Alkyl Mercury chloride compounds-induced genotoxicity in human blood cultures and corrective role of Ascorbic acid (Vitamin C)

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Abstract: Methyl mercury chloride is a xenobiotic metal that is a highly deleterious environmental pollutant. The biotransformation of mercuric chloride ($HgCl_2$) into methyl mercury chloride (CH_3HgCl) in aquatic environments is well-known and humans are exposed by consumption of contaminated fish, shellfish and algae. The genotoxicity induced by mercury compounds remains controversial. Therefore we have investigated the genotoxic effect of methyl mercury chloride (MMC; CH_3HgCl) at two concentrations (100, and 1000 µg/L) and the role of ascorbic acid (Vitamin C) at a single concentration of (9.734 mm) on MMC-treated short-term human lymphocyte cultures. We assessed the chromosomal aberrations (CAS), sister chromatid exchange (SCE) and COMET assay in control and MMC-treated lymphocyte cultures with and without Vitamin C supplementation. The results showed that MMC has increased the frequency of CAS and SCE/cell in a dose-dependent manner than control values. CH_3HgCl also, induced DNA damage in determined by COMET assay. These effects were prevented by the addition of Vitamin C to MMC-treated lymphocyte cultures. Data revealed that, mutagenic activity of MMC and the protective role of Vitamin C on mercury compounds-induced genotoxicity in human lymphocyte cultures is probably due to its strong antioxidant and nucleophilic nature.

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Introduction

Mercury, one of the most widely diffused and hazardous organ-specific environmental contaminants, exists in a wide variety of physical and chemical forms, each of which with unique characteristics of target organ specificity (Aleo *et al.,* 2002). Different forms of mercury found naturally; include the metallic form, inorganic compounds as well as alkyl, alkoxy and aryl mercury compounds. Once introduced into the environment, mercury compounds can undergo a wide variety of transformations. In sediments, inorganic mercury ($HgCl_2$) may be converted into methyl (CH_3HgCl) and dimethyl (CH_3CH_2HgCl) forms by methanogenic bacteria. This biotransformation constitutes a serious environmental risk, given that CH_3HgCl is the most toxic of the mercury compounds that accumulates in the aquatic food chain, eventually reaching human diets (Tchounwou *et al.,* 2003).

Efforts were made to find therapeutic agents capable of minimizing genotoxicity of various natural and man-made compounds. The genotoxicity induced by mercury compounds remains controversial (Rao, 1997).The three modern "faces" of mercury are our perceptions of risk from the exposure of billions of people to CH_3HgCl in fish, mercury vapor from amalgam tooth fillings and CH_3CH_2HgCl in the form of thimerosal added as an antiseptic to widely used vaccines (Clarkson, 2002).

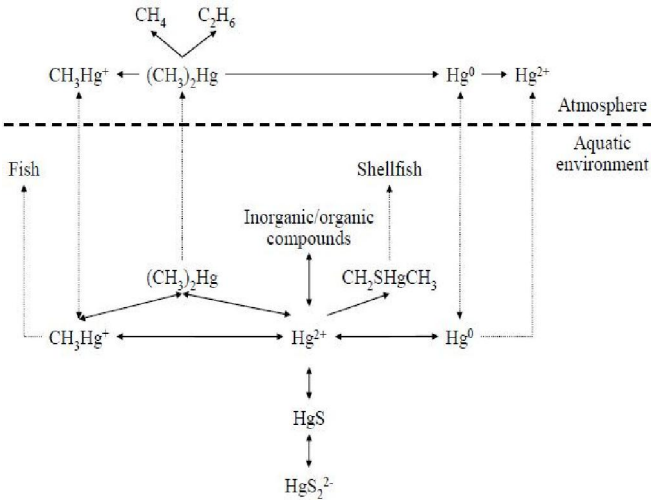
Mercury genotoxicity has been usually attributed to its ability to react with the sulfhydryl groups of tubulin, impairing spindle function and leading to chromosomal aberrations and polyploidy (De Flora *et al.,* 1994). Another important mechanism of mercury genotoxicity is its ability to produce free radicals that can cause DNA damage (Schurz *et al.,* 2000; Ehrenstein *et al.,* 2002).

In vivo studies have demonstrated a clastogenic effect of mercury on people exposed to this element in their work environment, through the consumption of contaminated food, or accidentally, Increased numbers of chromosome alterations and micronuclei have been reported in people who consume contaminated fish (Franchi *et al.,* 1994; Amorim *et al.,* 2000) and in miners and workers of explosive factories (Anwar *et al.,* 1991; Al-Sabti *et al.,* 1992). Negative results were also obtained in some cases (Mabille *et al.,* 1984; Hansteen *et al.,* 1993), demonstrating that cytogenetic monitoring of peripheral blood lymphocytes in individuals exposed

to mercury from different sources may not be completely specific (Schurz *et al.,* 2000).

Most of *in vitro* studies with lymphocytes also used high doses (250 to 6250 µg/L) of mercury compounds in order to evaluate its clastogenic effects (Betti *et al.,* 1993; Ogura *et al.,* 1996)

Ascorbic acid (AA) is well known antioxidant. The antioxidant property can be helpful in reducing clastogenic, mutagenic and carcinogenic properties of certain chemical agents (Siddique *et al.,* 2007).



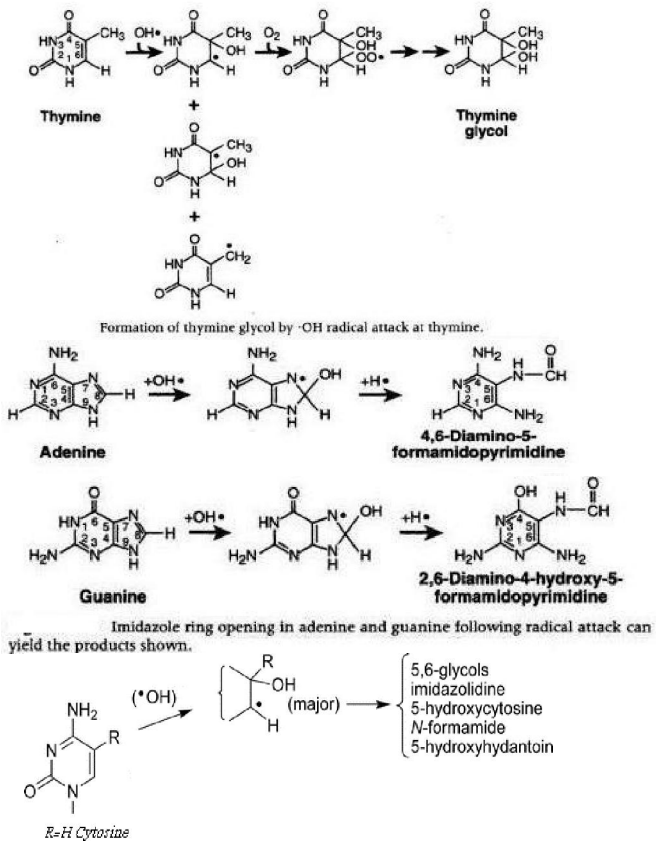
Ascorbic acid (AA) is involved in various biological activities, including free radical scavenging, and it has been demonstrated that treatment with AA significantly reduced the genotoxicity of well-known mutagens (Odin, 1997; Cabrera, 2000; Kaya *et al.,* 2002; Siddique *et al.,* 2005).

In this context, an anticlastogenic activity of ascorbic will be studied against the genotoxic damage induced in human lymphocyte cultures *in vitro* by an organic mercury compound; Methyl mercury chloride (CH_3HgCl). Low doses were used in order to assess genotoxic and cytotoxic effects of these compounds at concentrations normally found in nature (40 to 500 µg/L in the earth' crust environment). Also, low doses of ascorbic acid will be used because AA has both antioxidant and pro-oxidant activities (Bijur *et al.,* 1997).

In DNA, there are four different nucleobases, the pyrimidines Thy and Cty, and the purines Gua and Ade. In their free-radical chemistry, these nucleobases have many properties in common There are, however, also considerable differences which strongly affect the various reaction pathways. In nucleoside and nucleotides, free-radical attack mainly occurs at the base moiety. These reactions largely involve addition reaction. Only the sugar moiety and the methyl group in Thy can act as H-donors. (Mathews and Holde, 1996)

There is good evidence that H_2O_2 and O_2 radicals do not react directly with DNA. The main species responsible for oxidative DNA damage in cells appear to be hydroxyl radicals that are generated by a Fenton reaction involving the reduction of H_2O_2 by transition state metal ions e.g. Fe^{+2} or Hg^{+2} (Henle and Linn,1997). Hydroxyl radical-induced oxidation of DNA is complex, leading to a multitude of modifications at the level of DNA bases (thymine, cytosine, adenine, guanine, and 5-methylcytosine) (Breen and Morphy , 1995; Cadet *et al.,* 1997) .Such reactions can be generalized as follows:

DNA $[Hg^{2+}]$ + H_2O_2 → DNA $[Hg^{2+}]$ + $^{\bullet}OH$ + OH^{-}



The COMET assay is a method used to measure DNA damage in individual cells was done for the first time by Singh and his colleagues in 1988. The idea was to combine DNA gel electrophoresis with fluorescence microscopy to visualize migration of DNA strands from individual agarose-embedded cells. If the negatively charged DNA contained breaks, DNA supercoils were relaxed and broken ends were able to migrate towards the anode during a brief electrophoresis. If the DNA was undamaged,

the lack of free ends and large size of the fragments prevented migration. Determination of the relative amount of DNA that migrated provided a simple way to measure the number of DNA breaks in an individual cell (Olive and Banáth, 2006).

Much of the interest in this method comes from its potential applications in human biomonitoring and in ecological assessment of sentinel organisms exposed to environmental contaminants (Speit and Hartmann, 2005; Azqueta *et al.,* 2009).

Material and Methods

1- Subjects

The present study was carried out In Human Cytogenetics Department, National Research Centre, Cairo, Egypt. Blood samples were obtained from 20 healthy nonsmokers, among cytogenetic staff, 10 females and 10 males, aged 25-50 years, with no recent history of exposure to mutagens. The donors had given an informed written consent to participate in the study.

2- Methods

Chemical reagents

a) CH3HgCl

Two concentrations of CH_3HgCl were used in the cytogenetic tests (D1=1000 µg/L and D2=100 µg/L) corresponded to levels observed normally in nature (low doses). These CH_3HgCl concentrations and durations of exposure were evaluated from various earlier studies (Verschaeve *et al.,* 1984; Tchounwou *et al.,* 2003). Cells treated only with distilled water were used as controls.

b) Ascorbic acid (Vitamin C)

Ascorbic acid (Vitamin C) was used at a single concentration of 8.6 µg /5 ml culture medium (9.734 mm).

***In vitro* test with peripheral blood lymphocytes**:

Peripheral blood lymphocyte micro-cultures were performed according to standard methods (Moorehead *et al.,* 1960; Hungerford, 1978). For differential staining of sister chromatids, 0.26 mm bromodeoxyuridine (BrdU; Sigma) was added to the cultures, mixed gently and cultures were incubated at 37 °C for 72 h in complete darkness.

After 24 h, the treatment of CH_3HgCl (100 and 1000 µg/L) was given separately and along with a single concentration of 8.6 µg /5 ml culture medium of ascorbic acid. Cells were cultured for another 48 h at 37°C in an incubator.

- For chromosomal aberrations and SCE (sister chromatid exchange):

Cells were collected by centrifugation, then exposed to hypotonic solution (0.075 m KCl) for 45 min and fixed three times in acetomethanol (1:3). A few drops of this concentrated cell suspension were placed on clean and chilled slides and allowed to air dry.

- Slides were stained with solid Giemsa stain, GTG banding, SCE.

Scoring of slides

For chromosomal aberrations, Giemsa-stained and GTG- banded slides were used and a total of 50 metaphase plates were counted from each culture of an individual following the standard classification (Lisa *et al.,* 2009).

A minimum of 25 well-spread metaphases from each culture of an individual were scanned to calculate SCE/cell and SCE/chromosome at the magnification of 100X.

- For COMET assay:

- Cultured lymphocytes were isolated according to the procedures of Sierens *et al.,* (2001) with some modifications using Ficol-Hypague (separating medium). Samples were then centrifuged at 1400 ×g at 25°C for 15 min.
- Lymphocytes were apparent as a layer directly above the Ficoll-Hypague in the tube, and was carefully aspirated.
- Lymphocytes were then washed 3 times with PBS (50 mM, pH 7.4), and then centrifuged for 5 min at 180 × g. Cell pellets were resuspended in 50 mM PBS solution.
- After centrifugation, tubes were decanted and the alkaline COMET assay procedure on isolated lymphocytes was performed according to (Singh *et al.,* 1988; Woznika and Blasiak 2003).
- 110 µl of 0.5% agarose solution was added to a fully frosted microscope slide, immediately covered with a No. 1 coverslip and allowed to solidify. Next, lymphocytes were mixed with 75 µl of 0.5% LMPA (kept at 37°C), the coverslip was removed carefully and the cell mixture added.
- The coverslip was replaced and the slide placed on a tray kept on ice packs to solidify the LMPA. Next, the coverslip was removed and a top layer of 75 µl 0.5% LMPA was added. The coverslip was replaced and the slide returned onto the cold tray. After the top agarose layer had solidified, the coverslip was removed and the slide immersed in a jar containing cold lysing solution (2.5 M NaC1, 100 mM EDTA, 1% SLS and 10 mM Tris, pH 10.0, to which 1% Triton X-100 and 10% DMSO were added fresh). The slides were left at 4°C for at least 1 h.

- Electrophoresis and staining:

Slides were removed from the lysing solution and placed on a horizontal electrophoresis box. The unit was filled with an alkaline buffer (300 mM NaOH and 1 mM EDTA, pH 13.0) to a level ~ 0.25 cm above slides. Cells were exposed to alkali for 20, 40 or 60 minutes to allow for DNA unwinding and expression of alkali-labile damage. To electrophorese the DNA, an electric current of 25 V and 300 mA was applied for either 20 or 40 minutes using a compact power supply.

- All of these steps were conducted under yellow light to prevent the occurrence of additional DNA damage. After electrophoresis, the slides were placed horizontally and Tris buffer (0.4 M Tris, pH 7.5) was added gently to neutralize the excess alkali. Slides were allowed to sit for 5 minutes and this was repeated 3 times. Finally, 50 µl EtBr (2 µg/ml) was added to each slide and covered with a coverslip. After 2-5 minutes, coverslips were removed and the slides rinsed in distilled water to remove excess EtBr. Slides were covered again with coverslips, placed in a humidified air-tight container to prevent drying of the gel, and analyzed within 24 h.

Statistical analysis:

Results of chromosomal aberrations, and SCE, were compared with controls by Student's t-test. Probability values of 0.05 or less were considered significant. For COMET assay multiple comparisons between groups were done by means of multifactor ANOVA.

Results:

- Chromosomal aberrations:

Cytogenetic analysis of all subjects using GTG banding revealed that all karyotypes of all subjects under investigation were as shown in Table (1).

Methyl mercury chloride at both concentrations was able to induce structural aberrations at the chromosome level. Table (2) summarizes the results of analysis of chromosomal aberrations in human peripheral lymphocytes in culture following treatment with two different concentrations of CH_3HgCl and their negative controls (Photo: 1). Data obtained from 500 metaphases analyzed per treatment (50 metaphases / individual) showed a significant increase in the frequency of chromosomal gaps and chromosomal breaks for Dose1 (D1) of CH_3HgCl concentration alone compared with D1 and Vit.C. Meanwhile a significant increase in the frequency of chromosomal breaks was occurred when comparing Dose2 (D2) of CH_3HgCl concentration alone and with D2 and Vit.C. and the comparison between D1 and D2 alone revealed significant increase in chromosomal gaps only (Table 3). No significant variation was noted with respect to numerical aberrations throughout this study.

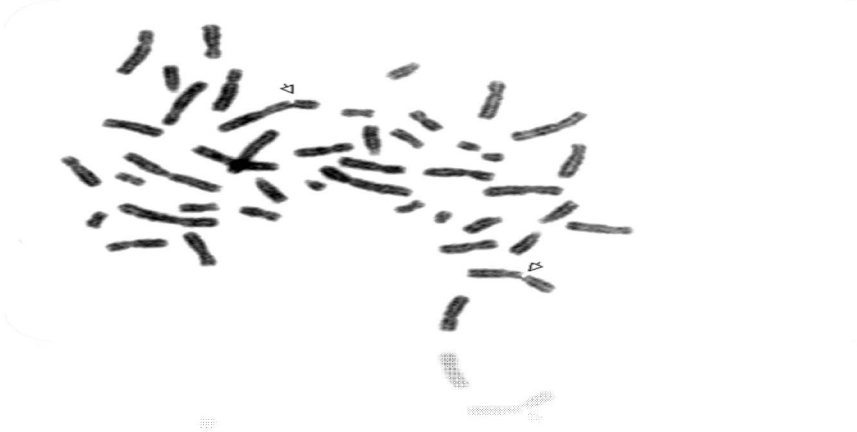


Photo 1: Metaphase spread stained with solid Giemsa stain showing chromosome and chromatid breaks.

Table (1): Age, sex, and karyotype results of subjects

Subject no.	Age	Gender	Karyotype
1	28	female	46,XX N
2	30	male	46,XY N
3	43	male	46,XY N
4	31	female	46,XX N
5	39	female	46,XX N
6	30	female	46,XX N
7	31	female	46,XX N
8	32	female	46,XX N
9	40	male	46,XY N
10	51	female	46,XX N
11	28	female	46,XX N
12	50	female	46,XX N
13	32	male	46,XY N
14	28	male	46,XY N
15	29	male	46,XY N
16	22	female	46,XX N
17	30	male	46,XY N
18	32	male	46,XY N
19	35	male	46,XY N
20	○27	male	46,XY N

Table (2): Frequency of cells with gaps, and breaks after exposure to two different doses of Methyl Mercury chloride alone and with Vit.C.

S	D1[a]		D1+ Vit. C		D2[b]		D2+ Vit. C[c]	
	Break	Gab	Break	Gab	Break	Gab	Break	Gab
1	10	2	8	0	4	0	1	0
2	9	0	-	0	2	0	2	0
3	8	0	7	0	0	0	1	0
4	14	6	6	0	3	0	1	0
5	9	5	9	0	0	0	0	0
6	8	0	7	0	5	2	3	1
7	12	2	8	0	4	0	4	0
8	9	0	7	0	7	0	5	0
9	5	3	3	0	4	0	0	0
10	8	0	2	0	1	1	2	0
11	7	0	-	0	1	0	3	0
12	13	1	8	0	5	0	0	0
13	8	0	6	0	4	2	2	3
14	10	5	8	0	5	0	3	1
15	9	0	7	1	6	0	4	3
16	13	6	11	1	9	4	5	6
17	9	0	7	3	6	1	0	0
18	11	3	5	0	0	0	0	0
19	7	0	4	0	0	0	0	0
N	19	19	17	19	19	19	19	19
Mean ± SD	9.42±2.32	1.74±2.26	6.65±2.21	0.26±0.73	3.47±2.65	0.53±1.07	1.89±1.76	0.74±1.59

a) MMC (D1) = methyl mercury chloride treated culture with higher dose.
b) MMC (D2) =methyl mercury chloride treated culture with lower dose.
c) Vit.C = Vitamin C (Ascorbic acid).

Table (3): Comparison of the 2 doses alone and with Vit. C

Culture types	Break	Gap
D1 Vs D1+ Vit. C	0.0008**	0.0167*
D1 Vs D2	4.7680	0.0373*
D2 Vs D2+ Vit. C	0.0053**	0.3597

*P < 0.05 Significant, **P ≤ 0.005 Highly significant.

- SCE values:

There was a significantly (P<0.05; P<0.005) higher number of SCE in CH_3HgCl treated cultures at lower and higher concentrations than in either the control or ascorbic acid alone added cultures except in control Vs D2+ Vit. C it was non significant "P >0.05" (Photo: 2, Tables: 4 and 5).

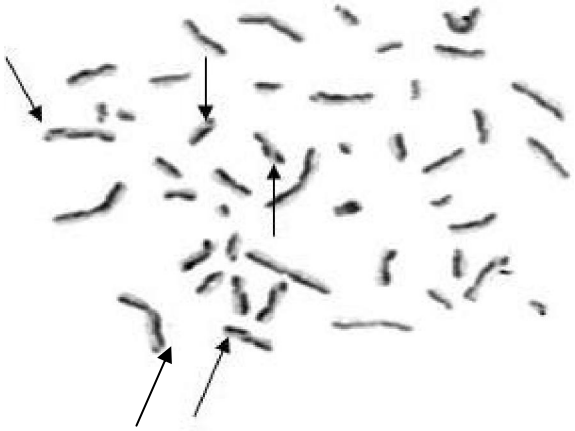


Photo 2: Metaphase spread showing increased Sister Chromatid Exchange (SCE).

Table (4): Values of Sister Chromatid Exchanges (SCEs) in human blood cultures

S	Control	D1	D1+Vit. C	Control	D2	D2+Vit. C
1	2.6	8.2	3.2	2.6	3.5	4.8
2	2.5	2.3	1.9	2.5	3.4	3.2
3	4.8	2.4	5.2	4.8	6.2	4.5
4	5.5	8.6	3.2	5.5	4.3	5.4
5	4.2	7.9	6.2	4.2	6.3	5.6
6	3.2	8.2	6.2	3.2	2.5	5.6
7	5.4	2.8	4.8	5.4	3.6	1.9
8	2.3	7.4	3.7	2.3	4.8	2.7
9	5.6	8.5	5.8	5.6	6.2	5.6
10	5.2	8.7	6.2	5.2	6.1	5.6
11	2.5	2.3	3.2	2.5	3.4	1.2
12	4.8	3.2	2.1	4.8	2.5	1.2
13	3.2	6.2	4.3	3.2	5	2.1
14	4.8	7.4	5.3	4.8	6.2	4.4
15	4.2	8	5.8	4.2	6.1	3.5
16	5.7	8.3	6.1	5.7	6.4	5.2
17	4	7.8	5.3	4	5.9	4.2
18	3.8	6.2	5.8	3.8	4.5	5.5
19	2.5	7.4	5.3	2.5	5.1	5.6
20	5.2	6.8	5.7	5.2	4.9	3.2
N	20	20	20	20	20	20
Mean ±SD	4.10±1.198	6.40±2.38	4.80±1.40	4.10±1.198	4.80±1.32	4.00±1.56

Table (5): Comparison of results of SCEs of 2 doses alone and with Vit. C versus control.

Control Vs D1	**0.0005****
Control Vs D1+ Vit. C	**0.0529***
Control Vs D2	**0.0254***
Control Vs D2+ Vit. C	**0.9003**
D1 Vs D1+ Vit. C	**0.0112***
D1 Vs D2	**0.0142***
D2 Vs D2 + Vit. C	**0.0316***

*P < 0.05 Significant, **P ≤ 0.005 Highly significant.

COMET assay:

A significant DNA damage was observed for the applied two concentrations of CH_3HgCl alone when compared with the control and no significant damage when comparing the two concentrations of CH_3HgCl and the added vitamin C (Photo: 3, Tables 6 and 7).

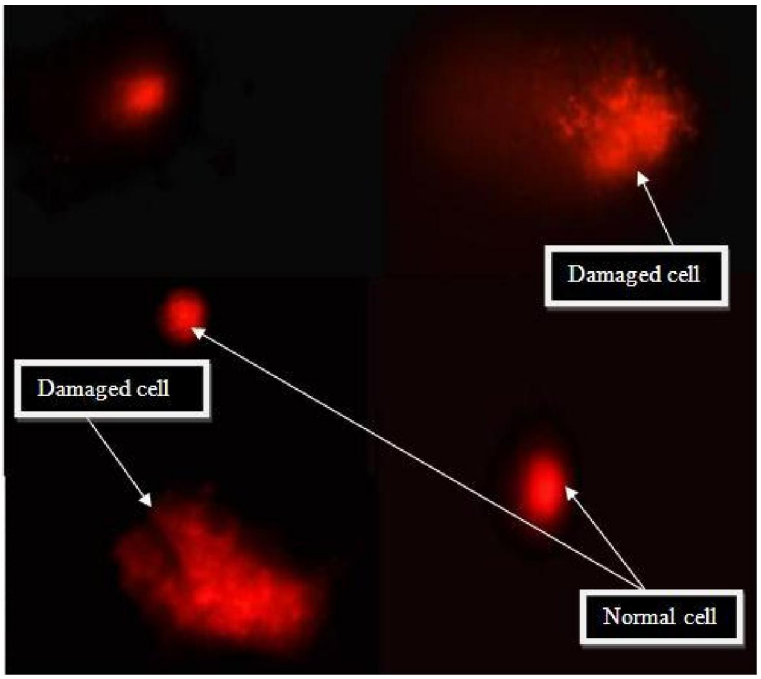


Photo 3: Typical COMETs from lymphocytes treated with CH_3HgCl to induce breaks. Damage categories were indicated.

Table (6): Percentage of DNA damage estimated by COMET assay

S	Control (%)	D1 (%)	D1+Vit. C (%)	D2 (%)	D2+Vit. C (%)
1	7.00	90.00	12.00	25.00	8.00
2	4.00	45.00	15.00	30.00	18.00
3	5.00	45.00	5.00	13.00	3.00
4	3.00	79.00	42.00	30.00	7.00
5	4.00	30.00	10.00	17.00	5.00
6	5.00	65.00	28.00	24.00	17.00
7	4.00	78.00	40.00	55.00	32.00
8	6.00	58.00	28.00	22.00	19.00
9	3.00	88.00	52.00	60.00	30.00
10	4.00	62.00	32.00	28.00	8.00
11	4.00	26.00	8.00	19.00	6.00
12	3.00	48.00	15.00	25.00	8.00
13	5.00	62.00	28.00	40.00	10.00
14	3.00	58.00	30.00	25.00	8.00
15	6.00	75.00	38.00	30.00	12.00
16	3.00	62.00	28.00	18.00	8.00
17	4.00	65.00	34.00	25.00	12.00
18	3.00	74.00	51.00	30.00	12.00
19	5.00	85.00	42.00	26.00	15.00
20	4.00	74.00	51.00	30.00	12.00
N	20	20	20	20	20
Mean ±SD	4.25±1.16	63.45±0.18	29.45±0.15	28.60±0.12	12.50±0.08

Table (7): Percentage of DNA damage estimated by COMET assay

DNA damage (%)	Control	D1	D1 + Vit. C	D2	D2 + Vit. C
	4.20±1.060	63.50± 17.90 [a,b]	29.50±14.70	28.60±11.50 [c,d]	12.50±7.70

a,b= statistical significant difference between control and D1 alone or with Vitamin C. c,d= statistical significant difference between control and D2 alone or with Vitamin C

Discussion

Most of or almost all carcinogenic and mutagenic agents take the DNA molecule as a target site. So, many of these agents are cytotoxic to cell because they damage this DNA molecule. Mercury has been found to be a causative agent of various disorders including neurological, nephrological, immunological, cardiovascular, musculoskeletal, reproductive and even genetic. Organic mercury is considered to be more toxic as it can pass via blood brain barrier (Zahir *et al.,* 2005; Zahir *et al.,* 2006).

Our investigated agent Methyl Mercury Chloride (MMC; CH_3HgCl) is an interesting example to study the cytogenetic effect of one of mercury compounds. The present study also, showed a protective role of vitamin C on mercury-induced genotoxicity *in vitro*.

Mercury compounds induce a general collapse of antioxidant mechanisms in the cell by binding to the sulfhydryl groups of glutathione, a radical scavenger. Such a collapse results in cell degeneration, loss of membrane integrity and finally cell necrosis (Schurz *et al.,* 2000). Necrosis can be indicated by a decrease in mitotic index, as

shown by the present results. A decrease in mitotic index followed by an increase in the generation of reactive oxygen species was detected in human blood lymphocytes exposed to CH_3HgCl (Ogura *et al.,* 1996). Another mechanism that may contribute to cell death induced by mercury compounds is apoptosis. Shenker *et al.,* (2000) reported that, CH_3HgCl caused a significant increase in cytochrome *c* in the cytosol of T cells. This effect may justify the higher cytotoxic action of CH_3HgCl, as observed in the present study. Previous obtained results support the higher cytotoxic effect of CH_3HgCl (Ogura *et al.,* 1996; Bahia *et al.,* 1999).

A number of *in vitro* studies on genotoxic effects of mercury and its compounds based on cytogenetic tests have been published. Their results suggest that ,organic compounds are generally more active, in terms of genotoxicity, than inorganic compounds (Gebhart and Rossman 1991; De Flora *et al.,* 1994; Ogura *et al.,* 1996; Bahia *et al.,* 2000). The binding of this metal to sulfhydryl groups of glutathione blocks its function as a free radical scavenger (Schurz *et al.,* 2000). Thus, free radicals become available to cause DNA damage (Ogura *et al.,* 1996). These mechanisms can lead to "double-strand breaks" that can be visualized as the chromatid gaps observed in the present study (Harvey *et al.,* 1997) and/or give rise to more evident chromosome alterations such as breaks, rearrangements, and so on (Morgan *et al.,* 1998).

In the present study, a significant dose related increase in the number of cells showing chromosome aberrations was observed after treatment with CH_3HgCl (Table1 and 2). However, such increase was not linearly related to dose. This effect can be explained by the fact that CH_3HgCl acts similarly to X-rays (Betti *et al.,* 1993), increasing DNA damage in a dose-dependent manner until a plateau is reached, with a decrease in damage being observed eventhough the doses continue to increase.

There was a significantly ($P<0.05$; $P<0.005$) higher number of SCE in CH_3HgCl -treated cultures at lower and higher concentrations than in either the control or ascorbic acid added cultures except in control Vs D2+ Vit.C it was non significant ($P>0.05$).

Sister chromatid exchange (SCE) frequency is a commonly used index of chromosomal stability in response to environmental or genetic mutagens. Induction of the frequency of SCE/cell was significantly higher and found to be dose related in our study, indicating the genotoxic nature of CH_3HgCl in human blood cultures. Other inorganic and organic mercury compounds are also known to increase induction of SCE/cell in human (Betti *et al.,* 1993) this supports our data.

This effect reflects two different phenomena: a dose-dependent increase in the proportion of normal cells whose DNA is damaged and a dose-dependent decrease of the probability that such cells can survive higher exposures (Hall, 2000). Our results showed that, exposure of peripheral blood lymphocytes to low doses of CH_3HgCl is sufficient for the expression of evident genotoxic and cytotoxic effects.

The mechanism of genotoxicity induced by mercury compounds is quite complex. Heavy metals meet directly with DNA as metals are positively charged ions or electrophiles and easily form complexes with DNA by binding with negatively charged centres or nucleophilic sites to cause mutagenesis (Gebhart and Rossman, 1991). Further, metals including mercury can react with sulfhydryl (SH) groups of proteins (Burton *et al.,* 1995) associated with DNA replication and alter genetic information and replication fidelity. Another mechanism by which mercury damages DNA molecule is via its probable involvement of reactive oxygen species (ROS) or oxygen free radicals (OFRs) and induces DNA strand breaks (Cantoni *et al.,* 1984; Aust *et al.,* 1985). Further, OFRs are known to reduce glutathione (GSH) levels (Shenker *et al.,* 1993) or inhibit GSH synthesis (Chang *et al.,* 1999), leading to a decline in intracellular thiol levels. Moreover, mercury alters DNA base pairs by binding with DNA and also forms cross-links between DNA strands, causing mutations (Guille *et al.,* 1981).

Previous results suggested that, the COMET test was highly effective in revealing DNA-damaging caused by environmental mutagens such as MMC (Olive and Durand, 2005).

In the present work it was confirmed that, COMET assay is more sensitive than chromosomal aberrations and SCE in revealing the damaging effect of MMC.

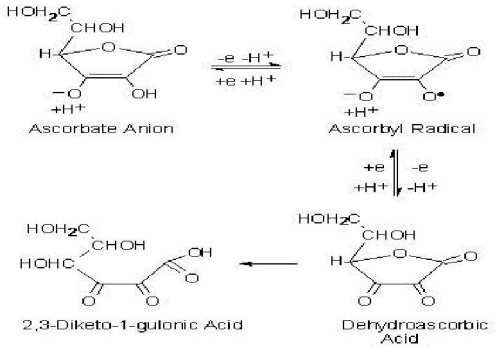
Ascorbic acid is known to provide antimutagenic and anticlastogenic properties against a number of chemicals in a variety of systems including man. In the present study also, addition of ascorbic acid to CH_3HgCl -treated blood cultures prevented such genotoxic and mitotis disturbing effects induced by mercury, revealing the protective nature of vitamin C. Vitamin C is a strong antioxidant having nucleophilic properties and binds to mercury ions (Hg^{2+}) to reduce mercury- induced DNA damage

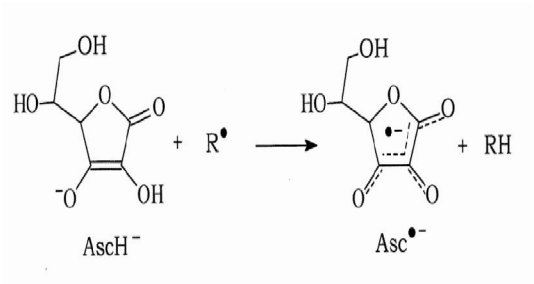
(Sato *et al.,* 1997; Rao, 1997; Alpsoy *et al.,* 2009). It further manifests its detoxification effect by removing or minimizing free radicals produced by mercury (Herbaczynska *et al.,* 1995). Ascorbic acid also protects DNA from oxidative damage (Antunes and Takahashi, 1999), reduces DNA damage exerted by irradiation (Green *et al.,* 1994) and also reduces micronucleus (MN) frequencies in polychromatic erythrocytes of bone marrow in rodents exposed to heavy metals and irradiation (Konopacka *et al.,* 1998), in support of our data.

Ascorbic acid was extremely unstable *in vitro*. It reduces other radicals including the hydroxyl radical, organic alkoxyl and peroxyl radicals, urate radical, tocopherol radical, and the ferric and cupric ions. It readily oxidizes to dehydro-ascorbate in a two step reaction. When the ascorbate anion loses one electron, it becomes an ascorbate radical, $A^{\bullet}$ .This ascorbate radical has several possible fates *in vitro*. It can loose another electron and become dehydro-ascorbate, gain an electron and proton to regenerate the ascorbate anion and react with another radical and form a stable compound, or two ascorbate radicals can dismutate to form one ascorbate anion and one dehydro-ascorbate molecule. (Noctor and Foyer, 1998; Deutsch, 2000; Smirnoff, 2005)

Ascorbic acid, or ascorbate, is an antioxidant because of the high reducing potential of its carbon-carbon double bond (Buettner, 1993) .Each step of ascorbate oxidation is reversible, and this permits recycling back to ascorbate. (Mehlhorn *et al.,* 1991; Coassin *et al.,* 1991; Buettner *et al.,* 1993). Loss of the second electron results in dehydroascorbic acid, which is not an acid. This contrasts with ascorbate, which carries a negative charge at physiologic pH (the pK_a of the carbon-3 hydroxyl is 4.2). DHA is quite unstable at physiologic pH and temperature, with a half-life of about 6 minutes (Drake *et al.,* 1942; Winkler, 1987). With hydrolysis of the lactone ring, DHA is converted to 2,3-diketo-1-gulonic acid (Chatterjee,1970 ; Bode *et al.,* 1990). This last step is probably irreversible in cells, although it can be reversed by mercaptoethanol *in vitro* (Deutsch *et al.,* 1996). Loss of ascorbate through decomposition of DHA is obviously wasteful, and cells such as the erythrocyte have redundant mechanisms to recycle DHA back to ascorbate.

Vitamin C (ascorbate, $AscH^-$), for example, can donate a hydrogen atom to a free radical molecule ($R^{\bullet}$) thereby neutralizing the free radical while becoming an ascorbate radical itself ($\bullet Asc^-$, or $Asc^{\bullet -}$, in different notation). But the $^{\bullet}Asc^-$ free radical is very stable because of its resonance structure (shown by the dashed lines in the illustration). Moreover, $AscH^-$ is readily regenerated from $^{\bullet}Asc^-$ with NADH or NADPH-dependent reductases (Hossain, 1985; Menon *et al.,* 2008).





Thus it is evident from our study that ascorbic acid prevents mercury compounds induced-genotoxicity in blood cultures due to its probable nucleophilic and detoxifying nature. Hence, supplementation of vitamin C through the diet might be useful to the population occupationally exposed to heavy metal poisoning.

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