CHAPTER IV
DISCUSSION

PART I- IN VIVO STUDIES

As a naturally occurring metalloid, arsenic is ubiquitously present in the environment. Epidemiological data gathered for more than a century have shown that arsenic is a potent human carcinogen.

The *in vivo* study on effect of arsenic trioxide (As$_2$O$_3$) was carried out in two regions of brain - cerebral hemisphere and cerebellum as well as in serum and whole blood of male albino mice (*Mus musculus*) followed by ameliorative effect of vitamin A on arsenic induced toxicity. The selected doses in the present study are 0.5 and 1 mg/kg body weight (based on the LD$_{50}$ of arsenic) while that of vitamin A (0.2 mg/kg body weight) is based on the earlier work done in our laboratory (Rajvanshi, 2002). Oral mode of administration was selected since drinking water is the major source of arsenic contamination. Various parameters studied at the end of treatment were body and organ weights, antioxidant and haematological indices, metabolites and arsenic retention.

Cerebral hemisphere of brain is related to most important functions viz., thought, voluntary movement, language, reasoning and perception, while cerebellum is concerned with movement, balance and posture. Moreover, since not much information is available on brain, long-term (chronic) effect of trivalent arsenic has been investigated on cerebral hemisphere and cerebellum of the adult mouse brain.
Gravimetric studies: Body and Organ weights

A marked reduction in the body weights of the arsenic treated mice was evident followed by a decrease in the weights of cerebral hemisphere and cerebellum of brain. In support of our data, decreased body and brain weights and food consumption in male Wistar rats were also confirmed by Nagaraja and Desiraju (1994), Wagstaff (1978) and Tamura (1978). These reduced gravimetric values thus indicated the lethal nature of the toxicant (Avani and Rao, 2006; Rao and Avani, 2006)

Antioxidant parameters

In the present study, levels of glutathione, activities of superoxide dismutase (SOD) and catalase significantly reduced, whereas lipid peroxide levels were enhanced in the brain of adult mice following low and high doses of arsenic exposure; which is in agreement with the earlier reports (Chinoy and Shah, 2004; Chattopadhyay et al., 2002; Chaudhuri et al., 1999). Recent studies on the mechanism of arsenic toxicity report participation of hydroxyl radicals in arsenic induced disturbances in the CNS (Garcia-Chavez et al., 2003).

A working model on the induction and pathways of ROS and NOS (Nitric oxide species) mediation of the genotoxicity of arsenic in mammalian cells has been proposed in a review by Hei and Filipic (2004). Trivalent sodium arsenite induces ROS formation and lipid peroxidation within minutes of entering cells, leading to an increase in the intracellular oxidative stress, causing mitochondrial damage. This damage could result in the leakage of superoxide anions into the cytosol and the subsequent production of
peroxynitrite anions. Arsenic induced $\text{H}_2\text{O}_2$ can react with $\text{Cl}^-$ (catalysed by myeloperoxidase) to produce hypochlorous acid which also causes DNA damage (Rossman, 2003). Because of high ATP demand, brain consumes $\text{O}_2$ rapidly and is thus susceptible to interference with mitochondrial function, which in turn leads to increased $\cdot\text{O}_2^-$ formation (Casarett and Doull, 2001).

GSH is a versatile protector and executes its neuro-protective function through free radical scavenging, restoration of damaged molecules by hydrogen donation, reduction of peroxides and maintainace of protein thiols in reduced state (Bump and Brown, 1996). Trivalent arsenicals react \textit{in vitro} with thiol containing molecules such as glutathione forming (GS)$_3$As(III) complex (Carter, 1995; Pi et al., 2002) and this property is considered to be one of the main mechanism of actions by which arsenic exerts its toxic effects. GSH, the most abundant non protein sulphydryl (NPSH) component in most cells acts as a nucleophillic scavenger of numerous compounds and their metabolites via enzymatic and chemical mechanism and plays an important role in protection against oxidative damage caused by ROS. The fundamental role of GSH is the protection of thiol groups present in the tissue from oxidative stress. Its depletion could impair cell's defence against toxic actions of many compounds leading to cell injury and death (Pi et al., 2002). GSH has been implicated as an inhibitor of As$_2$O$_3$-induced cell death either by conjugating with As$_2$O$_3$ or by sequestering reactive oxygen species induced by As$_2$O$_3$ (Grad et al., 2001).

\textbf{Ascorbic acid}

Decreased total (TAA) and reduced ascorbic acid (RAA) levels were reported after arsenic treatment in mice brain, while the dehydro form (DHA)
recorded a significant increase in our study. The decreased ascorbic acid levels in our study are concomitant with that of Ramanathan et al. (2002, 2003a,b) in animals exposed to arsenic. Under arsenic induced stress, ascorbic acid gets rapidly oxidized and gets converted to the reduced form. Subsequently, DHA did not get convert to the reduced form due to lack of GSH (which is required for ascorbate recycling), thus explaining accumulation of DHA and decreased levels of TAA and RAA. Earlier work done in our laboratory (Chinoy and Shah, 2004) using ascorbic acid in mice brain has documented comparable results with that of the present data. It is further indicated that arsenic affected ascorbate turnover pattern in the brain. Its low levels in the brain are justified by the fact that ascorbate has critical functions in the brain; by inhibiting peroxidation of membrane phospholipids and scavenger of free radicals (Agus et al., 1997; Shankaran et al., 2001). Further, ascorbate is involved in several oxidation-reduction processes of the tissue by acting as a strong antioxidant and detoxicant in the tissue like liver, etc (Nair et al., 2004a). Hence, its conversion from dehydro form to reduced form is altered due to low levels of GSH in the brain of arsenic exposed mice.

**Protein levels**

A decrease in protein levels occurred following arsenic exposure, with a marked decrease especially in the high dose in both the parts of brain and in the serum. These results are similar to that obtained by Chinoy and Shah (2004) of our laboratory. Decrease in protein levels could be attributed to their damage by singlet oxygen, often due to oxidation of essential amino acids, viz., methionine, tryptophan, histidine or cysteine residues (Halliwell and Gutteridge, 1985). Interactions between As(III) and thiol containing proteins
and peptides have generally been regarded as a basis for the effects of arsenic on the structure and function of these molecules (Serves et al., 1995). Binding of iAs\textsuperscript{III} to proteins is reported and it plays an important role in As metabolism by binding to cytosolic proteins (Styblo et al., 1995). Aldehydes, e.g., malonaldehyde formed during lipid peroxidation can also react with -SH group of proteins to damage them (Halliwell and Gutteridge, 1985), eventually leading to their depletion.

**Total sulphydryl groups**

A steep decline in the levels of total thiol (sulphydryl) groups was noted after arsenic exposure in cerebral hemisphere and cerebellum of brain and in the serum. Trivalent arsenic reacts *in vitro* with sulphydryl groups of glutathione, haemoglobin, proteins, amino acids or enzymes forming As-SH complexes (Pi et al., 2002; Delnomdedieu et al., 1994; Lagerkvist and Zetterlund, 1994) and this property of arsenic is generally considered to be its mechanism of action by which it exerts toxicity. Sulphydryl groups are also prone to be damaged by free radicals resulting in their oxidation (Halliwell and Gutteridge, 1985) due to arsenic poisoning. It has been shown that neuronal produced peroxynitrite may diffuse into bloodstream where it induces oxidative damage by decreasing levels of thiol groups (Kumura et al., 1996; Vasquez-Vivar et al., 1996). This fact could also be correlated with arsenic which is also known to generate peroxynitrite (Pi et al., 2002). Halliwell (1988) and Rozenshstein (1970) have reported decreased whole blood and serum sulphydryl group content and histological changes respectively in brain of albino rats exposed to As\textsubscript{2}O\textsubscript{3} as aerosol.
Metabolic parameters

Arsenic inhibited the activities of carbohydrate metabolism enzymes - adenosine triphosphatase (ATPase), succinic dehydrogenase (SDH), phosphorylase; while increased the deposition of glycogen in the brain tissue of mice. Such results have been reported by Chinoy (2002) of our laboratory after mice were exposed to arsenic trioxide for 30 days. Arsenite inhibits SDH activity and also uncouples oxidative phosphorylation thereby decreasing ATP content in cell (Casarett and Doull, 2001). Shobha Rani et al. (2000) have also reported a decrease in SDH activity in fish brain, in support of our data. Repetto et al. (1994) has also documented inhibition of SDH activity by As(III).

Mitochondria is known to accumulate arsenic and respiration mediated by NAD-linked substrates is particularly sensitive to arsenic. The dwindling in SDH activity also affects conversion of succinate to fumarate during Krebs cycle thereby reflecting its slow rate, thus resulting in decreased synthesis of high energy phosphate reserves such as ATP and ADP (Szinicz and Forth, 1988). Contrarily, an increase in glycogen levels in brain could be attributed to the fact that arsenic inhibits phosphorylase activity, so glycogen breakdown to glucose might decrease, resulting in increased accumulation of glycogen. Similar results have been reported in earlier work on brain and other organs such as liver and gastrocnemius muscle in support of our work (Nair et al., 2004a; Jhala et al., 2004).

Cholesterol and Total Lipids

The present study demonstrates strict depletion of both cholesterol and total depletion after inorganic As$_2$O$_3$ exposure in brain regions together with
serum. The nervous system, particularly the brain is rich in unsaturated fats, which is prone to oxidation by free radicals (Halliwell, 1992). Oxidation of cholesterol to 5-α-hydroperoxide during lipid peroxidation has been shown to damage arterial walls (Halliwell and Gutteridge, 1985). Yet another reason that could be attributed to arsenic induced decrease in cholesterol and total lipids could be due to decrease in the rate of TCA cycle (Szinicz and Forth, 1988) (due to inhibition of SDH and other important enzymes of this pathway), thus resulting in lesser production of acetyl CoA, the final product of Krebs cycle. Acetyl CoA, required for fatty acid synthesis. Hence, the reduced levels of cholesterol and total lipids in brain might be explained in mice exposed to arsenic.

**Cholinesterase (ChE) activity and Arsenic retention**

This enzyme is an important index for neurotoxicity and is widely used for assessing neurotoxic nature of numerous aspects like mercury, pesticides and fungicides (Rao, 1997). Oral administration of arsenic brought about a significant decline in cholinesterase activity in both the brain regions and serum; and this decrease was more precipitous in high dose treated groups of mice. This indicates that arsenic is a potent anti-ChE agent. ChE activity was completely inhibited by arsenic in rat brain (Tripathi et al., 1997; Nagaraja and Desiraju, 1994). Repetto et al. (1994) also have reported low cholinesterase activity in neuroblastoma cells after sodium arsenite exposure.

These noxious effects on various enzymes and metabolites seemed to be correlated to arsenic retention in both the regions of brain and whole blood in a dose dependent manner, as presented in our data. Hence, arsenic
retention exaggerated the toxic effects in tissue studied in the present investigation in the light of other data (Avani and Rao, 2005).

**Blood cell counts and haemoglobin**

Haematological indices - RBC, WBC and haemoglobin also exhibited a severe upturn in their mean values after arsenic exposure. In any living tissue toxic influences exert their effects first at the molecular and then at biochemical levels. The alterations in haematological changes serve as the earliest indicators of toxic effects on the tissue (Paprikar and Sharma, 2003). Decrease in haemoglobin content is correlated with that of RBC count, which in turn might be due to effect of arsenic in haemopoietic organs. Arsenic-induced bone-marrow suppression has been reported by ATSDR (1989). It is worth noting that a significant depletion in erythrocyte number was found after exposure to higher doses of arsenic (1 mg/kg body weight), which is a possible indication of haemolysing power of arsenic. In a study carried out by Delnomdedieu et al. (1995) upon rabbit erythrocytes, 20% of total erythrocyte As (III) burden is associated with protein fraction particularly with haemoglobin. In analogy to our data, Rozenshtein (1970) has also reported eosinophilia resulting from arsenic exposure. A significant decline in the total blood counts - RBC and WBC was reported by Kannan et al. (2001) after treatment in rats and guinea pigs.

**Amelioration by vitamin A**

Retinoid is one of the most promising substances for chemoprevention against certain cancers. In the present study, when vitamin A (as retinyl
palmitate) was supplemented along with both - low and high doses of arsenic, a remarkable resurgence was observed in the body and organ weights, antioxidant enzymes - SOD and catalase, together with the levels of glutathione, lipid peroxidation, and ascorbic acid. Brain and serum levels of protein, total -SH groups, cholesterol, lipids, cholinesterase enzyme and the carbohydrate metabolism indices (ATPase, SDH, phosphorylase and glycogen) along with arsenic retention in the vitamin A supplemented groups were comparable to the control value. Similar type of results have been previously shown by us (Avani and Rao, 2005).

The antioxidant activity of vitamin A against lipid peroxidation induced by other test chemical in rat tissues in vivo is known (Ciaccio et al., 1993; Martin et al., 1996; Chen et al., 1998). This vitamin also acts as an antioxidant by decreasing peroxidation products, scavenging reactive oxygen species and inhibiting the activation of promutagen (Gülkaç et al., 2004). In vivo mitigating studies of doxorubicin by vitamin A (as retinol palmitate) in rat brain have indicated increased resistance of membrane lipids to peroxidation, both endogenously produced and induced in vitro. These results indicate that vitamin A may act as a physiological antioxidant in cell membranes where it is localized. (Ciaccio et al., 1993).

Grosse et al.(1997) have indicated vitamin A as an efficient \( \cdot \text{O}_2^\cdot \) scavenger; infact the antioxidant action of vitamin A is based on their singlet oxygen quenching property, which is also its best documented action (Krinsky,1998). It can be thus proposed that scavenging of free radicals by vitamin A could lead to the protection of -SH groups, thereby the proteins, enzymes; and glutathione, as evident from these data.
Part II: *In vitro* studies

Peripheral lymphocytes have been classically used for detecting genotoxic effects in a great number of studies, since they are considered to be adequate for detecting general exposure. In addition, these cells are in non-proliferative stage (G₀) and have a long half-life (about 3 years) (Pastor et al., 2001). Genetic toxicology end points used as biomarkers include sister chromatid exchanges, aberrations and micronuclei.

Arsenic is a recognized human multisite carcinogen, presently affecting millions of people world-wide as an environmental contaminant. Earlier studies have shown that arsenicals inhibit the proliferation of cultured human cells under *in vitro* conditions. The aim of this study was to evaluate the exposure of a variety of doses of arsenic trioxide upon human peripheral blood lymphocytes

**Sister Chromatid Exchange**

Sister chromatid exchanges (SCEs) are a consequence of the interchange of replicating DNA between chromatids at apparently homologous loci. They are thought to be a result of DNA breakage and reunion. In the present study, mean SCE together with SCE/plate and SCE/chromosome showed a dose-dependent significant increase in the arsenic treated cultures. Arsenic has been identified as a potent clastogen, capable of inducing SCE in both human and rodent cells in culture (Hei and Filipic, 2004). Rudel et al. (1996) have reported increased SCE/cell and SCE/metaphase in peripheral lymphocytes and Chinese hamster ovary (CHO) cells after exposure to different concentrations of arsenic. Inhabitants of West Bengal, India and a
number of other countries affected by chronic hydroarsenicism have reported higher incidence of SCE in peripheral lymphocytes (Jha et al., 1992; Basu et al., 2004; Mahata et al., 2003; Lerda et al., 1994). Appreciable increase in the SCE frequencies have also been observed after sodium arsenite (SA) treatment in experiments with human peripheral lymphocytes (HPL), Syrian Hamster Embryo (SHE) and Chinese Hamster Ovary (CHO) cells (Gebel, 2001). Arsenic compounds are potent clastogens in many cell types and induce SCE in both humans and in rodent cells in culture (Kessel et al., 2002). Earlier work done in our laboratory have reported elevated SCE/cell and SCE/chromosome after As(III) exposure (Nair et al., 2004b).

**Cell cycle proliferative Index (CCPI)**

Lymphocytes cultured for 72h after being stimulated by phytohemagglutinin give a mixture of cells, that have divided once (M₁), twice (M₂) thrice (M₃) or more times (Perry and Wolff, 1974).

In the present study, inhibition of proliferation was observed more in the high dose arsenic treated cultures than low and medium doses, as a result of which, cell cycle proliferative index declined when compared to control. These data are in agreement with the data of Nair et al. (2004b), who have reported decreased CCPI after arsenic treatment. Thus, As₂O₃ caused a lag in the cell cycle kinetics. It is known that presence of toxic agents could greatly extend G₁; or cause the cells to enter G₀ temporarily and thus increase the length of cell cycle. A number of reports exist on the cell cycle arrest and mitosis disturbance induced by arsenic treatment (Yih et al., 1997, 2002; Huang and Lee, 1998). Cell cycle analysis using elutriated synchronous cell
populations revealed that intermediate concentrations of arsenite delays both G\(_1\) and G\(_2\) transit. G\(_2\) cells appear to be more sensitive to arsenite and transit through G\(_2\)/M phase is more delayed than transit through G\(_1\) and apoptosis is induced in these cells as they emerge from aberrant G\(_2\)/M phase (McCabe et al., 2000). Lymphocyte cultures from individuals exposed to high levels of hydroarsenicism had showed slower cell cycle kinetics. A dose-related inhibition of proliferation was observed when lymphocytes were exposed to different concentrations of arsenite during the last 24h before harvesting (Gonsebatt et al., 1992).

**Percentage of M\(_1\), M\(_2\) and M\(_3\)**

Percent M\(_1\) increased significantly, while M\(_2\) decreased with the increase in the dose of arsenic. However, M\(_3\) plates were observed only in the untreated control cultures. This is anticipatable, since arsenic caused a delay in the cell cycle.

**Effect on Average Generation Time (AGT)**

Cell division tracking provides a unique opportunity for the analysis of cell growth kinetics. These include the average time between successive divisions, the proportion of cells that survive and the proliferation per division. The survival of cells is directly related to the proportion of cells that enter the next cell generation. The proportion of time in which the cells reside is directly related to the proliferation per generation.

AGT revealed significant increase in a dose dependent manner in the arsenic treated cultures. In a pilot study on individuals chronically exposed to
arsenic in Mexico, AGT was longer in highly exposed group (Ostrosky-Wegman et al., 1991). Many investigators (Chatterjee and Giri, 1998; Pacchierotti et al., 2002) have reported increased average generation time in vivo in mouse/human affecting delay in cell cycle progression with different test chemicals.

**Effect on Population Doubling Time (PDT)**

Population doubling time is the time required to increase the total cell number to two fold. This shows how much the carcinogen is affecting the growth rate of cells, including the cell cycle kinetics. This analysis provides specific and quantitative information about cell cycle arrest induced by an agent that can be used to assess the contribution of cell cycle arrest effect to the overall growth inhibition.

In our studies, arsenic added cultures exhibited amplified values for mean PDT. Such disparities in the population doubling time for various test chemicals has been reported by Liou et al. (1998) and Torricelli et al. (1997).

**Chromosomal aberrations (CAs)**

In the present study, chromatid breaks/gaps, chromosome breaks/gaps and acentric fragments have been considered under chromosomal aberrations. The results of this index indicate significant increase in the frequency of structural aberrations with the increase in the dose of arsenic in the peripheral lymphocytes. These data are supported by earlier reports that have shown elevated incidence of chromosomal aberrations after chronic arsenic exposure through its contamination in drinking water (Ostrosky-
Increased incidences of CAs have been reported in other human population studies exposed to arsenic through its contamination in ground water in India and elsewhere and these human biomonitoring studies have proved that arsenic is an active inducer of CAs. In fact a positive correlation between CAs and arsenic concentrations in drinking water have been confirmed in a study in West Bengal (Mahata et al., 2003; Basu et al., 2004). Sodium arsenite has been demonstrated to induce CAs like chromatid gaps, fragments, endo-reduplications and chromosomal beaks (Jha et al., 1992; Gebel, 2001). Paldy et al. (1991) has detected significantly higher incidence of aberrant cells, most common being acentric fragments and dicentrics in children exposed to high concentration of arsenic. Arsenic induced augmentation in the frequency of structural chromosomal aberrations has been demonstrated by Avani and Rao (2006a).

**Micronuclei**

Micronuclei (MN) are extracellular bodies in the cytoplasm of cell that form when acentric fragment or whole chromosomes are left behind the main nucleus at telophase. Other reasons for its formation might be spindle fibre defects or damaged kinetochores.

In the present study, a significant increase was observed in the occurrence of binucleates with MNs. This increase was highest in high dose arsenic cultures. Arsenite induced micronuclei has been reported in X-Ray sensitive CHO cells (Wang and Huang, 1994) and in peripheral lymphocytes (Gonsebatt et al., 1997; Avani and Rao, 2006a). Micronuclei has also been used as a biomarker
of carcinogen exposure in populations exposed to arsenic through drinking water in West Bengal, India (Basu et al., 2004) in three cell types - human peripheral lymphocytes, oral mucosa and urothelial cells.

**Aneuploidy**

Mitotic defects, such as those of spindle function may lead to inaccurate distribution of chromosomes between daughter nuclei, resulting in cells with chromosome number that deviate from the normal diploid complement, called aneuploidy.

A dose dependent increase in the yield of aneuploidy (hypoploidy) in lymphocytes grown in presence of arsenic trioxide was observed in the present study. Arsenite induced aneuploidy has also been observed in the CHO cells and human fibroblast cells (HFW) ultimately leading to chromosome loss (Yih et al., 1997; Radha and Natarajan, 1998). Accumulated evidence has shown that arsenic exercises its action on cells which are about to divide, possibly by disrupting spindle microtubule dynamics. The dearrangement of spindle assembly results in apoptosis. The spindle disturbance can produce aneuploidy (Sciandrello et al., 2002) in these cells.

**Mechanism of arsenic induced in vitro toxicity**

Although how inorganic arsenic induces genetic injury is not fully elucidated, oxidative damage is likely involved in the arsenite-induced DNA strand breaks, chromatic breaks, MN and even apoptosis in a variety of cell systems (Ho et al., 2000). Reactive oxygen species (ROS) including
superoxide anion, hydroxyl radicals and hydrogen peroxide have been associated with the genotoxicity of iAs$^{III}$. ROS such as hydroxyl radicals are known to induce DNA base lesion such as 8-oxo 2-deoxyguanosine (8-OHdG) which is an oxidised DNA base and has been shown to be a mutagenic DNA lesion (Kessel et al., 2002; Hei and Filipic, 2004). In fact, antioxidants such as SOD, glutathione peroxidase, glutathione, catalase, NAC (precursor of GSH) and squalene are able to reduce arsenite induced chromosomal aberrations such as SCE and MN. These results imply that arsenite induced oxidative damage could play a role in genotoxicity (Yih and Lee, 1999, Hei et al., 1998). Studies by Liu et al. (2001) and Hei and Filipic (2004) have documented that arsenite induces within 5 minutes after treatment a dose dependent increase of intracellular oxyradical production and superoxide driven hydroxyl radicals in these cells, through its leakage from the mitochondria. Both mitochondrial membrane damage and induction of lipid peroxidation contributes to the genotoxicity of arsenic. In addition, heme oxygenase, an oxidative stress protein and peroxidase are induced by sodium arsenite in various human cell lines (Hei and Filipic, 2004). Arsenic induced generation of nitric oxide free radical also leads to DNA damage (Gurr et al., 2003). Active oxygen species are involved in the induction of MN by arsenite in the XRS-5 cells (Wang and Huang, 1994).

The toxic effects of arsenite have generally been considered to result from its strong interaction with sulphydryl groups, particularly vicinyl sulphydryl groups of functional molecules such as cytoskeleton molecules and ubiquitin conjugation enzymes. Arsenic has been reported to inhibit several human DNA repair enzymes including DNA polymerase B, DNA ligase I and III, which
could be due to indirect effect caused by As-induced changes in cellular redox levels or alterations in signal transduction pathways (Hu et al., 1998). It is thus conceivable that As induces DNA synthesis dependent effects such as CA and SCE. In addition, several other mechanisms of arsenic genotoxicity and carcinogenicity such as modulation of DNA methylation status and inhibition of DNA repair enzymes have also been well noticed in the literature (Ho et al., 2000). Arsenic also inhibits mitosis due to perturbation of spindle apparatus and tubulin which are responsible for cytogenetic alterations (McCabe et al., 2000).

**Amelioration by vitamin A**

Since the speculation of ROS in the genotoxicity of arsenite has been perceived for long, use of various antioxidants in skirmishing arsenic toxicity comes into the picture.

Retinoids have been found to be antioxidant and a free radical scavenger in many *in vivo* and *in vitro* mammalian studies (Quiles et al., 2002). Studies have indicated that vitamin A is a potent inhibitor of lipid peroxidation (Chen et al., 1998) and it also diminishes the level of DNA damage and frequency of chromosomal aberrations (Robichová et al., 2004).

Vitamin A is one of the most important nutrients essential for normal growth and differentiation. It protects cells from injury by free radicals, decreases the expression of certain oncogenes and inhibits growth of certain carcinomas (Gülkaç et al., 2004). Vitamin A also scavenges genotoxic oxygen species, modulates signal transduction pathways, inhibits cell transformation induced by physical and chemical agents and facilitates intercellular
communication inhibited by genotoxic compounds. In this way, vitamins have a protective role in cancer initiation and in the pathogenesis of mutation-related diseases (De Flora et al., 1999).

In the present study, vitamin A supplements to the cultures along with high dose arsenic trioxide (24 h exposure), a notable protective effect was manifested as observed from the mean frequencies of SCE, CCPI, AGT, PDT, %M₁ and %M₂, MN, CAs and aneuploidy. Experiments by AlDosari et al. (1996) have revealed that retinoids significantly reduced the frequency of micronucleus formation in bone marrow of benzo[a]pyrene (BaP) treated mice.

Studies were also carried out where vitamin A supplementation to low and high arsenic exposed cultures caused mitigation effects with respect to the genotoxicity of arsenic. Though this study indicated mitigation against arsenic induced genotoxic effects, its protection exhibited variation with respect to various genotoxic indices. This data thus signifies that vitamin A has a definitive protective action against arsenic exerted genotoxicity in our study. Further studies are necessary.

Role of kalmegh

As ROS induced oxidative stress has been implicated in the pathogenesis of a wide variety of arsenic related disorders, potential antioxidant therapy should therefore include either natural free radical scavenging antioxidant enzymes or agents which are capable of augmenting the activity of these enzymes.
The traditional use of kalmegh (*Andrographis paniculata*) leaf extract has long been known to the people of India. The leaf extract has the reputation of being a febrifuge, prevent cold etc. (Caceres et al., 1998). Diterpene lactone andrographolides are bioactive compounds from the medicinal plant *Andrographis paniculata* Nees. This plant extract has been reported to have antioxidant properties in diabetic rats (Zhang and Tan, 2000). Andrographolide, the bitter principle of the plant extract prevents oxygen radical and H$_2$O$_2$ production in human neutrophils, thus suggesting that ROS could be modulated by it (Shen et al., 2002). Findings by Singh et al. (2001) and Wang et al. (1997) have also suggested the antioxidant potential of this plant on Swiss albino mice and rabbits.

In a pharmacological study, Kapil et al. (1993) demonstrated that andrographolides protected rat liver against hepatotoxins carbon tetrachloride ($\text{CCl}_4$) and tert-butyl hydroperoxide (tBHP) by reducing the levels of lipid oxidation product malondialdehyde (MDA) and by maintaining high levels of the reduced form glutathione (GSH). They suggested that inhibition of malondialdehyde formation revealed the free radical scavenging properties of diterpene lactone andrographolides.

More recent studies on *Andrographis paniculata* have reported its suppressive effects on nitric oxide (NO) production in mouse peritoneal macrophages (Batkhuu et al., 2002). Rajgopal et al. (2003) have concluded from their studies that andrographolide treatment inhibited *in vitro* proliferation of different tumour cell lines, representing various kinds of cancer. The compound thus exerts direct anticancer activity on cancer cells by cell-cycle arrest at $G_0/G_1$ phase through induction of cell-cycle inhibitory protein $p27$ and
decreased expression of cyclin-dependent kinase 4 (CDK-4). Furthermore, they have also concluded that andrographolide can increase the proliferation of lymphocytes.

*In vitro* use of kalmegh in this study at the dose of 0.01 μg/7ml culture media along with high dose of arsenic brought about substantial decrease in the genotoxic endpoints, as observed from the data. A remarkable reduction in the mean SCE (SCE/cell and SCE/chromosome), CCPI, %M₁ and %M₂, AGT, PDT, MN, CAs together with aneuploidy was observed after supplementation of this herbal extract. The constructive role of kalmegh upon arsenic intoxicated lymphocyte cultures on SCE, CCPI, MN, CA and aneuploidy has been earlier reported by Avani and Rao (2006b) in support to the above data. But variation in amelioration in relation to genotoxic indices exerted by As occurred in comparison to control. Work was also done using kalmegh on lower doses of arsenic, which resulted in better mitigation.

It is known that *Syzygium cumini* (Jamun) reduces radiation-induced DNA damage in cultured human peripheral lymphocytes (Jagetia and Baliga, 2004). Numerous other herbal preparations having antioxidant property have known to inhibit chromosome damage and micronuclei in bone marrow of mice (Jagetia and Ganapathi, 1990; Jagetia and Aruan, 1997). Similar mechanism might have been possible for *Andrographis paniculata* as observed in the present study.

Pharmacological studies have demonstrated that andrographolides have improved antioxidant status of intoxicated and diabetic animals, suggesting that they functioned as chain-breaking antioxidants. They are simple, b-unsaturated lactones; hence conceptual hydrogen transfer would be
thermodynamically favoured for allylic hydrogen on carbon C-11 of andrographolide. Such hydrogen transfer results in andrographolyl radical, curtailing lipid peroxide formation. It is thus proposed that besides inhibiting lipid peroxidation by chain breaking, these could also scavenge other free radicals, mainly superoxides (Kamdem and Ho, 2002), thus exerting antioxidant potential on arsenic genotoxicity. Though exact mechanism of action of Andrographis paniculata is not known, however, based on the above reports it is plausible that scavenging of free radicals and hydrogen transfer by andrographolide might play an important role in providing protection against arsenic induced oxidative and genotoxic damage in our study. Other studies are underway for its elucidation.