Chapter 5

DISCUSSION
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5. DISCUSSION

5.1 General
Arsenic is a naturally occurring element in the earth's crust and is very widely distributed in the environment. The toxicity caused by arsenic is also well known. Arsenic toxicity is a global health problem affecting many millions of people. Contamination is caused by arsenic from natural geological sources leaching into aquifers, contaminating drinking water and may also occur from mining and other industrial processes. Arsenic is present as a contaminant in many traditional remedies.

Over the centuries, arsenic has been used for a variety of purposes. Arsenic was a constituent in cosmetics, and used more extensively than at present in agriculture to protect crops from pests. Arsenic as copper acetoarsenite was a pigment in paints, the best known being "Paris green". Before electricity was used for illumination, hydrogen liberated from coal fires and from gas for lighting combined with arsenic in the Paris green used in wallpaper to form arsine, a toxic gas. A fungus Scopulariopsis brevicalis present in damp wallpaper also metabolised the arsenic in Paris green to arsine. In industry, arsenic is used to manufacture paints, fungicides, insecticides, pesticides, herbicides, wood preservatives, and cotton desiccants. Arsenic is an essential trace element for some animals, and is used as an additive in animal feed. Gallium arsenide or aluminium gallium arsenide crystals are components of semiconductors, light emitting diodes, lasers, and a variety of transistors.

In the present investigation, we have therefore tried to study-

❖ The effect of arsenic on human in vivo.

- To evaluate the toxic effects of arsenic first of all arsenic content was measured from the water, hair and nails of the exposed individuals taken from the arsenic affected areas and were compared with the control individuals.
• Complete haemogram from the control and exposed group was studied.
• The effect of arsenic on human leucocyte culture was analysed from both exposed and control individuals.
• The effect of arsenic in induction of micronuclei in lymphocyte culture using cytokinesis block micronucleus assay for rapid quantitative analysis was made.
• Micronuclei assay from buccal smear of the exposed and control group was carried out.

❖ The effect of arsenic compounds on human in vitro.

• To evaluate the toxic effects of arsenic compounds, two different arsenic salts (sodium arsenite and sodium arsenate) were tested using human leucocyte culture.

❖ The effect of arsenic compounds on Mus musculus in vivo.

• To evaluate the toxic effects of arsenic compounds, two different arsenic salts (sodium arsenite and sodium arsenate) were tested using bone marrow chromosome study.

❖ Dietary protectant screened against arsenic compounds on Mus musculus in vivo.

• To evaluate the protection against arsenic compounds two dietary protectants were screened namely black tea and beta-carotene. Black tea infusion was given twice daily in doses simulating human consumption for short duration (7days) and long duration (15days) and was tested against sodium arsenite and sodium arsenate. Beta-carotene was given once daily in doses simulating human consumption for short duration (7days) and long duration (15days) and was tested against sodium arsenite and sodium arsenate. In case of black tea and beta-carotene both, control sets were maintained on distilled water.
5.2. Cytotoxic Effects Of Arsenic:

In humans, arsenic is a chromosomal mutagen (an agent that induces mutations involving more than one gene, typically large deletions or rearrangements). Arsenic appears to have limited ability to induce point mutations. However, elevated frequencies of micronuclei, chromosomal aberrations and aneuploidy have been detected in the peripheral lymphocytes or urothelial cells, or both, of people exposed to elevated levels of arsenic (Chakraborty et al., 2006, IARC, 2004, Vega et al., 1995). Inorganic arsenic induces sister chromatid exchanges, chromosomal aberrations and DNA-protein cross-links in human lymphocytes and fibroblasts. These effects are dose dependent, and sodium arsenite is more potent than sodium arsenate (Kosnett, 1994). Clastogenic activity has also been consistently demonstrated in vivo when inorganic arsenic compounds have been investigated in the bone marrow of mice (WHO, 2001). Hu (1989) recorded an increased frequency of chromosomal aberrations and micronuclei in cultured lymphocytes from smelter workers in Yunnan Tin Corporation and attributed to exposure to arsenic. Similar results were found by Paiva et al. (2006). Oppenheim and Fishbein (1965) obtained chromosomal changes after adding potassium arsenite to a culture of human leucocytes. Similar results were also found from the study of Paton and Allison (1972) when they exposed human diploid cells to arsenic salts. Burgdorf et al. (1977) found an elevated sister chromatid exchange rates in the lymphocytes of 6 patients treated with Fowler's solution. Beaudoin (1974) showed that in rats, 5-12 mg As/kg body weight of arsenate given as a single intraperitoneal injection on day 7 to 12 of gestation produced malformations such as eye defects, exencephaly, renal and gonadal agenesis.
5.2.1 Human study:

5.2.1.1 Effects of arsenic in directly exposed population:

5.2.1.1.1 Arsenic exposure study:

Of the various sources of arsenic in the environment, drinking water probably poses the greatest threat to human health. Drinking water is derived from a variety of sources depending on local availability: surface water (rivers, lakes, reservoirs and ponds), groundwater (aquifers) and rainwater. These sources are very variable in terms of arsenic risk. Alongside obvious point sources of arsenic contamination, high concentrations are mainly found in groundwaters. High levels of arsenic may be found in water from wells, notably in Bangladesh, West Bengal in India, Taiwan and Hungary. The permissible limit of arsenic concentration in ground water given by WHO (1981) was 0.05mg/l. The WHO guideline value for As in drinking water was provisionally reduced in 1993 from 0.05 mg/l to 0.01 mg/l. Whereas, in the affected areas of West Bengal, the level was reported to be 0.2mg/l to a maximum concentration of 3.7mg/l in the highly affected areas (Chowdhury et al. 2000, Mandal et al. 1996).

Airborne arsenic, particularly through occupational exposure, has also given rise to known health problems in some areas. Workers at smelting plants or residents nearby may be exposed to higher than normal levels of arsenic. Individuals sanding or burning wood preserved with inorganic arsenic may come into contact with vapours or dusts containing inorganic arsenic. Systemic toxicity may occur after inhalation, ingestion or topical exposure to inorganic arsenic.

Dietary arsenic is the key source of intake for most of the population. Arsenic in the diet is predominantly organic, which is much less toxic than inorganic arsenic. Total arsenic (organic and inorganic species) in fish and shellfish may be higher than most foods, however, inorganic arsenic constitutes less than 1% of this total value. Several authors have found a higher arsenic content in
the hair and nail samples of the arsenic exposed group who are drinking arsenic contaminated water (Borgono et al., 1971, Goldsmith et al., 1972, Paldy et al., 1991).

In the present investigation a comparison of arsenic content in water, hair and nails of the arsenic exposed group and control individuals have been given in tables 4.1.1. under Observations of this thesis.

The present study showed that the amount of arsenic in the drinking water of the exposed group was 66.75 ± 2.50 µg/l which was significantly higher than the control group (6.44± 0.21 µg/l), and was also higher than the permissible limit as given by WHO (<10 µg/l). The arsenic content of hair (1871.87±709.56 µg/kg) and nail samples (2135.48±984.55 µg/kg) of the exposed group were also significantly higher than the hair (383.75±112.36 µg/kg) and nail samples (273.67±152.47 µg/kg) of the control group.

Therefore it can be concluded from the present investigation that the arsenic exposed individuals in this study have got significantly higher percentage of arsenic in the body than the control group.

5.2.1.1.2 Haemogram study:

Exposure to arsenic can result in anemia and leukopenia, which may be because arsenic can cause bone marrow suppression. Several studies have found that anaemia, leukopenia and thrombocytopenia are common effects of arsenic poisoning in humans following acute (Armstrong et al., 1984) and chronic oral exposures (Tay and Seah, 1975) at doses of 50 µg/kg/day or more. Chronic exposure can produce decreased hematocrit (Chakraborty et al., 2003).

In the present investigation a comparison of haemogram of the arsenic exposed group and control individuals have been given in tables 4.1.1.2. under Observations of this thesis.
In the present study the average haemoglobin level for the exposed group, vary non-significantly from the control group. Moreover, the exposed individuals were found to be non-anaemic. Other blood parameters like TC, MCV, MCH, MCHC do not show any significant variation between the two groups.

Therefore it can be concluded that, the present investigation does not reveal any change in haemogram due to arsenic exposure.

5.2.1.1.3 Chromosomal study:

Several studies have indicated an effect of inorganic arsenic on human chromosomes \textit{in vivo}. Mahata \textit{et al.} (2003) in West Bengal found a statistically significant increase in chromosomal aberration (CA) frequency in arsenic symptomatic individual in compare to the control group. Maki-Paakkanen \textit{et al.} (1998) in his study in Finland found no statistically significant increase in CA, both including and excluding gaps in exposed group in compare to that of the control group. Nordenson \textit{et al.} (1978) examined chromosomal aberrations in lymphocytes from 39 employees at a Swedish copper smelter with high levels of airborne arsenic. In the arsenic-exposed workers, the frequency of chromosomal aberrations was significantly higher than in the controls. A study was carried out in Mexico for evaluation of cytogenetic effects in blood lymphocytes in two populations, exposed to arsenic via drinking water (Gonsebatt \textit{et al.}, 1997). The incidence of chromosome aberrations was found to be significantly higher in the high exposure group. Ostrosky-Wegman \textit{et al.} (1991) in a pilot study in Mexico found that persons chronically highly exposed to well water arsenic showed a non-significantly higher CA frequency in comparison to the respective control.

The present investigation was undertaken to study the effects of arsenic on human chromosomes \textit{in vivo}, using frequencies of total chromosomal aberrations (CA), and Mitotic Index (MI) as endpoints.
The data obtained based on year of exposure to arsenic have been given in table 4.1.1.3 under Observations of this thesis.

In the present study a significant increase in chromosomal aberrations was found in the exposed group in compare to control ($P \leq 0.01$). Increase in chromosomal aberrations is associated with increase year of exposure. Chromosomal aberration for the control group was found to be $0.86 \pm 0.76$. Whereas for less than 20 years exposure group it was $5.17 \pm 1.71$, for 20 to 30 years exposure group it was $5.61 \pm 1.58$, and for more than 30 years exposure group it was found to be $6.44 \pm 1.47$. Among all type of CA found, chromatid breaks were the main CA observed. Chromosome type aberrations such as chromosomal breaks, dicentrics were also observed. Beside this spindle disturbances like polyploidy were observed in few cases. Gaps were not included while calculating CA.

A significant reduction in mitotic index was found in the exposed group in compare to control, which gradually decreases with increase year of exposure. It suggests a slower progression of lymphocytes from S to M phase of the cell cycle (Gonsebatt et al., 1994) as every known clastogen disturbs the orderly progression of cells towards division. Arsenic is a known clastogen (Biswas et al., 2001) and as all the cells are harvested at similar time period so this effect may be due to chronic exposure to arsenic.

Therefore it can be concluded from the present study that arsenic can induce chromosomal aberrations in human lymphocyte culture.

5.2.1.1.4 Cytokinesis block micronuclei assay from lymphocyte culture:

In Argentina, a study was carried out by Dulout et al. (1996) who evaluated micronuclei (MN) in binucleated cells in peripheral blood lymphocytes from 22 Andean women and children exposed to arsenic in drinking water. In the exposed group a highly significant increase in the frequencies of micronuclei was observed in compare to control. Basu et al. (2004) found significantly
increased level of micronuclei frequency in lymphocytes of arsenic exposed group in West Bengal in compare to control.

The present investigation was undertaken to study the effects of arsenic in induction of micronuclei on human lymphocyte culture from individuals exposed to arsenic.

The data obtained from the investigation based on year of arsenic exposure have been given in table 4.1.1.4 under Observations of this thesis.

It was found from the present study that a statistically significant increase in micronuclei was found in the exposed group in compare to control ($P< 0.01$). Micronuclei percentage for the control group was found to be $0.11\pm 0.06$. Whereas for less than 20 years exposure group it was $0.43\pm 0.17$, for 20 to 30 years exposure group it was $0.49\pm 0.11$, and for more than 30 years exposure group it was found to be $0.51\pm 0.14$. Therefore increase in micronuclei percentage is associated with increase year of exposure.

5.2.1.1.5 Micronuclei assay from buccal smear.

Gonsebatt et al. (1997) showed a significant increase in the frequency of micronuclei (MN) in oral cells in persons exposed to arsenic in Mexico. Chakraborty et al. (2006) had shown a significant increase in buccal smear micronuclei percentage in individuals chronically exposed to arsenic. Tian et al. (2001) found an increase in MN in the exposed populations who were drinking arsenic contaminated water. However, the study carried out by Martinez et al. (2005) in northern Chile found elevated but no statistically significant increase in MN frequency in exposed population in compare to the control individuals.

The present investigation was undertaken to study the effects of arsenic in induction of micronuclei on buccal smear from individuals exposed to arsenic.
The data obtained from the investigation based on year of arsenic exposure have been given in table 4.1.1.5 under Observations of this thesis.

It was found from the present study that a statistically significant increase in micronuclei was found in the exposed group in comparison to control ($P \leq 0.01$). Micronuclei percentage for the control group was found to be 0.29±0.07, whereas for less than 20 years exposure group it was 0.83±0.33, for 20 to 30 years exposure group it was 0.91±0.36, and for more than 30 years exposure group it was found to be 0.93±0.28.

As the highest micronuclei percentage was found in the highest exposure group in this study, therefore it could be concluded that buccal smear micronuclei assay is an efficient biomarker in monitoring the harmful effects of arsenic specially where arsenic is drunk in water.

Comments: Measurement of inorganic arsenic in the urine is the best way to determine recent exposure (within the last 1 to 2 days), while measuring inorganic arsenic in hair or fingernails may be used to detect high-level exposures that occurred over the past 6-12 months (Agahian et al., 1990, Buchet et al., 1997). Blood arsenic is a useful biomarker only in the case of acute arsenic poisoning or stable chronic high-level exposure (WHO, 2001). In the present investigation, exposed individuals in the study have got significantly higher percentage of arsenic in the body than the control group.

Though changes in haemogram was observed in arsenic exposed individuals but in the present investigation no significant changes had been found in the exposed individuals.

Arsenic is a potent carcinogen, but the exact mechanism of arsenic-induced carcinogenicity still remain unclear; however short term assays indicate that arsenic does not induce point mutations but rather it acts as a clastogen, that causes breaks in the chromosomes (Biswas et al., 1999). Majority of the exposed population who had an exposure history for a relatively longer period
showed higher incidences of micronuclei formation (Dulout et al., 1996) and chromosomal aberrations (Ostrosky-Wegman et al., 1991). A micronucleus (MN) is a small extranucleus separated from the main nucleus, generated during cellular division by a whole lagging chromosome or by an acentric chromosome fragments. The micronucleus test is used as an indicator of genotoxic exposition, since it is associated with chromosome aberrations (Roberts, 1997). Moore et al. (1997) found higher incidence of micronuclei from blood was found in the arsenic exposed group. Smith et al. (1993) came to the conclusion that the exfoliated oral cells MN frequencies serve as an appropriate index to monitor the genotoxicity induced by arsenic because these cells are in direct contact with the carcinogen. Chromosomal aberrations (CA) in peripheral blood lymphocytes have long been used as a biomarker of early effects of genotoxic carcinogens (Hagmar et al., 2004).

In the present investigation a significantly higher percentage of chromosomal aberration and micronuclei from both lymphocyte culture and buccal smear was observed in the exposed group in comparison to control.

Considering the widespread effects of arsenic induced toxicity in human beings, from the present study it can be concluded that both CA and MN can be used as biomarker to assess the effect of arsenic in the exposed group.

5.2.1.2 Effects of arsenic compounds in vitro:

5.2.1.2.1 Effects of sodium arsenite:

Several studies have indicated an effect of inorganic arsenic on human chromosomes in vitro. The effects of sodium arsenite on human peripheral blood lymphocytes in vitro, were studied by Jha et al. (1992). Sodium arsenite was found to inhibit cell cycle progression and induced chromatid-type aberrations and sister chromatid exchanges (SCEs) in a positive dose related manner. In another study, normal human lymphocytes from three subjects were treated in vitro with 1-2 μM arsenite (Wiencke and Yager, 1992). A significant increase in SCEs were observed. It has been observed that, an in
vitro addition of sodium arsenite to lymphocyte culture from healthy subjects induced the same chromosomal changes as in vivo study (Mahata et al., 2004). Vega et al. (2005) treated human lymphocyte cultures with arsenite in vitro and found a dose dependent increase in hyperploid cells.

The present investigation was undertaken to study the effects of sodium arsenite on human chromosomes in vitro following exposure to different concentrations, using frequencies of total chromosomal aberrations (CA), Damaged cells (DC), and Mitotic Index (MI) as endpoints.

The data obtained following three different concentration of sodium arsenite ($1.6 \times 10^{-7}M$, $1.6 \times 10^{-8}M$, $1.6 \times 10^{-9}M$) screened after 48 hours of treatment have been given in tables 4.1.2.1.1.1 under Observations of this thesis. The doses correspond to $1/15^{th}$, $1/150^{th}$, and $1/1500^{th}$ fraction of minimum toxic dose.

The degree of clastogenic effects induced was directly proportional to the concentrations of chemicals used. The frequencies of total chromosomal aberrations and damaged cells after exposure to all concentrations differ significantly from distilled water. Chromosomal aberration for the control group was found to be nil, whereas for the three treatment groups it was $6.8 \pm 0.84$, $5.6 \pm 0.55$, and $4.6 \pm 0.55$ respectively. Damaged cell percentage for the three treatment groups were $5.8 \pm 1.3$, $4.8 \pm 0.84$, and $4.0 \pm 0.71$ respectively. Chromatid breaks were the most frequent type of structural chromosomal aberration found for all three concentrations used. Beside this chromosome breaks and rearrangements were found in two higher concentrations, but it was absent in the lowest concentration. Among numerical aberration aneuploidy were the most frequent type of changes found and polyploidy was only present in the second highest concentration of salts. Mitotic index was gradually increased in two lower doses then reduced in the highest concentrations.

One-way ANOVA was performed to analyse the effects of individual doses of sodium arsenite. The frequencies of total CA% induced by the different concentrations of the salt were found to differ significantly between the sets ($P \leq 0.01$).
The results indicate that different concentrations of sodium arsenite induce chromosomal aberrations in human lymphocyte culture. However, arsenic as a metal stimulates mitosis as has been seen in linearly in the two lower doses but in the highest dose mitotic index reduced, so the highest dose was found to be mitostatic because arsenic also has an effect on spindle assembly.

5.2.1.2.2 Effects of sodium arsenate:

Crossen (1983) investigated the effects of pentavalent arsenic compounds on sister chromatid exchanges (SCE) in both G0 and 5 BrdU substituted chromosomes. The results showed that pentavalent arsenic could induce SCEs in human lymphocytes. Beside this, considerable variations in response among individuals were also observed. Peters et al. (1977) observed human skin biopsies, treated with sodium arsenate after irradiation with a xenon lamp. The effectiveness of the dark repair enzyme system was found to be significantly reduced in arsenic treated cells. Okui and Fujiwara (1986) investigated the repair inhibiting effects of pentavalent sodium arsenate in normal human and xeroderma pigmentosum (XP) fibroblasts. The results showed that sodium arsenate was found to be effective in preventing repair events.

The present investigation was undertaken to study the effects of sodium arsenate on human chromosomes in vitro following exposure to different concentrations, using frequencies of total chromosomal aberrations (CA), Damaged cells (DC), and Mitotic Index (MI) as endpoints.

The data obtained following three different concentration of sodium arsenite (4.8x10^{-7}M, 4.8x10^{-8}M, 4.8x10^{-9}M) screened after 48 hours of treatment have been given in tables 4.1.2.1.2.1 under Observations of this thesis. The doses correspond to 1/15th, 1/150th, and 1/1500th fraction of minimum toxic dose.

The degree of clastogenic effects induced was directly proportional to the concentrations of chemicals used. The frequencies of total chromosomal aberrations and damaged cells after exposure to all concentrations differ
significantly from distilled water. Chromosomal aberration for the control group was found to be nil, whereas for the three treatment groups it was 3.8± 0.84, 3.2 ± 0.45, 2.0 ± 0.71 respectively. Damaged cell percentage for the three treatment groups were 3.2± 1.09, 2.8± 0.84, 1.8± 0.84 respectively. Chromatid breaks were the only type of structural chromosomal aberration found for all three concentrations used. Among numerical aberration aneuploidy was the only type of change found for all concentrations used. Mitotic index was gradually increased in two lower doses then reduced in the highest concentrations.

One-way ANOVA was performed to analyse the effects of individual doses of sodium arsenate. The frequencies of total CA% induced by the different concentrations of the salt were found to differ significantly between the sets (P< 0.01).

The results indicate that different concentrations of sodium arsenate induce chromosomal aberrations in human lymphocyte culture.

**Comment:** It was evident from the present study that both arsenic salts namely sodium arsenite and sodium arsenate induces chromosomal aberrations in human lymphocyte culture in a dose dependent manner.

A comparison of the similar doses (1/15\textsuperscript{th}, 1/150\textsuperscript{th}, and 1/1500\textsuperscript{th} fraction of minimum toxic dose) of sodium arsenite and sodium arsenate showed that for all concentrations of salts used, the effects were higher in case of sodium arsenite than sodium arsenate.

So it can be concluded from this observation that sodium arsenite is more toxic to human system than sodium arsenate at similar dose and exposure time.
5.2.2 Animal study:

5.2.2.1 Direct effects of arsenic compounds on Swiss albino mice (*Mus musculus*) chromosomes *in vivo*:

5.2.2.1.1 Effects of sodium arsenite:

Several studies have indicated that sodium arsenite is a potent clastogen on mice *in vivo* (Poddar, 2002, Poma et al., 1987). Li and Rossman (1989a,b) demonstrated that in Chinese hamster V79 cells, arsenite treatment inhibits nuclear DNA ligase II activity.

The present investigation was undertaken to study the effects of sodium arsenite on chromosomes, in mice *in vivo*, using frequencies of total chromosomal aberrations (CA), and Damaged cells (DC) as endpoints.

The data obtained following single concentration of sodium arsenite (2.5mg/kg body weight, 1/4\(^{th}\) LD\(_{50}\)) screened after 24 hours have been given in table 4.2.1.1.1 under Observations of this thesis.

A single exposure to sodium arsenite, observed after 24 hours, was found to induce chromosome damage. The frequencies of total chromosomal aberrations and percentage of damaged cells differ significantly from distilled water. Chromosomal aberration for the control group was found to be 0.6±0.55, whereas for the treatment group it was 9.2±0.45. The percentage of damaged cell for the control group was 0.6±0.55, whereas for the treatment group it was 8.4±0.55. The breaks induce were mainly chromatid breaks, indicating damage at the G2 phase of the cell cycle.

The results indicate that a single exposure to sodium arsenite induces chromosomal aberrations in mice bone marrow cells. The present study also indicates that arsenic, as sodium arsenite, can induce chromosome damage in mammalian system.
5.2.2.1.2 Effects of sodium arsenate:

In cultured Chinese Hamster Ovary (CHO) cells arsenate at higher dose levels ($10^{-4}$M), was found to induce chromosomal aberrations (Kochhar et al., 1996). The study carried out by Hodge and Embree (1977) showed that, when sodium arsenate was given to male mice in a single dose intraperitoneally, it did not elicit a dominant lethal effect.

The present investigation was undertaken to study the effects of sodium arsenate on chromosomes, in mice in vivo, using frequencies of total chromosomal aberrations (CA), and Damaged cells (DC) as endpoints.

The data obtained following single concentration of sodium arsenite (10mg/kg body weight, $1/4^\text{th}$ LD$_{50}$) screened after 24 hours have been given in table 4.2.1.1.2 under Observations of this thesis.

A single exposure to sodium arsenate, observed after 24 hours, was found to induce chromosome damage. The frequencies of total chromosomal aberrations and percentage of damaged cells differ significantly from distilled water. Chromosomal aberration for the control group was found to be $0.6\pm0.55$, whereas for the treatment group it was $6.2\pm0.84$. The percentage of damaged cell for the control group was $0.6\pm0.55$, whereas for the treatment group it was $5.8\pm0.84$.

The results indicate that a single exposure to sodium arsenate induces chromosomal aberrations in mice bone marrow cells. The present study also indicates that arsenic, as sodium arsenate, can induce chromosome damage in mammalian system.

Comments: It was evident from the present study that both arsenic salts namely sodium arsenite and sodium arsenate induces chromosomal aberrations in mice bone marrow cells and both of them are toxic to mammalian system.
A comparison of the similar doses (1/4th LD₅₀) of sodium arsenite and sodium arsenate following exposure for 24 hrs was presented in Table 4.2.1.1.3. The results showed that chromosomal aberrations percentage induced by sodium arsenite was 9.2±0.45, which was significantly higher than sodium arsenate 6.2±0.84 (P< 0.01).

So it can be concluded from this observation that sodium arsenite is more toxic to mammalian system than sodium arsenate at similar dose and exposure time.

5.2.2.2 Effects of black tea infusion given alone as dietary supplement in Swiss albino mice (Mus musculus) in vivo:

Gupta et al. (2001) investigated black tea and its two polyphenols (Theaflavin and Thearubigins) against chemically induced genetic damage as measured by chromosome aberrations and sister chromatid exchanges in mice. The results showed that black tea has anticlastogenic effect. Tea has been advocated as a protectant against metal induced chromosome damage by many researchers (Mukherjee et al. 1999, Shiraki et al. 1994, Taniguchi et al. 1992).

In the present investigation, the data obtained after dietary administration of tea infusion daily for 7, 15 days, have been given in tables 4.2.1.2.1, 4.2.1.2.2 under Observation of this thesis.

The total percentage of chromosomal aberration induced by the tea infusion at 7 and 15 days were significantly higher (P< 0.05) than those of distilled water for the same period. Chromosomal aberration for the 7 days control group was found to be 0.6±0.55 and for 15 days control group it was 0.4±0.55, whereas for the 7 days treatment group it was 1.4±0.54 and 15 days treatment group 1.2±0.45. The percentage of damaged cell for the control group was 0.6±0.55 and 0.4±0.55 for 7 days and 15 days respectively, whereas for the 7 days and 15 days treatment group it was 0.8±0.45.
However, total chromosome breaks did not differ significantly from the control sets indicating that it is nonclastogenic as well. The percentage of damaged cells was non-significantly higher than the respective control sets after 7 and 15 days of exposure. Therefore, tea infusion, taken as a dietary supplement for 15 days has not induced chromosome damage to a significant level.

5.2.2.3 Dietary administration of black tea infusion in modifying the clastogenic effects of arsenic compounds in Swiss albino mice (Mus musculus) in vivo:

5.2.2.3.1 Black tea infusion in different duration against single dose of sodium arsenite:

Administration of black tea infusion alone, when fed to mice for once daily for 7 days and 15 days did not induce higher level of chromosome damage, when compared with the negative control set.

Sodium arsenite, as shown in earlier experiment was clastogenic. The mean values of total CA percentage, and DC percentage after exposure to the single concentration of sodium arsenite (1/4\text{th} of LD\textsubscript{50}) were 9.2±0.45 and 8.4±0.55 respectively.

In the present investigation, the data obtained after dietary administration of black tea infusion twice daily for 7, 15 days, followed by single dose of sodium arsenite (1/4\text{th} of LD\textsubscript{50}) have been given in table 4.2.1.2.3.1 under Observation of this thesis.

A significant reduction of total CA% and DC% was observed in the animals primed with black tea infusion for 7 days and 15 days and then exposed to the single concentration of sodium arsenite on day 7 and day 15, one hour after priming.
After 7 days and 15 days priming followed by sodium arsenite, the total CA\% were 3.8±0.84, 3.2±0.84 and DC\% were 3.2±0.45, 2.4±0.55 respectively. A comparison of the effect of black tea infusion given once daily for seven days and fifteen days followed by sodium arsenite showed that, the protective action against sodium arsenite was higher when given for 15 days in compare to tea infusion given for 7 days.

The reduction in clastogenicity was found to be statistically significant as shown by one-way ANOVA ($P<0.01$).

Therefore black tea when gavaged to mice for 7 days and 15 days, followed by exposure to single dose of sodium arsenite, reduced significantly the clastogenic activities of sodium arsenite.

5.2.2.3.2 Black tea infusion in different duration against single dose of sodium arsenate:

Administration of black tea infusion alone, when fed to mice for once daily for 7 days and 15 days did not induce high level of chromosome damage, when compared with the negative control set.

Sodium arsenate, as shown in earlier experiment was clastogenic. The mean values of total CA percentage, and DC percentage after exposure to the single concentration of sodium arsenate ($1/4^{th}$ of LD$_{50}$) were 6.2±0.84 and 5.8±0.84 respectively.

In the present investigation, the data obtained after dietary administration of black tea infusion twice daily for 7, 15 days, followed by single dose of sodium arsenate ($1/4^{th}$ of LD$_{50}$) have been given in table 4.2.1.2.4.1 under Observation of this thesis.

A significant reduction of total CA\% and DC\% was observed in the animals primed with black tea infusion for 7 days and 15 days and then exposed to the single concentration of sodium arsenate on day 7 and day 15, one hour after
priming. After 7 days and 15 days priming followed by sodium arsenate, the total CA% were $3.0 \pm 1.0$, $2.6 \pm 0.55$ and DC% were $2.8 \pm 0.84$, $2.4\pm 0.55$ respectively.

A comparison of the effect of black tea infusion given once daily for seven days and fifteen days followed by sodium arsenate showed that, the protective action against sodium arsenate was higher when given for 15 days in compare to 7 days protection.

The reduction in clastogenicity was found to be statistically significant as shown by one-way ANOVA ($P< 0.01$).

Therefore black tea infusion when gavaged to mice for 7 days and 15 days, followed by acute exposure to sodium arsenate, reduced significantly the clastogenic activities of sodium arsenate.

**Comments:** Dietary supplementation by plant antioxidants has been advocated for the last few decades to minimize the effects of environmental pollutants in human (Daniel and Wenzel, 2003, Hussain et al., 1990, Nandi et al., 1997, Newmark, 1996, Sharma, 1995, Sharma and Talukder, 2000).

Plant products have been shown to protect against genotoxic effects of various environmental pollutants at extracellular, intracellular level or both (Sharma and Talukder, 2000).

Antioxidants like polyphenols, vitamin C, vitamin E are well known anticlastogenic and anticarcinogenic agents. In has been found that rather than purified synthetic agents, general crude dietary plant extracts given orally are much more effective (Sarkar et al.1996b, Sharma 1995). A few plants contain large amounts of antioxidants and act as protectants like garlic, cabbage and *Allium sativum*. They have been found to be an effective anticlastogen against several metals including arsenic (Das et al. 1993a,b, 1996, Roy Choudhury et al. 1993, 1996, 1997).
The present investigation was undertaken to study the protective action of black tea infusion against clastogenic activity of sodium arsenite and sodium arsenate. The observations made here show that these compounds are potent clastogen and can induce significantly high levels of chromosomal aberrations and damaged cells following a single exposure. The protection afforded by black tea infusion, when given daily by gavaging to the mice for 7 days and 15 days followed by single dose of sodium arsenite and sodium arsenate alone on day 7 and day 15 respectively, reduce chromosomal aberration percentage and damaged cell percentage significantly.

A comparison of the protective action of black tea infusion given twice daily for 7 days and 15 days followed by single and similar dose (1/4th LD50) of sodium arsenite and sodium arsenate alone on day 7 (Table 4.2.1.2.5.1) and day 15 (Table 4.2.1.2.6.1) showed that the protection of black tea was higher for sodium arsenite than sodium arsenate, whereas total chromosomal aberrations was found to be lesser in case of sodium arsenate. One-way ANOVA shows that the changes were statistically significant.

The protective activity of black tea infusion against sodium arsenite and sodium arsenate shown here, can be attributed to the combined activity of different polyphenols present namely epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) (Chen and Ho, 1995, Pillai et al., 1999, Vinson et al., 1995). This work is of importance as black tea can be used as a natural dietary supplement to counteract the cytotoxic effects of exposure to arsenic.

5.2.2.4 Effects of beta-carotene prepared from crude leaf of Indian Spinach given alone as dietary supplement in Swiss albino mice (Mus musculus) in vivo:

Spinach leaves contain high amount of carotene including beta-carotene (see Review of Literature, Beta-carotene, Table 1). Effects of beta-carotene were observed on different toxic metal pollutants (Agarwal et al. 1992, Mukherjee et
al. 1991). Crude extract of Indian spinach (*Beta vulgaris* L. var. *benghalensis*) leaf had been observed earlier to protectant against clastogenic effects of lead salts and chromium salts, when administered daily as a dietary supplement (Sarkar et al., 1996 a, 1997).

In the present investigation, the data obtained after dietary administration of beta-carotene from spinach leaves, daily for 7, 15 days, have been given in tables 4.2.1.3.1, 4.2.1.3.2 under Observation of this thesis.

The total percentage of chromosomal aberration induced by beta-carotene at 7 and 15 days were non-significantly higher than those of distilled water after same period. Chromosomal aberration for the 7 days control group was found to be 0.6±0.55 and for 15 days control group it was 0.4±0.55, whereas for the 7 days treatment group it was 1.2±0.84 and 15 days treatment group it was 1.0±0.71. The percentage of damaged cell for the control group was 0.6±0.55 and 0.4±0.55 for 7 days and 15 days respectively, whereas for the 7 days and 15 days treatment group it was 0.8±0.45.

Moreover, chromatid breaks did not differ significantly from the control sets indicating that it is nonclastogenic as well. The percentage of damaged cells was non-significantly higher than the respective control sets after 7 and 15 days of exposure. Therefore, beta-carotene prepared from spinach leaf taken as a dietary supplement for 15 days has not induced chromosome damage to a significant level.
5.2.2.5 Dietary administration of beta-carotene in modifying the clastogenic effects of arsenic compounds in Swiss albino mice (*Mus musculus*) in vivo:

5.2.2.5.1 Beta-carotene in different duration against single dose of sodium arsenite:

Administration of beta-carotene alone, when fed to mice for once daily for 7 days and 15 days did not induce high level of chromosome damage, when compared with the negative control set.

Sodium arsenite, as shown in earlier experiment was clastogenic. The mean values of total CA percentage, and DC percentage after exposure to the single concentration of sodium arsenite (1/4\textsuperscript{th} of LD\textsubscript{50}) were 9.2±0.45 and 8.4±0.55 respectively.

In the present investigation, the data obtained after dietary administration of beta-carotene once daily for 7, 15 days, followed by single dose of sodium arsenite(1/4\textsuperscript{th} of LD\textsubscript{50}) have been given in table 4.2.1.3.3.1 under Observation of this thesis.

A significant reduction of total CA% and DC% was observed in the animals primed with beta-carotene for 7 days and 15 days and then exposed to the single concentration of sodium arsenite on day 7 and day 15, one hour after priming. After 7 days and 15 days priming followed by sodium arsenite, the total CA% were 3.4±0.5, 2.8±0.45 and DC% were 2.6±0.55, 2.2±0.45 respectively.

A comparison of the effect of beta-carotene given once daily for seven days and fifteen days followed by sodium arsenite showed that, the protective action against sodium arsenite was higher when given for 15 days in compare to beta-carotene given for 7 days.

The reduction in clastogenicity was found to be statistically significant as shown by one-way ANOVA (P≤ 0.01).
Therefore beta-carotene when gavaged to mice for 7 days and 15 days, followed by acute exposure to sodium arsenite, reduced significantly the clastogenic activities of sodium arsenite.

5.2.2.5.2 Beta-carotene in different duration against single dose of sodium arsenate:

Administration of beta-carotene alone, when fed to mice for once daily for 7 days and 15 days did not induce high level of chromosome damage, when compared with the negative control set.

Sodium arsenate, as shown in earlier experiment was clastogenic. The mean values of total CA%, and DC% after exposure to the single concentration of sodium arsenate (1/4th of LD50) were 6.2±0.84 and 5.8±0.84 respectively.

In the present investigation, the data obtained after dietary administration of beta-carotene once daily for 7, 15 days, followed by single dose of sodium arsenate (1/4th of LD50) have been given in table 4.2.1.3.4.1 under Observation of this thesis.

A significant reduction of total CA% and DC% was observed in the animals primed with beta-carotene for 7 days and 15 days and then exposed to the single concentration of sodium arsenate on day 7 and day 15, one hour after priming. After 7 days and 15 days priming followed by sodium arsenate, the total CA% were 2.2 ± 0.45, 1.8±0.45 and DC% were 2.0 ± 0, 1.6 ± 0.55 respectively.

A comparison of the effect of beta-carotene given once daily for seven days and fifteen days followed by sodium arsenate showed that, the protective action against sodium arsenate was higher when given for 15 days in compare to 7 days protection.

The reduction in clastogenicity was found to be statistically significant as shown by one-way ANOVA (P≤ 0.01).
Therefore beta-carotene when gavaged to mice for 7 days and 15 days, followed by acute exposure to sodium arsenate, reduced significantly the clastogenic activities of sodium arsenate.

Comments: It has been found earlier by our group that, regular dietary supplementation with crude extracts of plants including *Phyllanthus emblica* L. fruit and bulb of *Allium sativum*, can reduce the effects of clastogens significantly in mice *in vivo* (Das *et al.*, 1993a, Dhir *et al.*, 1990, Roy *et al.*, 1992). Experiments carried out using *Phyllanthus emblica* (Agarwal *et al.*, 1992, Dhir *et al.* 1990, 1993, Ghosh A *et al.* 1993c, Roy *et al.* 1991, 1992, Sharma and Talukder 2000) have shown that when crude extract of the fruit was used as a dietary supplement for a prolonged period, it markedly reduce the cytogenecity of several metals like aluminium, nickel and cesium.

Earlier studies have shown that aqueous crude extract of spinach beet can reduce clastogenic effects of cyclophosphamide (Abraham *et al.*, 1986) and chromium (Sarkar *et al.*, 1993).

The present investigation was undertaken to study the protective action of beta-carotene obtained from spinach leaves against clastogenic activity of sodium arsenite and sodium arsenate. The observations made here show that these compounds are potent clastogen and can induce significantly high levels of chromosomal aberrations and damaged cells following a single exposure. The protection afforded by beta-carotene obtained from crude spinach leaf, when given daily by gavaging to the mice for 7 days and 15 days followed by single dose of sodium arsenite and sodium arsenate alone on day 7 and day 15 respectively, reduce chromosomal aberration percentage and damaged cell percentage significantly.

A comparison of the protective action of beta-carotene given once daily for 7 days and 15 days followed by single and similar dose (1/4th LD50) of sodium arsenite and sodium arsenate alone on day 7 (Table 4.2.1.3.5.1) and day 15...
(Table 4.2.1.3.6.1) showed that the protection of beta-carotene infusion was higher for sodium arsenite than sodium arsenate, whereas total chromosomal aberrations was found to be lesser in case of sodium arsenate. One-way ANOVA shows that the changes were statistically significant.

5.2.2.6 Histopathological studies

The effect of sodium arsenite and sodium arsenate salts alone and in combination with tea and beta-carotene were observed in liver, kidney, spleen, intestine and stomach of mice in vivo.

5.2.2.6.1 Effects of sodium arsenite alone: Following exposure to sodium arsenite liver shows fatty degeneration and mixed inflammatory cells infiltrates including lymphocytes and neutrophils. Kidney shows mild chronic inflammatory cells infiltrates. Stomach shows mixed inflammatory cell infiltrates extending upto the muscularis. Intestine shows chronic inflammatory cell infiltrates with hyperplasia of Payer's patch. No significant abnormalities were found in spleen. Therefore it can be concluded that single dose of sodium arsenite given alone is capable of producing alterations in liver, kidney, stomach and intestine in mild to moderate degree.

5.2.2.6.2 Effects of sodium arsenate alone: Following exposure to sodium arsenate liver shows mixed inflammatory cells infiltrates and focal vacuolar degenerations. Kidney shows fair amount of chronic inflammatory cells infiltrates. Spleen shows mild hyperplasia of white pulp region. Stomach shows mild inflammatory cell infiltrates. Intestine shows chronic inflammatory cell infiltrates with hyperplasia of Payer's patch. Therefore it can be concluded that single dose of sodium arsenate given alone is capable of producing alterations in liver, kidney, stomach, spleen and intestine in mild to moderate degree.

5.2.2.6.3 Effects of black tea alone: Following exposure to black tea alone stomach shows mild chronic inflammatory cell infiltrates with lymphoid aggregate. Intestine shows mild mixed inflammatory cell infiltrates. Liver,
kidney and spleen show normal architecture. So it can be concluded that except some mild changes in stomach and intestine black tea has not produced any significant changes in the organ studied.

5.2.2.6.4 Effect of black tea followed by sodium arsenite: Following exposure to black tea for 7 days followed by sodium arsenite on day 7, liver shows mild inflammatory cells infiltrates, but it is relatively mild in compare to the set where sodium arsenite was given alone. Kidney and stomach show mild chronic inflammatory cells infiltrates. Intestine shows mild inflammatory cells infiltrates. Spleen shows no significant abnormalities. So it can be concluded that black tea has got some protective role against sodium arsenite.

5.2.2.6.5 Effect of black tea followed by sodium arsenate: Following exposure to black tea for 7 days followed by sodium arsenate on day 7, liver shows mild inflammatory cells infiltrates and relatively less amount of infiltrates seen in comparison to arsenate. Kidney and stomach show mild chronic inflammatory cells infiltrates. Intestine shows mild mixed inflammatory cell infiltrates. Spleen shows no significant abnormalities. So it can be concluded that black tea has got some protective role against sodium arsenate.

5.2.2.6.6 Effects of beta-carotene alone: Following exposure to beta-carotene alone, intestine shows superficial ulceration with few chronic cell infiltrations. Stomach shows superficial ulceration in few areas. Liver, kidney and spleen show normal structure. So it can be concluded that except some mild changes in stomach and intestine beta-carotene has not produced any significant alterations.

5.2.2.6.7 Effect of beta-carotene followed by sodium arsenite: Following exposure to beta-carotene for 7 days followed by sodium arsenite on day 7, liver shows normal lobular pattern but central veins in some areas are dilated with congestion of blood. Kidney, spleen, intestine and stomach show normal
structure. So it can be concluded that beta-carotene has got some protective role against sodium arsenite.

5.2.2.6.8 Effect of beta-carotene followed by sodium arsenate: Following exposure to beta-carotene for 7 days followed by sodium arsenate on day 7, liver shows dilated and congested central vein. Intestine shows superficial epithelial ulceration with inflammatory cell infiltration. Kidney, spleen and stomach show normal structure. So it can be concluded that beta-carotene has got some protective role against sodium arsenate.

Comment: Straub et al. (2007) found that when mice were exposed to 0 or 250 parts per billion (ppb) of As(III) in their drinking water for 5 weeks, there was significant vascular remodeling with increased sinusoidal endothelial cell (SEC) capillarization, vascularization of the peribiliary vascular plexus (PBVP), and constriction of hepatic arterioles in As(III)-exposed mice. Mazumder (2005) showed that prolong drinking of arsenic-contaminated water is associated with hepatomegaly in mice. Liver histology showed fatty infiltration and hepatic fibrosis. Roy and Bhattacharya (2006) exposed Channa punctatus (Bloch) for 14 days to nonlethal concentrations (1/20 LC50 and 1/10 LC50) of As2O3. Tissue disorientation, peliosis, and vacuolization accompanied by karyolysis, apoptosis, and necrosis of hepatocytes were significant. In the kidney shrinkage of the glomerulus and increase in the Bowman’s space were observed. Irregularities in the renal tubule including apoptotic and necrotic cells were also common. Decreased intertubular space and enlargement of the height of the brush border cells were noteworthy. Blair et al. (1990) exposed B6C3F1 mice to arsine gas at a concentration of 0.5, 2.5, or 5.0 ppm. Increased hemosiderosis and extramedullary hematopoiesis in spleen were noted.

Sasser et al. (1996) exposed Sprague-Dawley rats to either 0, 0.01, 0.1, 0.5, 1.0, or 2.0 mg/kg doses of lewisite [dichloro(2-chlorovinyl)arsine] a potent toxic vesicant that reacts with the sulfhydryl groups of proteins through its arsenic group. A treatment-related lesion was detected in the forestomach of both sexes at 2.0 mg/kg. These lesions were characterized by necrosis of the
stratified squamous epithelium accompanied by infiltration of neutrophils and macrophages, proliferation of neocapillaries, hemorrhage, edema, and fibroblast proliferation. Mild acute inflammation of the glandular stomach was also observed in some cases at 1.0 and 2.0 mg/kg.

In the present histopathological studies it was found that when sodium arsenite and sodium arsenate is given alone in mice it produces mild to moderate changes in liver, kidney, spleen, intestine and stomach. Black tea and beta-carotene alone produce non-significant changes to the above-mentioned organ. When black tea and beta-carotene was given in combination with sodium arsenite and sodium arsenate, they are found to be protective against these salts as we observed relatively mild changes or no changes in the above-mentioned organ in comparison to the set where sodium arsenite and sodium arsenate was given alone.

5.3. Conclusions:

- Arsenic is a potent clastogen and it causes cytotoxic damage on long term exposure as reflected by statistically significant increase in MN frequency and CA in the exposed population, proportional with the period of exposure.
- Arsenic salts namely sodium arsenite and sodium arsenate exerts similar type of cytotoxic effects, but for similar doses effects were higher in case of sodium arsenite than sodium arsenate.
- In Swiss albino mice (*Mus musculus* L.2n=40) arsenic salts (sodium arsenite and sodium arsenate) have cytotoxic effects reflected by significant increase in CA in bone marrow chromosomes in compare to control. Most prevalent aberration was chromatid break and CA was higher in case of sodium arsenite than sodium arsenate.
- When animals were primed by tea and beta-carotene separately, in doses simulating human consumption for short duration (7 days) and long duration (15 days) followed by administration of arsenic salts (sodium arsenite and sodium arsenate) singly and similar dose, the cytotoxic damages were found to be low. The preventive effect was more when primed for long duration than short duration.