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Genotoxic Effect of Benzene on Male Mammalian Germinal tissue (spermheads)And Its Comparative Minimization By Phyllanthus emblica, Allium sativum 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 of benzene on germinal tissue (spermhead abnormality / spermatotoxicity) in 10-15 week old albino rats (*Rattus rattus*). This aspect of benzene toxicity is of greater importance as any effect on germinal tissue not only effects the fertility of the individual but also there are increased chances of abnormalities in the future generations. Many medicinal plants and vitamins are known to have antioxidant and anticlastogenic properties. Therefore, vitaminC 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 / spermatotoxicity of benzene. Germinal genotoxicity of benzene was investigated at doses of 1/40, 1/20, 1/10, 1/5 oral LD50. Antioxidants vitamin C (10mg/kg b.wt) and crude medicinal plant extracts (*P. emblica*=1000mg/kg b.wt., and *A. sativum*=1000mg/kg b.wt.) were tested for their ability to minimize genotoxic effects of benzene at 1/10 LD50 dose. Observations were taken at pre, concurrent and post treatment levels of antioxidants. Statistical analysis was done by 'student t-test'. Benzene was observed to be strong sprematotoxic agents as it caused significant increase in percentage of abnormal sperms. *P. emblica*, *A. sativum* extracts, and vitamin C significantly reduced all the types of observed spermhead abnormalities induced by benzene. They showed best results during pre-treatment. Spermatotoxicity of benzene was best minimized by *A. sativum* extract. *P. emblica* and vitamin C showed more or less similar results. It was concluded that daily intake of mentioned antioxidants especially *A. sativum* extract might be beneficial in minimizing and providing protection against benzene caused genotoxicity to the germinal tissue / sperms.

Key Words: *Benzene; Genotoxicity;Spermatotoxicity; spermhead abnormality; VitaminC ; Phyllanthus emblica ; Allium sativum .*

1. Introduction

Benzene is also known as benzole and coal naphtha. It is a solvent of major industrial importance with chemical formula C_6H_6. Benzene is the parent hydrocarbon of aromatic group. It is produced in enormous amounts principally from coal tar distillation, from petroleum, from pyrolysis of gasoline and from hydro-dealkylation of toluene. It undergo electrophilic aromatic substitutions. **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. It was also reported to be present in unleaded gasoline and cigrarette smoke.[1]

What is more alarming is that consumers may be exposed unknowingly in the 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.

ISSN 2348 – 7968

Benzene was found to be absorbed via ingestion, inhalation, and skin application. Experimental data indicated that animals can absorb up to 95% of oral doses and humans can absorb up to 80% of inhaled benzene (5 min. exposure). Humans may absorb benzene vapours through skin as well as the lungs. Of the total dose absorbed by the two routes, 22-36% enters the body through the skin. Metabolism of benzene is required for its toxicity. The liver was observed the main site for the metabolism of benzene and the bone marrow a minor site. Phenol, hydroquinone, catechol and benzene oxide were the major metabolites. The metabolites likely to be responsible for toxicity included free radicals generated by oxidizing enzymes. Benzene was observed to be eliminated either unchanged in expired air or as metabolites in urine.[2]

Benzene is enlisted as industrial chemical carcinogen showing genotoxic effects, a high incidence of chromatid and chromosomal aberrations in rabbits, chromatid deletions in bone marrow chromosomes of rats after subcutaneous dosing with undiluted benzene.[3] A significant increase was found in CA, MN and comet tail length (DNA damage) in benzene exposed group.[4] 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.[5]

Studies for numerical aberrations of sex chromosomes have also been done. The numerical aberrations of sex chromosomes in interphase sperm were observed in workers exposed to benzene series using two colour fluorescence *in situ* hy bridization. Aneuploidy frequencies of X and Y chromosomes in X and Y sperms were also detected.[6] Numerical autosomal chromosome aberrations were also detected in sperms of workers exposed to benzene series by two-color fluorescence *in situ* hybridization (FISH) where the aneuploidy frequencies of 9 and 18 chromosomes were detected in sperms. This study showed that exposure to benzene at higher concentration may induce increase in aneuploidy frequency of sperm autosomal chromosome.[7]

Benzene at higher concentration may induce increases in frequencies of numerical and structural aberrations for chromosome 1 of sperms in exposed workers.[8] Frequency of numerical aberrations for chromosomes 7 and 8 in the sperms of workers exposed to benzene series were also investigated by some other workers using two-colour fluorescence *in situ* hybridization (FISH). A statistically significant increase in the frequency of overall numerical chromosome aberrations was seen in the exposed group. The results suggested that higher concentration of benzene may induce higher frequencies of numerical aberrations in the sperms of workers exposed to benzene series.Some workers have identified changes in global gene expression patterns in response to benzene metabolites in human peripheral blood mononuclear cells. Treatment with 1,2,4–benzentriol resulted in the suppression of gene related to regulation of protein expression and activation of genes that encode heat shock proteins and cytochrome P450 family members.[9]Some workers have identified changes in global gene expression patterns in response to benzene metabolites in human peripheral blood mononuclear cells. Treatment with 1,2,4–benzentriol resulted in the suppression of gene related to regulation of protein expression and activation of genes that encode heat shock proteins and cytochrome P450 family members.[10]At the molecular level, benzene exposure alters gene expression in peripheral blood cells and induces aneuploidy in hematopoietic progenitor cells.[11,12]Genotoxic effects are initiated by benzene and its metabolites, by directly reacting with DNA and byproducts of the cell during mechanism pathway.[13] A study observed a dose dependent increase in benzene induced chromosomal damage and estimated a benchmark concentration limit of 0.205 ppm benzene. A locus on Chr 10, that contained a pair of over-expressed sulfotransferases, which were inversely correlated with genotoxicity was identified.[14]

2. Medicinal Plant Extracts and Vitamin C

Antimutagenic or Anticlastogenic effects of **Vitamin-C** has been observed in various test systems.[9, 10] Vitamin-C was demonstrated in one study to neutralize the oxidative stress-related germ cell injury in Cd treated mice. Study also indicated the higher potentiality of vitamin-C in minimizing testicular Lipid peroxidation potential and thereby increased the sperm count level and reduced the percentage of morphologically abnormal sperms. This study emphasizes the possible role of the reactive oxygen species (ROS) in inducing sperm abnormality by way of altering specific gene loci in germ cell chromosomes. The probable role of the vitamin-C in relieving the ROS related injury to the germ cells was discussed.[15]Genoprotective effect of Vitamin-C was also observed against ethyl methane sulphonate (EMS) in a fish - *Anabas testudineus*. Several cytogenetical endpoints like chromosome aberrations, micronuclei, abnormal nuclei and sperm head abnormality at different time intervals were scored. All the three does of vitamin-C appeared to reduce the EMS-induced genotoxicity in this fish to a variable extent. Higher doses of vitamin-C appeared to give better protection.[16]Vitamin C is potentially involved in cancer and cardiovascular diseases prevention. Grosso *et al.* has summarize recent and well established advances in vitamin C research and its clinical implications. Since vitamin C has the potential to counteract inflammation and subsequent

ISSN 2348 – 7968

oxidative damage that play a major role in the initiation and progression of several chronic and acute diseases, it represents a practical tool to administer for the early prevention of these pathologic conditions.[17]

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.[18-23]Crude extract reduced the cytotoxic effect to a greater extent than Vitamin-C alone. Pre-treatment with amla (*Emblica officianalis*) fruitclearly indicted its protective effect against bio-effects of irradiation to Swiss albino mice.[24] Amla administration to rats increased their body level of protein responsible for regulating the transcription of genes involved in lipid and cholesterol metabolism.[25] While characterizing the antioxidant activity of amla, Khopde *et al.* concluded that amla is a more potent antioxidant than vitamin-C due to the presence of Ascorbic acid and other polyphenols in the natural formulation of amla.[26] Oral administration of *Emblica officinalis* fruit juice (500mg/kg b.wt.) for 8 days followed by single toxic dose of Cd as $CdCl_2$ (3mg/kg, b.wt. i.p.) considerably reduced mortality in rats. Pretreatment with amla also reduced histopathogical damage and lipid peroxidation in liver, kidney and testes. These results suggested cytoprotective potential of *Emblica* fruit in acute cadmium toxicity.[23] Also was reported the protective nature of *phythanthus* fruit extract in lead induced sperm head abnormalities.[27] Co-concurrent treatment of *Phyllanthus emblica* extract with adriamycin reduced the percentage of micronuclei in bone marrow erythrocytes in male mice.[28]Also has been mentioned in a review that amla nurtures the ovaries and sperms and enhances fertility.[29]

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*.[30,31]Four antioxidants (tetrahydro-beta-carboline derivatives) identified in the aged garlic extract, have shown strong hydrogen peroxide scavenging activities in *in vitro* assay.[32] Radioprotective effect of *Allium sativum* extract was also studied by some workers. A freshly prepared aqueous extract of garlic was tested in mice for its possible, *in vivo* protective effect against gamma–radiation-induced chromosomal damage. The sulfhydryl content and glutathione S-transferase activity registered significant increases after either pretreatment with the extract or irradiation. Significant reductions in sulfhydryl content and glutathione S-transferase activity were observed in extract treated irradiated animals.[33] In another study four antioxidants (tetrahydro-beta-carboline derivatives) were identified in the aged garlic which show strong hydrogen peroxide scavenging activities and were potent antioxidants.[32] Various aqueous garlic preparations were found to scavenge superoxide anion, hydrogen peroxide and hydroxyl radical. The heating before or after the garlic cutting was unable to eliminate the capacity of the extracts to scavenge all the three reactive-oxygen species.[34] Garlic extract was able to ameliorate schistosomal infection induced genetic alterations in DNA of mice to great extent.[35]

3. Materials and Methods

3.1 Animals: For the present investigation, Albino rats (*Rattus rattus*) in the age group of 10-15 weeks and weighing75-100gm were used as test animals. They werehoused 6 animals per cage, in the animal house of BiosciencesDepartment, H.P. University, Shimla at an optimumtemperature of 25±50C in sanitary cages. They weregiven standard pellet diet (Hindustan Lever Ltd.) andwater was given *ad libitum*. The material used for cytologicalstudy was testes and vas defferense for sperm or germinal tissue analysis.

3.2 Benzene Treatment: In the first set of experiments **spermatotoxicity** of **benzene** was investigated on the bases of abnormal sperms (spermhead abnormality). Test animals were divided into four groups (A,B,C&D). The different doses of benzene were given as1/40 ,1/20 ,1/10, 1/5 of the oral LD_{50} value (3800 mg/kg b wt) respectively.

3.3 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 (spermatotoxic) 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 LD50 dose of benzene was tested for minimization by the mentioned antioxidants.

3.4 Biochemical Investigations: All the animals were given i.p. 2.5 mg/kg body weight of colchicine prepared in HBSS (Concentration 0.7 mg/ml), 2½ hours before sacrificing them. The material used for the study of germinal cell toxicity was male germinal tissue i.e. sperm cells. Each animal was dissected out for testies after 1st, 3rd and 5th week of last benzene treatment for sperm slide preparations. Antioxidants were checked for their effectiveness at pre, concurrent and post-treatment levels. The doses of antioxidants were taken as- 1000mg/kg b.wt. for*Phyllanthus emblica&Allium sativum* (Grp-A&B) and 10 mg/kg b.wt. for Vitamin-C(grp-C). For sperm slide preparations animals were sacrificed on 5th week after the last treatment of the antioxidant. Protocol adopted for Preparation of Sperm slides were Wyrobek & Bruce[36] and Evans *et al.*[37] with some modifications. The slides were air-dried and

ISSN 2348 – 7968

stored in dust-free chambers.They were stained in 2% Giemsa stain and mounted in DPX and put in an oven (preheated) at 60^0C, for overnight. Observations & Photomicrographs from well spread sperm slides were made with the help of Research Binocular Microscope under magnifications (45 X 10) X and (100 X 10) X for sperm slides. Criteria for evaluation were spermhead abnormality at different time intervals and doses. 1000 sperms were studied per animal. Also observations after various treatments (pre, concurrent & post) of medicinal plant extract + industrial chemicals were taken to understand the anti-spermatotoxic or minimizing effect of antioxidants.

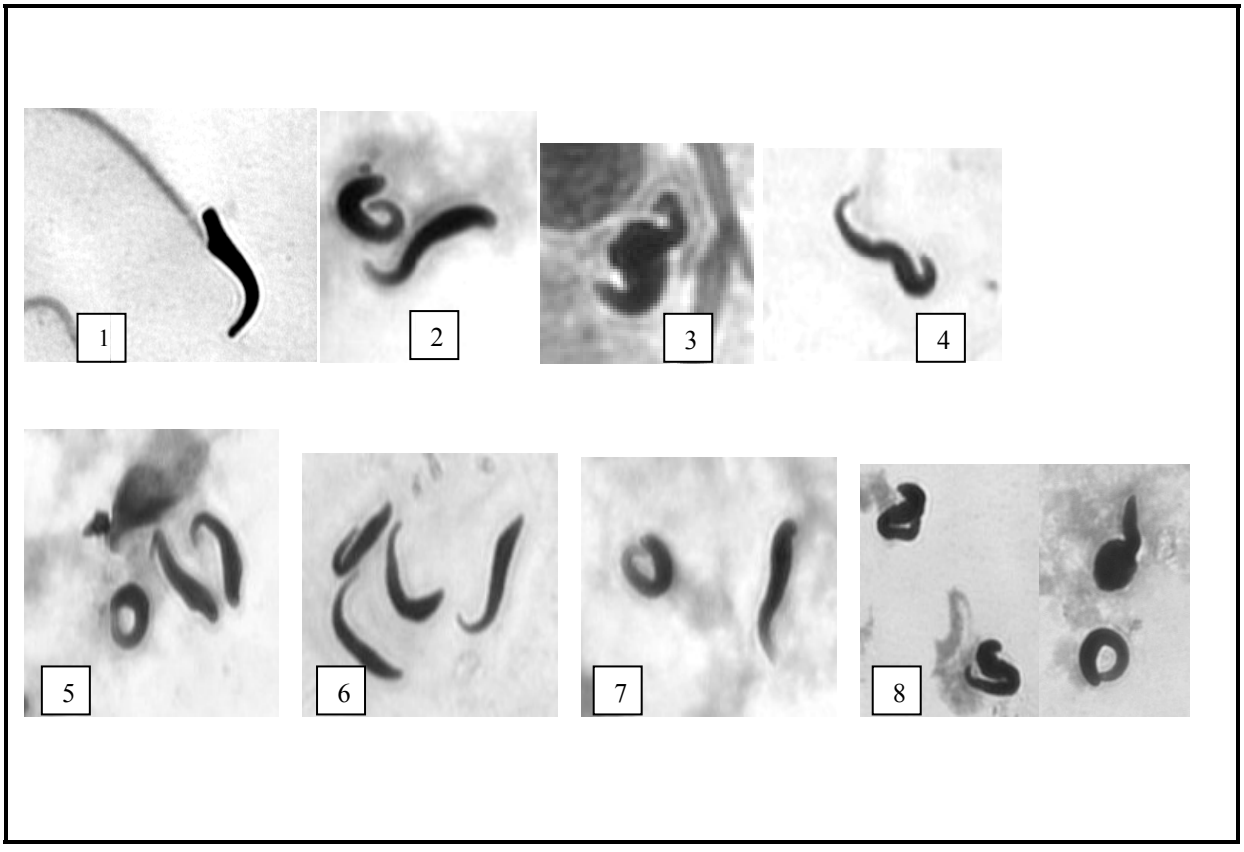
3.5 Statistical analysis: Calculation for percentage of abnormal sperms was done by 'student t-test' for chemical carcinogen as well as chemical carcinogen + medicinal plant extracts.

4. Results

4.1 Spermatotoxicity of Benzene:

Significant difference was found in the % abnormal sperm values of the test animals due to benzene treatment as compared to that of the control group (Table-1). Types of spermhead abnormalities observed were: lens-shaped, tadpole-shaped, ring-shaped, S-shaped and spermheads with varied shapes. Predominant type was the ring-shaped spermhead abnormality [Plates-1-8].

In all the groups (A-D with benzene doses ranging from 1/40 LD_{50} - 1/5 LD_{50}) there was observed increase in % abnormal sperms with increase in post-treatment interval (Table-1, Fig-1). But, in case of **group- D**(1/5 LD_{50} dose) % abnormal sperm was very high at the 5^{th} week of post-treatment and was found to be more than 50%, which confirms the high spermatotoxicity of benzene at this dose level (Table-1).



Plates – 1 to 8 :**1.** Normal Spermhead, **2, 5-7.** Ring & Lens shaped, **3 & 4.**Irregularly shaped, **8.**S-shaped spermheads.

ISSN 2348 – 7968

TABLE–1: Spermhead Abnormality induced by Benzene in Rat sperms (n=2)

Group/Dose (mg/kg b.wt)	Post treatment Weeks	No. of Sperms Studied per animal	% Abnormal Sperms ± S.E.
Control	5wk	1000	0.902±0.040
(A) 97	1wk	1000	12.000±0.100**
	3wk	1000	22.550±0.050**
	5wk	1000	26.250±0.050**
(B) 190	1wk	1000	14.750±0.150**
	3wk	1000	23.800±0.100**
	5wk	1000	31.250±0.150**
(C) 360	1wk	1000	22.450±0.050**
	3wk	1000	29.850±0.050**
	5wk	1000	40.250±0.150**
(D) 760	1wk	1000	27.600±0.100**
	3wk	1000	36.650±0.150**
	5wk	1000	51.050±0.150**

Student's t-test:* & ** superscripts indicate level of significance, * – $p < 0.05$, ** – $p < 0.01$.
b.wt. – Body weight, S.E.– Standard Error, wk.– week.

4.2 Antispermtotoxic effect against Benzene:

To study the antispermatotoxic effect against benzene, its 360 mg/kg b.wt.dose at the 5th week of post-treatment level was taken as the criteria of comparison. % Abnormal sperm value at this level was observed as 40.250±0.150. All the observations were taken at 5th week.

4.21 *Phyllanthus emblica* (Amla) extract (Group-A): (Table-2, Fig-2).

Treatment with crude extract of amla was able to reduce spermatotoxicity of benzene to a significant level in test animals. Pre-treatment gave the best result with lowest percentage of abnormal sperms, followed next by the concurrent treatment.

4.22 *Allium sativum* (Garlic) extract (Group-B): (Table-3, Fig-3).

Treatment with garlic extract was able to reduce spermatotoxicity of benzene to a better extent than that of amla extract. These values were lower than the corresponding values of the amla treated group. Pre-treatment of garlic was the most effective one followed next by the concurrent-treatment.

4.23 Vitamin-C(Group-C):(Table-4, Fig- 2).

Treatment with vitamin-C was also able to reduce spermatotoxicity of benzene to a significant level but less so than garlic treated group. To some extent, vitamin-C and amla extract had similar effect in reducing the spermatotoxic effect of benzene. Here also the pre-treatment proved to be most effective followed next by concurrent-treatment.

ISSN 2348 – 7968

Table – 2: Minimization of Spermhead Abnormality induced by Benzene (n=2) with *Phyllanthus emblica* (Amla) extract (n=5) in Rat Sperms.

Group-A Treatment	Dose (mg/kg b.wt.)	Post treatment Weeks	No. of Sperms Studied per animal	% Abnormal Sperms (Mean ± S.E.)
Benzene	360	5wk	1000	40.250±0.150
Phyllanthus	1000	5wk	1000	0.000±0.000
P→B	1000→360	5wk	1000	8.050±0.150**
B + P	360+1000	5wk	1000	17.050±0.150**
B→P	360→1000	5wk	1000	31.150±0.050**

Student's t-test: * & ** superscripts indicate level of significance, * – p <0.05, ** – p <0.01.
P → B – Pre – treatment, B + P – Concurrent – treatment, B → P – Post – treatment, P – Phyllanthus extract
B – Benzene, b.wt. – Body weight, S.E.– Standard Error, wk.– week

Table – 3: Minimization of Spermhead Abnormality induced by Benzene (n=2) with *Allium sativum* (Garlic) extract (n=5) in Rat sperms.

Group-B Treatment	Dose (mg/kg b.wt.)	Post treatment Weeks	No. of Sperms Studied per animal	% Abnormal Sperms (Mean ± S.E.)
Benzene	360	5wk	1000	40.250±0.150
Garlic	1000	5wk	1000	0.000±0.000
G→B	1000→360	5wk	1000	6.450±0.050**
B + G	360+1000	5wk	1000	15.400±0.200**
B→G	360→1000	5wk	1000	27.950±0.250**

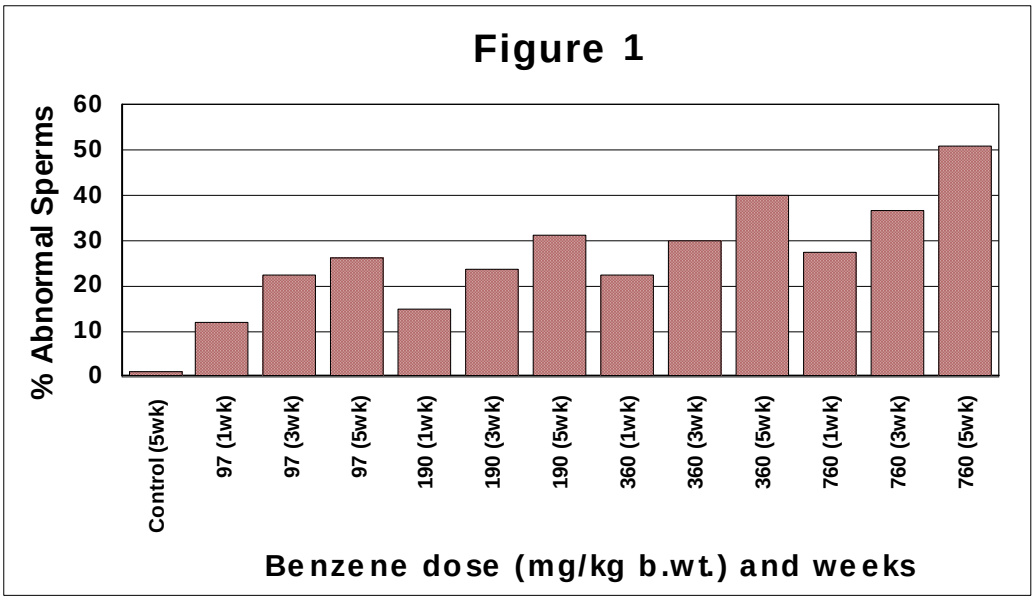
Student's t-test: * & ** superscripts indicate level of significance, * – p <0.05, ** – p <0.01.
G → B – Pre-treatment, B + G – Concurrent-treatment, B → G – Post-treatment, G – Garlic extract, B – Benzene b.wt. – Body weight, S.E.– Standard Error, wk.– week.

Table – 4: Minimization of Spermhead Abnormality induced by Benzene (n=2) with Vitamin-C (n=5) in Rat sperms.

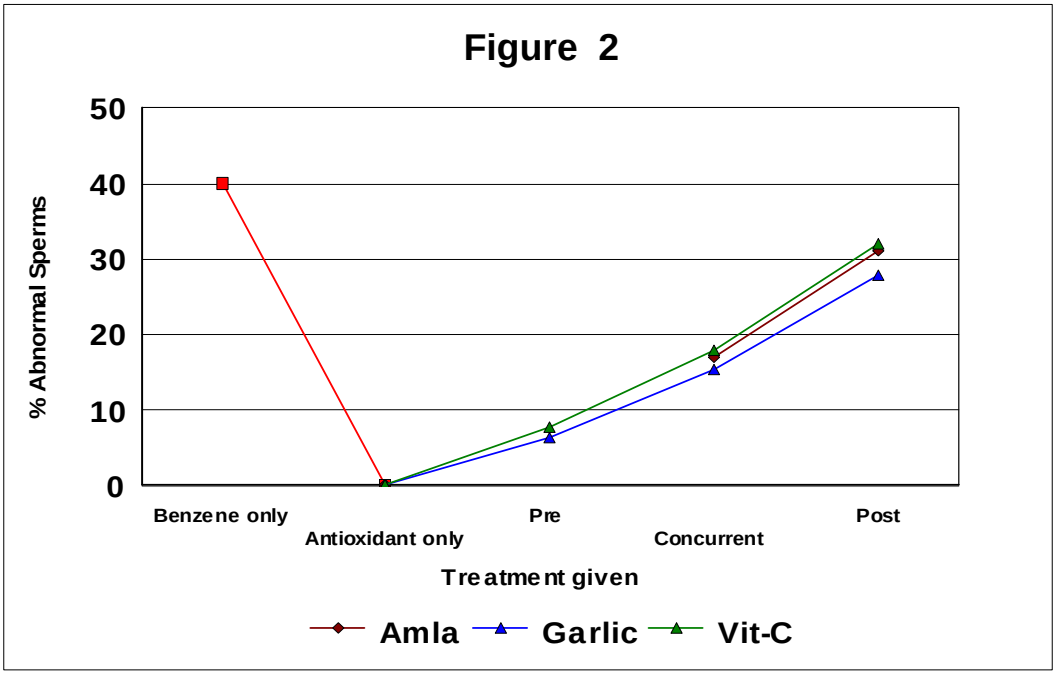
Group-C Treatment	Dose (mg/kg b.wt.)	Post treatment Weeks	No. of Sperms Studied per animal	% Abnormal Sperms (Mean ± S.E.)
Benzene	360	5wk	1000	40.250±0.150
Vit-C	10	5wk	1000	0.000±0.000
Vit-C→B	10→360	5wk	1000	7.700±0.200**
B + Vit-C	360+10	5wk	1000	17.950±0.050**
B→Vit-C	360→10	5wk	1000	31.950±0.050**

Student's t-test: * & ** superscripts indicate level of significance, * – p <0.05, ** – p <0.01.
Vit-C → B – Pre – treatment, B + Vit-C – Concurrent – treatment, B → Vit-C – Post – treatment,
Vit-C – Vitamin-C, B – Benzene, b.wt. – Body weight, S.E.– Standard Error, wk.– week.

ISSN 2348 – 7968



Figure–1: % of Abnormal Sperms induced by Benzene in Rat sperms (n=2)



Figure–2:Minimiztion in % of Abnormal Sperms by *Phyllanthus emblica* (Amla), *Allium sativum* (Garlic) andVitamin-Cin Rat (n=2)

ISSN 2348 – 7968

4.3Result summary

Benzene was observed to be strong sprematotoxic agents as it caused significant increase in percentage of abnormal sperms in relation to dose as well as post-treatment period. *Phyllanthus emblica* (Amla) and *Allium sativum* (Garlic) extracts and Vitamin-C, all proved to be efficient antispermatotoxic agents and significantly reduced the number of abnormal sperms induced by benzene. Sprematotoxicity of benzene was best minimized by *A. sativum* extract. *P.emblica* and Vitamin-C showed more or less equal results against benzene sprematotoxicity. All the antioxidants showed best results at their pre-treatment level of administration. Second best results were observed with concurrent-treatments.Effectiveness of Medicinal plant extracts and vitamin-C against benzene, in decreasing order of their effectiveness was - *A.sativum*>*P.emblica* ≥Vitamin-C.

5. Discussion

5.1 Genotoxicity of Benzene- In the present study benzene is observed to be a potent germ cell genotoxicant. It was observed to induce different types of chromosomal aberrations and spermhead abnormalities at all the dose levels of this study (Plates-1-8). It also showed dose dependent increase in percentage of abnormal sperms due to benzene in test animals (Table–1, Fig-1). These observations were well supported by the findings of Liu *et al.*[8] and Zhao *et al*.[9] that higher concentrations of benzene may induce higher frequencies of numerical aberrations in the sperms of workers exposed to benzene series. Some other workers also showed that exposure to benzene at higher concentration may induce increase in aneuploidy frequency of sperm autosomal and sex chromosomes, in exposed workers.[6] It was also found in a study that the optimized rate of metabolism of Benzene was almost twofold higher in male mice than in female mice. Further, the elimination of phenol from blood was significantly faster in male mice.[38]

Mechanism of action- Benzene is the simplest of the aromatic hydrocarbons. It metabolize in the body by the initial formation of an arene oxide.[39] Arene oxides of benzene react with a number of nucleophiles.[40]Benzene oxide, a predominant intermediate in the metabolic conversion of benzene, can damage DNA.[41] Benzene reduce DNA synthesis and might inhibit DNA repair in cultured human leucocytes.[42]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.[43] Benzene metabolize by cytochrome P450 2EI to various phenolic metabolites which accumulated 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

5.2 Minimization of genotoxicity by Medicinal plant extracts andVitamin-C

5.21 *Phyllanthus emblica* (Amla) extract

Present study showed that *P. emblica* (amla) extract was signficnatly effective in reducing the acute spermatotoxicity of benzene and that too at the pre-treatment level (Tables-2, Figs-2). Khandelwal *et al.*[23] also observed that pre-treatment with amla reduced histopathological damage and lipid peroxidation in liver, kidney and testes. Results of their study suggested cytoprotective potential of amla fruit against acute cadmium toxicity. In the earlier study[44] it was observed that *Phyllanthus emblica* extract was able to minimize the genotoxic effects of benzene to a highly significant level. In the investigation, pre-treatment of *P.emblica* extract was observed to be very effective in reducing or minimizing the genotoxic effect of benzene. Earlier study done by some workers, found that pre-treatment with the extract of *E.officinalis* was able to significantly reduce the cytotoxicity of sodium arsenite. There was significant decrease in damaged cells.[45]

Mechanism of action -Vitamin-C, tannins, polyphenolic compounds and ellagic acid are found to be among some of the important components of *Phyllanthus emblica*.[46] Ascorbic acid (Vitamin-C) and phenolic compounds alongwith ellagic and tannic acids are inhibitors and blocking agents against carcinogens, preventing formation of nitrosamines from secondary amines and nitrates in an acidic environment of stomach. Ellagic and tannic acids inhibited mutagenicity of direct acting N-nitroso compounds.[47,48] Ellagic acid may protect DNA from the attack of electrophllic species or free radicals by binding to its nucleophilic sites.[49] Ellagic acid was also found to inhibit cytochrome P450 and it is also a scavenging agent.[50] Polyphenolic antioxidants (e.g. ellagic acid) are scavengers of free radicals, antioxidants, chelating agents and modifiers of various enzymes.[51] 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.[52]

ISSN 2348 – 7968

5.22 *Allium sativum* (Garlic) extract-Present study showed that pre-treatment of *A.sativum* (Garlic) extract was most effective in reducing the percentage of abnormal sperms, due to benzene (Tables-3, Figs-2). A study by Singh *et al.*[33] supported these results very well. They studied freshly prepared *A. sativum* aqueous extract for its possible *in vivo* protective effect against gamma-radiation. Pre-treatment of the various concentrations of extract was observed to reduce gamma-radiation induced chromosomal damage in bone-marrow. In the earlier study it was observed that *A.sativum* extract was able to minimize the genotoxic effects of benzene to a significant level.[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*.[30,31,53]

Mechanism of action- Diallylsulphide, allylmethyl sulphide, quercetin (flavonoid) and riboflavin (Vitamin-B) are among the main components of *A.sativum*.[46,54] Dietary flavonoids and polyphenolic antioxidants are scavengers of free radicals, antioxidants, chelating agents and modifiers of various enzymes.[51] Quercetin was found to inhibit a number of cytochrome P450/P448 functions.[55,56] This could probably explain the better effectiveness of garlic extract against benzene genotoxicity as compared to other antioxidants in the present study because benzene is reported to be metabolized by P450. 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.[57-59] Glutathione-S-transferase has been observed as a major detoxification enzyme, which catalyses the binding of electrophiles to glutathione (GSH).[60] 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.[61] Various aqueous garlic preparations were found to scavenge superoxide anion, hydrogen peroxide and hydroxyl radical.[34]

5.23 Vitamin-C- In the present study vitamin-C was observed to significantly reduce the spermatotoxic effects of benzene. It was observed to reduce the percentage of abnormal sperms to a significant level in the test animals treated with benzene (Table-4 Figs-2). This can be well supported by the findings of the study done by Acharya *et al.*[15] where it was demonstrated that Vitamin-C neutralize the oxidative stress-related germ cell injury in Cd treated mice. Study also indicated the higher potentiality of vitamin–C in minimizing testicular lipid peroxidation potential and thereby increased the sperm count level and also reduced the percentage of abnormal sperms. These workers emphasized the possible role of reactive oxygen species (ROS) in inducing sperm abnormality. One study also showed that vitamin-C reduces spermhead abnormality induced by EMS in a fish-*Anabas testudineus*.[16] In the earlier study vitamin-C proved to be effective in minimizing genotoxicity of benzene. It was observed that pre and concurrent administrations of vitamin-C were more effective in reducing the spermatotoxic effects of benzene.[44] Its anticlastogenic property has been also found in *in vivo* mammalian systems.[62-64] Hoda *et al.*[65] in a study observed that concurrent treatment was 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.[66]

Mechanism of action- Vitamin-C was found to be primarily effect 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.[67,68] It was also observed to scavenge free radicals formed during preparation of the food, or during the metabolic process in the body.[69] In respiratory tract, it may react rapidly with air pollutants like O_3, cigarette smoke, and NO_2.70,71Vitamin-C was demonstrated in one study to neutralize the oxidative stress-related germ cell injury in Cd treated mice. Study also indicated the higher potentiality of vitamin-C in minimizing testicular Lipid peroxidation.[15]

6. Conclusion

Benzene proved to be an efficient genotoxic chemical, causing significant spermatotoxicity. *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. All the anticlastogens were most effective in minimizing the spermatotoxicity of the benzene at the pre-treatment level of administration followed by concurrent level of administration. It was concluded that benzene was highly spermatotoxic and daily intake of medicinal plant *A.sativum* extract proved to be more efficient in minimizing its spermatotoxic effects as compared to *P.emblica* and Vitamin-C .

ISSN 2348 – 7968

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