Original Paper

Genotoxicity of Benzene in Mammalian Cells (*Rattus Rattus)* and its Minimization by Medicinal Plant Extracts and Vitamin C

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ABSTRACT

Benzene is an enlisted industrial carcinogen with genotoxic effects. The present work was aimed at studying the genotoxic effects (chromosomal aberrations and mitotic index changes) of benzene on somatic (bone marrow) cells of 10-15 week old albino rats (*Rattus rattus*). Many medicinal plants and vitamins are known to have antioxidant and anticlastogenic properties. Therefore, vitamin C and crude extracts of fruits of medicinal plants *Phyllanthus emblica* (Amla) and *Allium sativum* (garlic) cloves were tested for their comparative effectiveness in minimizing the genotoxicity of benzene.

Genotoxicity of benzene was investigated at doses of 1/40, 1/20, 1/10, 1/5 oral LD_{50}. Antioxidants vitamin C (10 mg/kg b.wt) and crude medicinal plant extracts (*P. emblica*=1000mg/kg b.wt., *A. sativum*=1000mg/kg b.wt.) were tested for their ability to minimize genotoxic effects of benzene (at 1/10 LD_{50} dose), at pre, concurrent and post treatment levels. Statistical analysis was done by 'student t-test'.

Benzene was observed to cause significant increase in number of chromosomal aberrations, percentage of aberrated cells and depression in mitotic-index. *P. emblica*, *A. sativum* extracts, and vitamin C significantly reduced all the types of observed abnormalities induced by benzene. They showed best results during pre-treatment. Genotoxicity of benzene was best minimized by *A. sativum* extract. *P. emblica* and vitamin C showed more or less similar results.

Thus, the daily intake of *A. sativum* extract might prove to be beneficial in minimizing and providing protection against benzene genotoxicity.

Key Words: Benzene; Genotoxicity; Chromosomal aberration; Somatic cell; Antioxidant; Anticlastogen; Vitamin C, *Phyllanthus emblica;* Amla; *Allium sativum;* Garlic

Introduction

Benzene is generally used in laboratories, agriculture, hospitals, textile industries, home products, as a constituent in motor fuels; as a solvent for fats, waxes, resins, oils, inks, paints, plastics, and rubber; in the extraction of oils from seeds and nuts in photogravure printing, as a chemical intermediate, in the manufacture of detergents, explosives, pharmaceuticals, and dyestuffs.[1,2] It was also reported to be present in unleaded gasoline and cigrarette smoke.[3] Consumers may be exposed unknowingly at home through the use of commercial products, that may contain benzene in concentrations of 10 to 100%, such as rubber cement, brush cleaners, paint strippers and bicycle tire patching compounds.[4] Additional benzene containing consumer products are carburetor cleaners and art and craft supplies.

Environmental and occupational exposure to benzene may be due to its presence in emissions from burning coal and oil, motor vehicle exhaust, and evaporation from gasoline service stations and in industrial solvents.

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Benzene is enlisted as an industrial chemical carcinogen with genotoxic effects. Studies have demonstrated a high incidence of chromatid and chromosomal aberrations in rabbits, and chromatid deletions in bone marrow chromosomes of rats after subcutaneous dosing with undiluted benzene.[5] After intraperitoneal dosing of male rats with 0.5ml benzene/kg b.wt., in the bone marrow study, both chromatid and chromosome aberrations were significantly increased over the control value upto 8 days after dosing.[6] A significant increase was found in CA, MN and comet tail length (DNA damage) in benzene exposed group.[7] Results of a study suggested that benzene could induce a variety of DNA damage types such as single strand breaks (SSBs), double-strand breaks (DSBs) and oxidative base modification.[8]

Medicinal Plant Extracts and Vitamin C

Anticlastogens namely, vitamin C and crude extracts of *Phyllanthus emblica* (amla) and *Allium sativum* (garlic) were tested for their ability to minimize the genotoxicity of benzene.

Antimutagenic or anticlastogenic effects of vitamin C has been observed in various test systems.[9,10] Genoprotective effect of vitamin C was also observed against ethyl methane sulphonate (EMS) in a fish - *Anabas testudineus*.[11]

Crude extract of *Phyllanthus emblica* Linn. (amla), has been shown to reduce the chromosomal abnormalities induced by metanil yellow and zinc chloride, nickel and lead; cesium chloride; lead and aluminium; cadmium and chlordane in mice.[12-17] Crude extract reduced the cytotoxic effect to a greater extent than vitamin C alone. Pre-treatment with amla (*Emblica officinalis*) fruit clearly indicated its protective effect against bio-effects of irradiation to Swiss albino mice.[18] Amla administration to rats increased their body level of protein responsible for regulating the transcription of genes involved in lipid and cholesterol metabolism.[19]

Aqueous extract of garlic *(Allium sativum)* bulb has been found to inhibit the mutagenic effects of ionizing radiations and various clastogens in *Salmonella*, Chinese hamster cells and mouse *in vivo*.[20,21] Four antioxidants (tetrahydro-beta-carboline derivatives) identified in the aged garlic extract, have shown strong hydrogen peroxide scavenging activities in *in vitro* assay.[22] Spontaneous short-term fermentation of garlic was found to increase the anti-oxidative properties of garlic. It was attributed to the increased level of polyphenols.[23]

The present work was aimed at studying the genotoxic effects (chromosomal and cellular abnormalities) of benzene on somatic cells of rats at different doses and different exposure levels, followed by minimization of genotoxic effects by two medicinal plant extracts (*Phyllanthus emblica* and *Allium sativum*) and vitamin C at three different levels, i.e., pre-, concurrent and post-treatments to determine the scientific evidence of their curative values in the most effective period. Finally, comparative effectiveness of these two medicinal plant extracts and vitamin C for the chemical at the somatic tissue level was observed.

Materials and Methods

Animals: For the present investigation, Albino rats (*Rattus rattus*) in the age group of 10-15 weeks and weighing 75-100gm were used as test animals. They were housed 6 animals per cage, in the animal house of Biosciences Department, H.P. University, Shimla at an optimum temperature of 25±5^{0}C in sanitary cages. They were given standard pellet diet (Hindustan Lever Ltd.) and water was given *ad libitum*. The material used for cytological study was bone-marrow for somatic tissue analysis.

Benzene Treatment: In the first set of experiments, genotoxicity of benzene was investigated on the bases of chromosomal aberrations. Test animals were divided into four groups (A, B, C & D). Different doses of benzene were given as 1/40, 1/20, 1/10, and 1/5 of the oral LD_{50} value (3800 mg/kg bwt) respectively.

Plant Extracts and Vitamin C Treatment: In the second set of experiments, antioxidants, i.e., vitamin C and medicinal plant extracts (viz. *Phyllanthus emblica* vern. Amla and *Allium sativum* vern. Garlic) were tested for their ability to minimize genotoxic effects of benzene. Crude extracts of medicinal plants were prepared by boiling, and their concentrations were kept roughly according to daily human uptake recommended. Dose of vitamin C was decided according to its daily recommended therapeutic dose. 1/10 LD_{50} dose of benzene was tested for minimization by the mentioned antioxidants.

Biochemical Investigations: All the animals were given IP 2.5 mg/kg body weight of colchicine prepared in HBSS (concentration 0.7 mg/ml), 2½ hours before sacrificing them. Then each animal was dissected for femurs for bone marrow after 3, 5, 7 & 10 days of benzene treatment. Antioxidants were checked for their effectiveness at pre, concurrent and post-treatment

levels after 3 days of benzene treatment. The doses of antioxidants were taken as 1000 mg/kg bwt for *Phyllanthus emblica* and *Allium sativum* (Grp A & B) and 10 mg/kg bwt. for vitamin C (Grp C). The protocol adopted for chromosomal aberration assay for bone marrow was that of Scribner et al (1983)[24] with some modifications. The slides were air-dried and stored in dust-free chambers. They were stained with 2% Giemsa stain and mounted in DPX and put in an oven (preheated) at 60^0C overnight. Observations were made with the help of research binocular microscope under different magnifications. Readings of various aberrations were taken to know the extent of genotoxic effect. This was followed by readings of chromosomal aberrations after various treatments (pre, concurrent & post) of medicinal plant extract plus industrial chemicals, to understand the anticlastogenic or minimizing effect of antioxidants.

Statistical analysis: Calculation of mitotic-index and aberrant metaphases was done by 'student t-test' for both: in case of chemical carcinogen, as well as chemical carcinogen plus medicinal plant extracts.

Results

Clastogenicity of Benzene:

Metaphase plates from the animals treated with different doses of benzene showed significant differences in M.I., CA/cell and %AC as compared to the control values. Overall there was a decreasing trend in M.I. with increasing post-treatment period and dose of benzene, except at 3rd and 5th days in group-B (**Table 1, Fig 1a**). Also, there was a decrease in CA/cell and %AC values with increase in post-treatment days. But the opposite trend for CA/cell and %AC values was observed in relation to dose, as there was increase in both the aspects in relation to dose (**Table 1, Figs 1b, 1c**). Exception was seen at the highest dose level where CA/cell increased (instead of decreasing) at 7th day of post-treatment interval (**Table 1, Fig 1b**). CA/cell and %AC values were very high at the highest dose level which proves the toxicity of benzene (**Table 1**). All the groups showed maximum of chromosomal and chromatid deletions, minutes, end-to-end associations, clumping and hypoploidy, but very less or negligible number of polyploidy, hyperploidy and diploidy. Group-A animals treated with 97 mg/kg bwt (1/40 LD_{50}) of benzene showed constant decrease in M.I. from 5th to 10th day post-treatment level, but it was slightly less at the 3rd day of post-treatment interval (**Table 1, Fig 1a**). In group-B (190 mg/kg bwt or 1/20 LD_{50}), CA/cell and %AC seemed to decrease with increase in post-treatment interval except at 7th day where %AC increased slightly (**Table 1, Figs 1b, 1c**). Also, there was constant decrease in M.I. with increase in post-treatment days. For 3rd and 5th days of post-treatment, M.I. was higher than the control, but later it started decreasing (**Table 1, Fig 1a**). This shows irreversible damage to cells and their chromosomes due to benzene toxicity. In the case of group-C (360 mg/kg bwt or 1/10 LD_{50}), there was a decrease in M.I. value as the post-treatment interval increased, except at 7th day of post-treatment, where there was slight increase as compared to the previous level. But overall M.I. values were greater than that of group B (**Table 1, Fig 1a**). Metaphase plates from group-D (760 mg/kg bwt or 1/5 LD_{50}), at 3rd day level could not be procured due to death of test animals after dosing. Thus there was constant decrease in M.I. as the post-treatment period increased. All the M.I. and CA/cell values were far less than the control values (**Table 1, Fig 1a**). Thus benzene was observed to be an efficient genotoxic agent causing significant increase in number of chromosomal aberrations per cell, as well as in percentage of aberrated cells. There was depression in mitotic-index due to benzene, in the test animals.

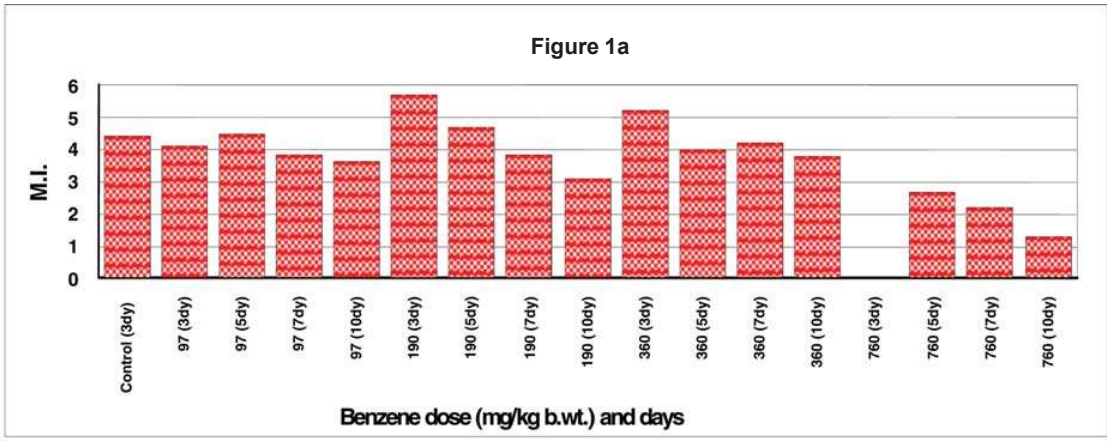
Anticlastogenicity against Benzene:

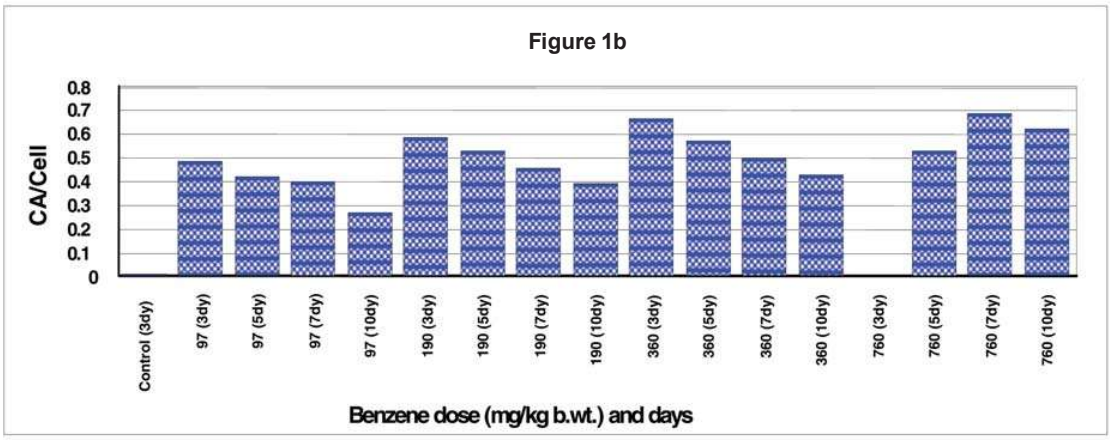
To study the anticlastogenicity against benzene, 360 mg/kg bwt dose at 3rd day post-treatment level was taken as the criterion of comparison.

Treatment with *Phyllanthus emblica* (Amla) extract was able to significantly reduce chromosomal aberrations caused by benzene treatment in test animals (**Table 2**). All the M.I, CA/cell and %AC values, after giving amla extract were significantly less than that of these values for benzene. Minimum values, i.e., best results were observed at the pre-treatment level.

Treatment with *Allium sativum* (Garlic) extract was also able to reduce the chromosomal aberrations caused by benzene to a significant level (**Table 3**), and the values observed were even less than that for the amla extract (**Figs 2a, 2b, 2c**). Thus, garlic extract proved to be a more efficient anticlastogen against benzene. Best results were found at the pre-treatment level (**Table 3**).

Vitamin C treatment was found to be effective in reducing chromosomal aberrations caused by benzene (**Table 4**), but not as efficiently as amla and garlic extract (**Figs 2a, 2b, 2c**). All these values were significantly lower than the values observed for animals treated with benzene, but were greater than those observed for amla or





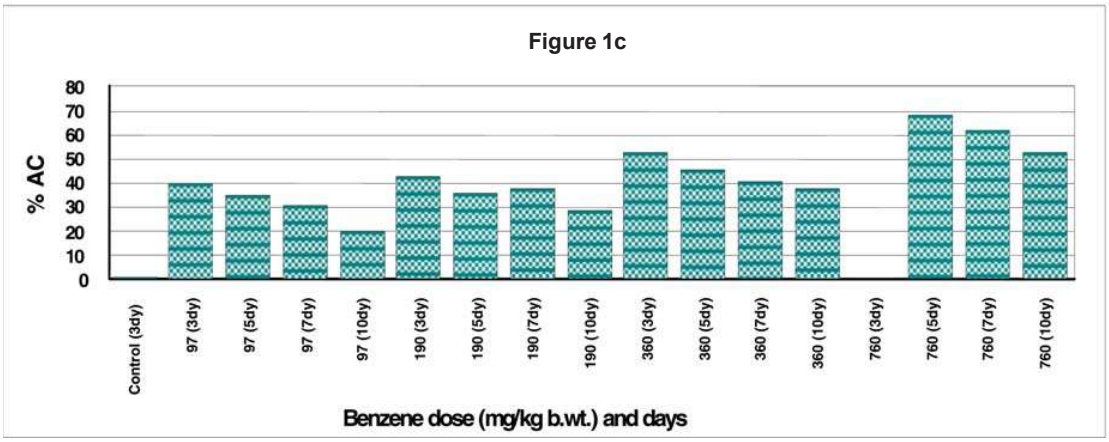
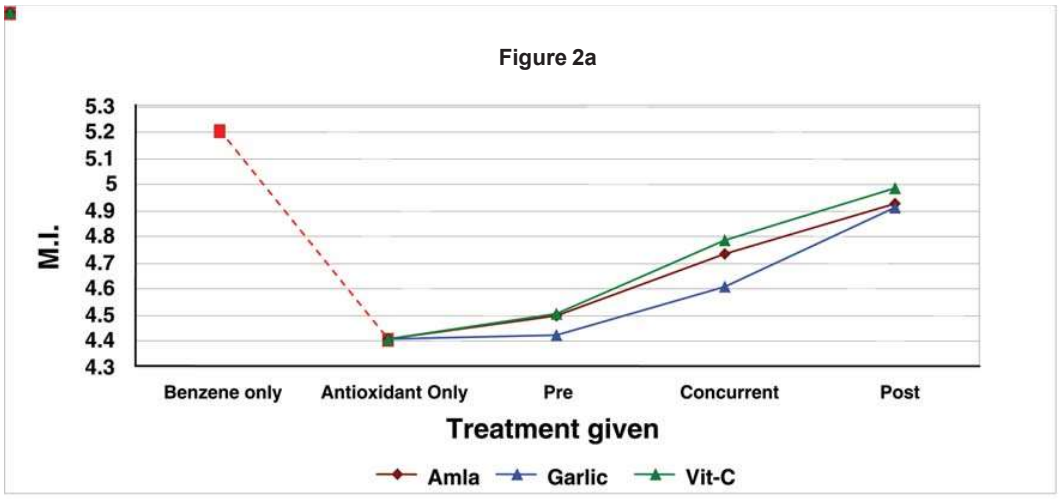
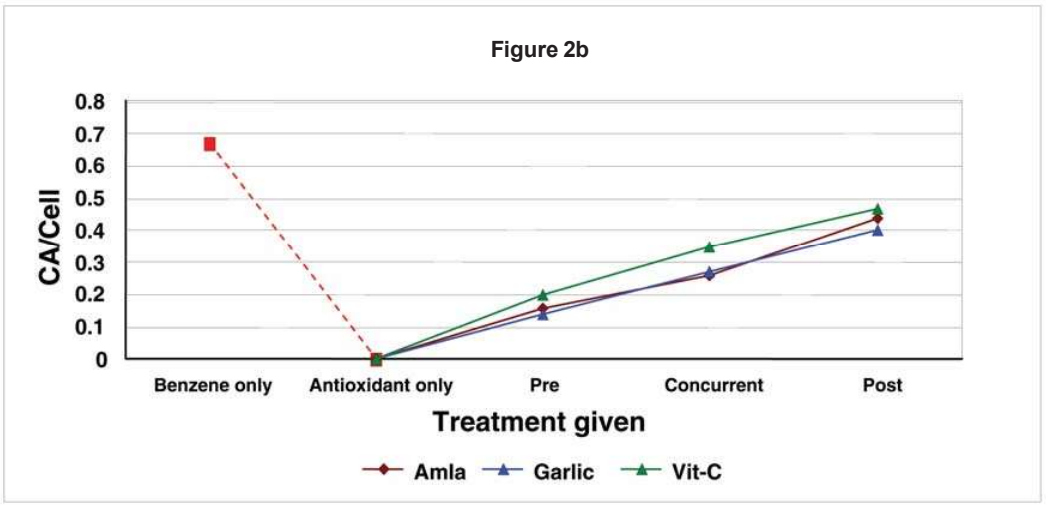


Fig 1a, 1b, 1c: Clastogenicity of benzene in bone marrow cells of rats





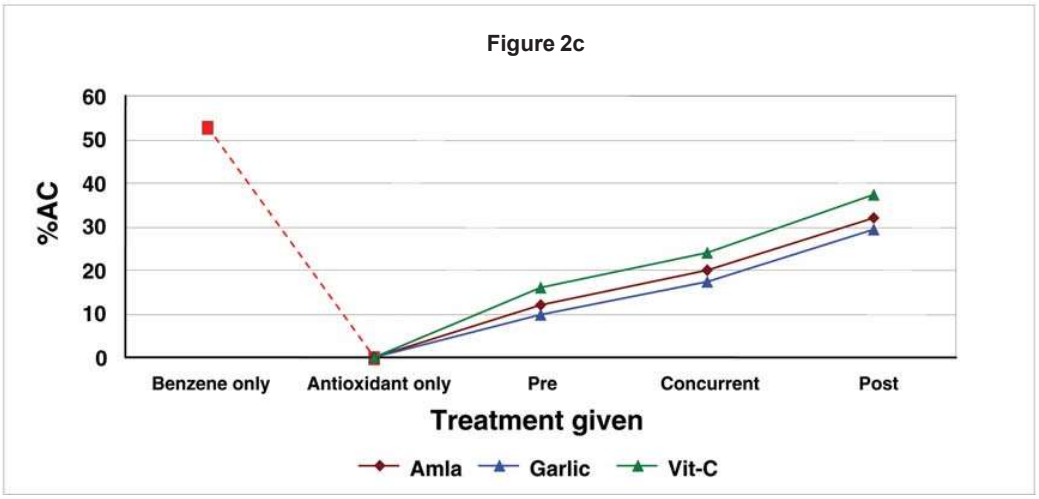


Fig 2a, 2b, 2c: Comparative minimization of benzene induced clastogenicity by amla, garlic & vitamin C (antioxidants)

Table 1 Chromosomal Aberrations Induced by Benzene in the Bone Marrow Cells of Rats (n=2)

Group/ Dose (mg/kg b.wt.)	Post-treatment Days	Chromosomal Aberrations															M.I	Mean ± S.E.	
		Structural Aberrations											Numerical Aberrations					CA/Cell	%AC
		ChBr	ChGa	ChFr	Pulv	RC	DC	TchtdDe	TchDe	M	Clump	EEA	Poly	Hyper	Hypo	Diplo			
Control	3dy	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	4.412	0.0100±0.0058	1.000±0.5774
A 97	3dy	4	5	2	0	2	6	6	7	4	4	3	0	0	6	0	4.136	0.4900±.0100**	40.00±0.000**
	5dy	1	4	3	2	3	6	7	6	3	2	2	0	0	3	0	4.464	0.4200±.0000**	35.00±1.000**
	7dy	1	2	2	1	3	5	5	4	6	3	4	0	0	4	0	3.842	0.4000±.0000**	31.00±1.000**
	10dy	1	3	4	0	4	4	2	4	1	1	1	0	0	2	0	3.647	0.2700±.0100**	20.00±0.000**
B 190	3dy	4	4	3	3	2	5	7	8	6	4	7	0	0	6	0	5.686	0.5900±.0100**	43.00±1.000**
	5dy	3	6	3	2	3	4	6	5	6	3	7	0	0	5	0	4.723	0.5300±.0100**	36.00±0.000**
	7dy	1	4	3	3	2	5	4	3	6	4	6	0	0	5	0	3.875	0.4600±.0000**	38.00±0.000**
	10dy	1	3	3	2	2	3	3	4	4	5	5	0	0	4	0	3.101	0.3900±.0100**	29.00±1.000**
C 360	3dy	3	3	5	7	5	7	5	9	9	5	1	0	1	7	0	5.208	0.6700±.0100**	53.00±1.000**
	5dy	3	2	5	5	4	4	5	5	8	6	5	0	0	5	0	4.016	0.5700±.0100**	46.00±0.000**
	7dy	2	2	2	2	4	4	8	7	4	5	5	1	0	4	0	4.244	0.5000±.0000**	41.00±1.000**
	10dy	1	2	1	1	4	3	6	7	5	4	5	0	0	4	0	3.786	0.4300±.0100**	38.00±0.000**
D 760	3dy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5dy	2	2	4	2	3	2	3	9	8	8	6	0	0	4	0	2.671	0.5300±.0100**	68.00±0.000**
	7dy	2	3	5	4	3	3	7	8	9	9	9	0	1	6	0	2.180	0.6900±.0100**	62.00±0.000**
	10dy	1	3	4	3	3	3	9	8	8	8	5	0	0	7	0	1.309	0.6200±.0200**	53.00±1.000**

Student's t-test: * & **superscripts indicate level of significance. * - $p < 0.05$, * * - $p < 0.01$.

M.I. – Mitotic-Index, CA– Chromosomal Aberration, AC–Aberrant cells, b.wt.– Body weight, ChBr – Chromosomal Break, ChGa – Chromosomal Gap, ChFr – Chromosomal Fragmentation,Pulv – Pulverization, RC – Ring Chromosome, DC – Dicentric Chromosomes, TchtdDe – Terminal Chromatid Deletion, TchDe – Terminal Chromosomal Deletion, M – Minute,Clump – Clumping, EEA – End to end association, Poly – Polyploidy, Hyper – Hyperploidy, Hypo – Hypoploidy, Diplo – Diploidy

Table 2 Minimization of Chromosomal Aberrations Caused by Benzene (n=2) with Phyllanthus emblica Extract (n=5) in Bone Marrow Cells of Rats

Group-A Treatment	Dose (mg/kg b.wt.)	Post-treatment Days	Chromosomal Aberrations															M.I	Mean ± S.E.	
			Structural Aberrations											Numerical Aberrations					CA/Cell	%AC
			ChBr	ChGa	ChFr	Pulv	RC	DC	TchtdDe	TchDe	M	Clump	EEA	Poly	Hyper	Hypo	Diplo			
Benzene	360	3dy	3	3	5	7	5	7	5	9	9	5	1	0	1	7	0	5.208	0.6700±.0100	53.00±1.000
Phyllanthus	1000	3dy	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	4.407	0.0040±0.0039	0.400±0.400
P→B	1000→360	3dy→3dy	1	2	2	4	2	2	5	6	7	4	1	0	0	3	0	4.499	0.1560±0.0040**	12.000±0.632**
B + P	360+1000	3dy	2	3	5	7	5	6	5	9	9	5	1	0	1	7	0	4.733	0.2600±0.0063**	20.400±0.748**
B→P	360→1000	3dy→3dy	7	7	8	10	7	6	11	10	12	13	5	0	1	12	0	4.927	0.4360±0.0075**	32.000±0.632**

Student's t-test:* & ** superscripts indicate level of significance, * – p <0.05, ** – p <0.01.
P à B – Pre-treatment, B + P – Concurrent-treatment, B à P – Post-treatment, P – *Phyllanthus emblica* extract, B – Benzene.
b.wt. – Body weight, S.E.– Standard Error.
M.I. – Mitotic-Index, CA– Chromosomal Aberration, AC–Aberrant cells, ChBr – Chromosomal Break, ChGa – Chromosomal Gap, ChFr – Chromosomal Fragmentation, Pulv – Pulverization, RC – Ring Chromosome, DC – Dicentric Chromosomes, TchtdDe – Terminal Chromatid Deletion, TchDe – Terminal Chromosomal Deletion, M – Minute, Clump – Clumping, EEA – End to end association, Poly – Polyploidy, Hyper – Hyperploidy, Hypo – Hypoploidy, Diplo – Diploidy.

Table 3 Minimization of Chromosomal Aberrations Caused by Benzene (n=2) with Allium sativum (Garlic) Extract (n=5) in Bone Marrow Cells of Rats

Group-B Treatment	Dose (mg/kg b.wt.)	Post-treatment Days	Chromosomal Aberrations															M.I	Mean ± S.E.	
			Structural Aberrations											Numerical Aberrations					CA/Cell	%AC
			ChBr	ChGa	ChFr	Pulv	RC	DC	TchtdDe	TchDe	M	Clump	EEA	Poly	Hyper	Hypo	Diplo			
Benzene	360	3dy	3	3	5	7	5	7	5	9	9	5	1	0	1	7	0	5.208	0.6700±.0100	53.000± 1.000
Garlic	1000	3dy	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	4.405	0.0000±0.0000	0.400±0.400
G→B	1000→360	3dy→3dy	1	3	2	3	2	2	3	5	6	4	0	0	1	3	0	4.422	0.1400±0.0063**	10.000±0.632**
B + G	360+1000	3dy	3	3	5	7	5	7	5	9	9	6	1	0	1	7	0	4.606	0.2720±0.0049**	17.600±0.400**
B→G	360→1000	3dy→3dy	6	6	8	9	8	7	11	12	11	9	2	0	1	10	0	4.910	0.4000±0.0063**	29.600±0.400**

Student's t-test:* & ** superscripts indicate level of significance, * – $p < 0.05$, ** – $p < 0.01$.
G à B – Pre-treatment, B + G – Concurrent-treatment, B à G – Post-treatment, G – Garlic extract, B – Benzene.
b.wt. – Body weight, S.E.– Standard Error,
M.I. – Mitotic-Index, CA– Chromosomal Aberration, AC–Aberrant cells, ChBr – Chromosomal Break, ChGa – Chromosomal Gap, ChFr – Chromosomal Fragmentation, Pulv – Pulverization, RC – Ring Chromosome, DC – Dicentric Chromosomes, TchtdDe – Terminal Chromatid Deletion, TchDe – Terminal Chromosomal Deletion, M – Minute, Clump – Clumping, EEA – End to end association, Poly – Polyploidy, Hyper – Hyperploidy, Hypo – Hypoploidy, Diplo – Diploidy.

Table 4 Minimization of Chromosomal Aberrations Caused by Benzene (n=2) with Vitamin C (n=5) in the Bone Marrow Cells of Rats

Group-C TREATMENT	Dose (mg/kg b.wt.)	Post-treatment Days	Chromosomal Aberrations															M.I	Mean ± S.E.	
			Structural Aberrations											Numerical Aberrations					CA/Cell	%AC
			ChBr	ChGa	ChFr	Pulv	RC	DC	TchtdDe	TchDe	M	Clump	EEA	Poly	Hyper	Hypo	Diplo			
Benzene	360	3dy	3	3	5	7	5	7	5	9	9	5	1	0	1	7	0	5.208	0.6700±.0100	53.00±1.000
Vit-C	10	3dy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.410	0.0000±0.0000	0.000±0.000
Vit-C→B	10→360	3dy→3dy	1	2	4	5	4	3	6	7	6	5	1	0	1	5	0	4.501	0.2000±0.0063**	16.000±0.632**
B + Vit-C	360+10	3dy	3	3	7	8	6	7	9	10	12	9	2	0	2	9	0	4.782	0.3480±0.0102**	24.000±0.632**
B→Vit-C	360→10	3dy→3dy	6	7	9	11	8	9	11	13	12	10	7	0	2	12	0	4.983	0.4680±0.0080**	37.600±0.400**

Student's t-test:* & ** superscripts indicate level of significance, * – $p < 0.05$, ** – $p < 0.01$.
Vit-C à B – Per-treatment, B + Vit-C – Concurrent-treatment, B à Vit-C – Post-treatment, Vit-C – Vitamin-C, B – Benzene.
b.wt. – Body weight, S.E.– Standard Error.
M.I. – Mitotic-Index, CA– Chromosomal Aberration, AC–Aberrant cells, ChBr – Chromosomal Break, ChGa – Chromosomal Gap, ChFr – Chromosomal Fragmentation, Pulv – Pulverization, RC – Ring Chromosome, DC – Dicentric Chromosomes, TchtdDe – Terminal Chromatid Deletion, TchDe – Terminal Chromosomal Deletion, M – Minute, Clump – Clumping, EEA – End to end association, Poly – Polyploidy, Hyper – Hyperploidy, Hypo – Hypoploidy, Diplo – Diploidy.

garlic treated groups (**Table 4, Figs 2a, 2b, 2c**). Minimum values were observed at the pre-treatment level of administration (**Table 4**).

Effectiveness of medicinal plant extracts and vitamin C against different industrial chemicals, in decreasing order of their effectiveness was - *A.sativum* > *P.emblica* > Vitamin C. *Phyllanthus emblica* (Amla) and *Allium sativum* (Garlic) extracts and Vitamin-C, all proved to be efficient anticlastogen and antispermatotoxic agents and significantly reduced the number of chromosomal aberrations and number of abnormal sperms induced by the chemical. Genotoxicity of benzene was best minimized by *A. sativum* extract, whereas *P.emblica* and vitamin C showed more or less similar results against benzene genotoxicity. All the antioxidants showed best results at their pre-treatment level of administration. Less effective results were observed with concurrent treatments.

Discussion

(A) Genotoxicity of Benzene:

In the present study, benzene was observed to cause both structural and numerical types of chromosomal aberrations in bone marrow cells of test animals. Forni (1979)[25] also observed both numerical and structural chromosome changes in bone marrow cells due to benzene haemopathy. In recent studies by Roma-Torres et al (2006)[7] and Chen et al (2007)[8], significant increase in chromosomal aberrations, micronuclei and variety of DNA-damage types (single strand breaks, double strand breaks and oxidative base modification) were observed in a group of workers exposed to benzene and isolated human lymphocytes respectively.

Benzene (880mg/kg) showed more activity in mice when administered orally by Ciranni et al (1988)[26], which was the mode of administering the chemical in the present study.

The present study showed a dose dependent increase in chromosomal aberrations per cell (CA/ Cell) as well as in percentage of aberrated cells(%AC). Mitotic-index showed overall decrease in its value with increase in dose of benzene. These observations were supported by an earlier study by Erexson et al (1985),[27] in which significant concentration-related increases in SCE (sister chromatid exchange) frequency, decreases in mitotic-indices and inhibition of cell cycle kinetics were observed.

In a study on rabbits treated with s.cu. dose of benzene, a high incidence of chromatid and chromosome type of aberrations were found in all the animals which persisted upto 60 days after the end of dosing with benzene.[28] This finding might probably explain for the presence of chromosomal aberrations till the 10th day of post-treatment of benzene in the present study, although their number decreased by that time.

A micronucleus assay in rats given different IP doses of benzene on each of two successive days showed that animals at high dose groups had significantly higher micronuclei counts after the final dose.[6] At still higher dose of benzene, chromatid and chromosome aberrations were significantly increased in bone-marrow, over control values upto 8 days after dosing. The same trend was seen in the present study with benzene, where-in at the highest dose level the CA/cell value was observed to keep on increasing till the 7th day level of post-treatment.

Eastmond et al (2001)[29] also indicated that benzene exposure is characterized by chromosome breakage, primarily within the euchromatin, and modest increases in aneuploidy. Similar to this finding, in the present study, at the highest two doses (but not at lower doses), the incidence of polyploidy and hyperpoidy appeared, although they were in very few numbers.

Mechanism of action: Benzene is metabolized in the body by the initial formation of an arene oxide, which reacts with a number of nucleophiles.[30,31] Benzene oxide, a predominant intermediate in the metabolic conversion of benzene, can damage DNA.[32] Benzene reduces DNA synthesis and might inhibit DNA repair in cultured human leucocytes.[33] Also was reported an irreversible covalent interaction of benzene metabolite with DNA *in vivo*, and binding benzene and some of its metabolites to both DNA and protein.[34] Benzene is metabolized by cytochrome P450 2EI to various phenolic metabolites which accumulate in the bone-marrow. Myloperoxide, which is present in very high levels in bone-marrow, was found to further catalyse the metabolism of these phenolic metabolites to reactive free radical species. Redox cycling of these free radicals produces active oxygen which may damage cellular DNA

(B) Minimization of Genotoxicity by Medicinal Plant (*Phyllanthus emblica* and *Allium sativum)* Extracts and Vitamin C:

(i) *Phyllanthus emblica* (Amla) extract:

In the present study it was observed that *Phyllanthus emblica* extract was able to minimize the genotoxic effects of benzene to a highly significant level. The number and types of chromosomal aberrations per cell as well as the percentage of aberrated cells were reduced significantly. A study by Giri and Banerjee (1986)[12] also showed that *P.emblica* extract reduced the chromosomal and structural abnormalities induced by metanil yellow and zinc chloride, and mitostatic properties of metanil yellow. *Emblica officinalis (P.emblica*) extract significantly reduced the clastogenic effect of 3,4-benzopyrene in mice.[35]

The results of the present study were also strongly supported by a recent investigative study regarding genoprotective efficiency of chayawanprash awaleha (90% bulk is *Phyllanthus amblica)* where bidi smokers showed significant decrease ($p<0.01$) in mitotic-index, chromosomal aberrations, sister chromatid exchanges and satellite associations.[36] The frequency of chromosomal aberrations came down to the level of controls.

In the present investigation, pre-treatment of *P.emblica* extract very effectively reduced the CA/Cell, % AC and Mitotic-index values near the control values. This is supported by an earlier study where crude aqueous extract of *E.officinalis* (*P. emblica*) was administered orally to mice over various time periods, after their treatment with sodium arsenite. Pre-treatment with the extract of *E.officinalis* was able to significantly reduce the cytotoxicity of sodium arsenite. There was significant decrease in chromosomal aberrations and damaged cells.[37] Khandelwal et al (2002)[16] also observed cytoprotective potential of amla fruit against acute cadmium toxicity.

Mechanism of action: Vitamin C, tannins, polyphenolic compounds and ellagic acid are found to be among some of the important components of *Phyllanthus emblica*.[38] Ascorbic acid (vitamin C) and phenolic compounds along with ellagic and tannic acids are inhibitors and blocking agents against carcinogens, preventing formation of nitrosamines from secondary amines and nitrates in the acidic environment of stomach. Ellagic and tannic acids inhibit mutagenicity of direct acting N-nitroso compounds.[39,40] Ellagic acid may protect DNA from the attack of electrophilic species or free radicals by binding to its nucleophilic sites.[41] Ellagic acid has also been found to inhibit cytochrome P450, and it is also a scavenging agent.[42] Polyphenolic antioxidants (e.g., ellagic acid) are scavengers of free radicals, antioxidants, chelating agents and modifiers of various enzymes[43] Pre-treatment with amla was observed to enhance the activity of various antioxidant enzymes, GST and GSH systems in the blood. Long-term administration of amla was also found to be capable of preventing dyslipidaemia and oxidative stress in ageing process.[19]

(ii) *Allium sativum* (Garlic) extract:

In the present study it was observed that *A.sativum* extract was able to minimize the genotoxic effects of benzene to a significant level and better than amla or vitamin C. The number and types of chromosomal aberrations, as well as the percentage of aberrated cells were reduced significantly. Depression or increase in mitotic-index was also reduced or returned near to the control values by *A.sativum* extract. When crude extract of garlic bulb was given orally to Swiss mice for 30 days, along with the cytotoxicant sodium arsinate, frequency of chromosomal aberrations was reduced significantly in animals.[44] Aqueous extract of garlic bulb has been found to inhibit the mutagenic effect of ionizing radiations and various clastogens in Salmonella, Chinese hamster cells and mouse *in vivo*.[20,21]

The present study also showed that pre-treatment of *A.sativum* (Garlic) extract was most effective in reducing the number of chromosomal aberrations per cell, as well as the percentage of aberrated cells due to benzene. In a study done earlier by Singh et al (1996)[45] for radioprotective effect of *A. sativum*, freshly prepared aqueous extract of garlic was tested in mice *in vivo* against gamma-radiation induced chromosomal damage. Pre-treatment with the extract for 5 days reduced gamma-radiation induced chromosomal damage in bone-marrow.

Mechanism of action: Diallylsulphide, allylmethyl sulphide, quercetin (flavonoid) and riboflavin (vitamin B) are among the main components of *A.sativum*.[38,46] Dietary flavonoids and polyphenolic antioxidants are scavengers of free radicals, antioxidants, chelating agents and modifiers of various enzymes.[43] Quercetin was found to inhibit a number of cytochrome P450/P448 functions.[47] Diallylsuphide, allylmethyl sulphide and diallyl trisulphide enhance the level of glutathione-S-transferase and accelerate the detoxification of the carcinogens. The allyl

groups were the effective groups.[48,49] Glutathione-S-transferase has been observed as a major detoxification enzyme, which catalyses the binding of electrophiles to glutathione (GSH).[50] GSH is known to suppress chemically induced chromosomal aberrations. It has been suggested that sulphydryl compounds analogous to GSH may be involved in the detoxification process.[51] Various aqueous garlic preparations were found to scavenge superoxide anion, hydrogen peroxide and hydroxyl radical.[52]

(iii) Vitamin C:

In the present study, vitamin C also proved to be effective in minimizing genotoxicity of benzene, and significantly reduces the number and all types of chromosomal aberrations per cell, as well as the percentage of aberrated cells due to benzene. Earlier studies also found a decrease in the incidence of carcinogen-induced gene mutations, sister-chromatid exchanges and chromosomal breakages *in vitro* conditions in various test-systems.[53,54] In human beings also, vitamin C has been reported to decrease chromosomal changes in peripheral lymphocytes of coal-tar workers occupationally exposed to polycyclic aromatic hydrocarbons and benzene.[55] It was found to prevent or reduce frequency of sister chromatid exchange (SCE) and chromosomal aberration, caused by mercuric chloride, in short-term human leucocyte cultures.[56]

The present study also observed that pre and concurrent administrations of vitamin C were more effective in reducing the genotoxic effects of benzene. Hoda et al (1991)[57], in a study observed that when vitamin C was given concurrently, before and after the organophosphorus pesticides it minimized the mitoinhibition and clastogeny caused by these pesticides. Concurrent treatment was observed to be a more effective mode of vitamin C supplementation. Concurrent administration of vitamin C was also found to be most effective in modulating genotoxicity of pesticides - endosulfan, phosphamidon and mancozeb.[58] In the present study, vitamin C was also observed to significantly reduce the spermatotoxic effects of benzene.

Mechanism of action: Vitamin C was found to primarily affect the nitrosation reaction, but it also inhibited the mutagenesis of the direct-acting carcinogen N-methyl-N-nitronitrosoguanidine (MNNG) and decreased its damage to DNA.[59,60] It was also observed to scavenge free radicals formed during preparation of the food, or during the metabolic process in the body.[61] In the respiratory tract, it may react rapidly with air pollutants such as O_3, cigarette smoke, and NO_2.[62,63] Vitamin C was demonstrated in one study to neutralize the oxidative stress-related germ cell injury in Cd treated mice. Studies have also indicated the higher potentiality of vitamin C in minimizing testicular lipid peroxidation.[64]

Conclusion

Benzene has been proved to be an efficient genotoxic chemical, causing significant chromosomal alterations and decrease in mitotic-index (mitotic-depression). All the anticlastogens studied were very effective in minimizing the genotoxicity of benzene at the pre-treatment level of administration, followed by concurrent level of administration. *Allium sativum* (garlic) extract proved to be a strong antigenotoxicant against benzene. It was observed to be more effective than *Phyllanthus emblica* (amla) and vitamin C. It can therefore be concluded that benzene is highly genotoxic, and daily intake of medicinal plant *A.sativum* extract can be more efficient in minimizing its genotoxic effects as compared to *P.emblica* and vitamin C.

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