR spectral studies indicate the probable presence of flavonoidal compounds and also the terpenoidal or steroidal glycosides in *Solanum nigrum* leaves. GC-MS spectral analysis also confirms the occurrence of phenyl rings in the samples. Schilling (1984) isolated 10 flavonoids from the leaf extracts of 11 species belonging to the section *Solanum*; they were all flavols with the predominant glycosidic moiety being glucose. The occurrence of the steroidal alkaloid solasodine and solasodine-like alkaloids in most species belonging to the genus *Solanum* has resulted in a number of phytochemical surveys of various taxa from different geographical regions throughout the world. The medicinal effects of these plants are generally attributed to these glycoalkaloids. Those so far identified in the blacknightshade include solanine, solasonine, solamargine and chaconine (Everist, 1974; Weller and Phipps 1979; Cooper and Johnson, 1984).

So far, few attempts have been made to isolate and identify the various chemicals responsible for the medicinal effects observed in species belonging to the *Solanum* section *Solanum*. The minimal work done on glycoalkaloids, which are said to be responsible for anticancer activity, indicated that solanine and solamargine, from leaves and unripe fruits, are the two most important components (Watt and Breyer-Brandwijk, 1962). It is reported that the these compounds are present in the greatest concentrations in green (i.e.unripe) berries, and that the actual concentration can be variable in accordance to the stage of plant development, as well as being affected by genetic, seasonal and environmental factors (Edmonds and Chweya, 1997).

Ikeda *et al.* (2000) investigated steroidal saponins from the whole plant of *Solanum nigrum* (L). El-Ashall *et al.* (1999) reported that the HPLC analysis revealed the presence of *Solasonin, Solamargine* and *Solanin.* These glycoalkaloids were shown to be distributed in the leaves, stems and roots (Ivanchenko and Tukalo, 1975).

The present investigation indicates the presence of various components having effective functional groups. Thus the BBL and RBL were found to contain biologically active secondary metabolites, which might be responsible for the antioxidant activity along with the other small antioxidant molecules (analysed in phase I) and also responsible for the anticancer activity.

The outcome of the present study, thus, scientifically validates and strengthens the utilization of BBL and RBL in the preparation of medicinal aids to cure a wide spectrum of diseases associated with oxidative stress.

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