EVALUATION OF ROS INDUCED GENETIC ABERRATION IN BENZENE INTOXICATED RAT BONE MARROW

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Abstract: Here we aimed to observe the dose dependent genotoxic potential of benzene which may trigger the leukomogenic activity in bone marrow of rat. We used Wistar rat as a model and benzene was administrated orally at the doses of 50mg/ml/day and 100mg/ml/day for 90 days which exhibited definite signs of genotoxicity which may initiate carcinogenesis. Cellular stress of bone marrow was evaluated through various ROS parameter which was resulted into significant decrease in the level of antioxidative enzyme activity. There was significantly increased frequency of micronuclei in bone marrow of rat especially in polychromatic erythrocyte and the ratio of polychromatic and normochromatic erythrocyte was depleted at the significant level. Structural chromosomal aberration was observed significantly higher at 100mg/ml exposure of rat. The extent of DNA damage was evaluated through comet assay. A significant increase in the tail length of comet was recorded in the benzene exposed rats. Furthermore, the analysis of DNA ladder assay and cell morphology study of bone marrow cell was revealed the apparent hike of apoptotic cell death due to benzene intoxication. Present finding indicate that benzene exposure develops cancer pathogenesis in rat bone marrow by modulating the oxidative stress parameters and apoptosis pathway.

Key words: Benzene, Oxidative stress, Genotoxicity

INTRODUCTION

Benzene is an important industrial chemical and hazardous pollutant. Exposure to benzene has been shown to lead to aplastic anemia and acute myelogenous leukemia in humans and multiple forms of cancer in rodents [1]. Benzene is metabolized in the liver by cytochrome P450 2E1 to various phenolic metabolites which accumulate in the bone marrow [2]. Myeloperoxidase and other heme-protein peroxidases present in the bone marrow may further convert the phenolic metabolites of benzene to semiquinone radicals [3]. This peroxidative metabolism and redox cycling of semiquinones can produce reactive oxygen species which may inflict oxidative damage on cellular DNA and induce genotoxic effects [4].

Oxidative metabolites of benzene cause its myelotoxicity and carcinogenicity [5], and CYP destruction [6,7]. Benzene treatment induces its own metabolism by CYP whereby it is oxidized to phenol, hydroquinone and other metabolites by CYP2E1 in rats, mouse and human [6,8]. DT-diaphorase [NADP (H)-quinone acceptor oxidoreductase] and ascorbate decreased metabolic activation of phenol to products that bind covalently to microsomal proteins, apparently because of inhibition to the more reactive benzoquinone [9]. It was suggested that OH* radicals were involved in the metabolism of benzene.
to genotoxic metabolite [10]. The reactive oxygen species (ROS) formed by CYP monooxygenase apparently oxidizes benzene outside the CYP active site [11,12]. Due to the participation of active oxygen species in benzene and its metabolite induced DNA damage in cell. However, the mechanism of cellular DNA damage through the generation of active oxygen species remains to be clarified [13].

Benzene exposure in humans and animals has been shown to result in structural and numerical chromosomal aberrations in lymphocytes and bone marrow cells, indicating that benzene is genotoxic [14]. According to Snyder et al. [15] benzene and its metabolites do not function well as mutagens but are highly clastogenic, producing chromosomal aberrations, sister chromatid exchanges and micronuclei. In several studies, increased levels of chromosomal aberrations in peripheral blood lymphocytes were correlated with a heightened risk of cancer, especially haematological malignancies. Thus, chromosomal aberrations may be a predictor of future leukaemia risk [16,17].

Benzene exposure increased DNA damage to bone marrow cell and leads apoptosis or mutation in cells. Apoptosis contributes to the pathogenesis of a number of diseases, including cancer. Cell deaths in response to DNA damage, in most instances, have been shown to result from apoptosis [18, 19]. Apoptosis is induced by many cytotoxic chemicals and ionizing radiation and characterized by morphological and biochemical changes such as chromatin condensation, nuclear fragmentation, formation of apoptotic bodies, and DNA fragmentation at internucleosomal sites [13].

To recognize the benzene as a carcinogen, we investigated ROS induced DNA damage in relation to apoptosis. Oxidative DNA damage was quantified by single cell gel electrophoresis. Mutagenic property of benzene as a carcinogen was examined by micronucleus assay and chromosome aberration. The induction of apoptosis was confirmed by internucleosomal cleavage of DNA which was assessed by agarose gel electrophoresis.

**MATERIAL AND METHODS**

**Chemicals:** Benzene, agarose, 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione, low melting agarose, Giemsa stain, phosphate buffer saline (pH 7.2), hydrogen peroxide (H₂O₂), ethidium bromide, bromophenol blue, RPMI 1640, trichloroacetic acid (TCA), thiobarbituric acid (TBA). All chemicals used were of analytical grade.

**Experimental protocol:** Sixty male Wistar rats weighing 250±20gms were used for present experiment. The animals were kept in neat cages in well ventilated animal house of department of Zoology at The M.S. University of Baroda, Vadodara. The animals were kept in 12 hrs dark and 12 hrs of daylight and were provided with standard rat feed (Pranav Agro Limited, India) and water ad libitum. Animals were divided into following four groups, containing 15 rats in each group: group 1, control; group 2, low dose (50mg/ml benzene) group 3, high dose (100 mg/ml benzene); group 4, vehicle control (corn oil). They were sacrificed 24 hrs after the 90 days of benzene administration. Immediately, both the femur bones were dissected out from each animal, bone marrow was aspirated with normal saline (0.9% NaCl), centrifuged at 2500 rpm and pellet was dissolved in RPMI 1640 medium. All the protocols for experiments were approved by IAEC of the department of Zoology according to CPCSEA, India.

**Determination of oxidative stress parameters:** The extent of lipid peroxidation in the bone marrow cells was determined according to the protocol of Janero [20] by measuring the level of TBARS formed. The results were expressed as MDA formed using an extinction coefficient of 1.56 × 10⁵ cm⁻¹M⁻¹. A deproteinized bone marrow sample in 10% TCA reacted with 2% TBA to form a pink colored complex that absorbed at 532nm.

The total glutathione (GSH) was measured at 412 nm according to Tietz method [21]. The thiol reagent, 5,5’-Dithiobis-2-nitrobenzoic acid (DTNB), reacts with GSH present in tissue sample forming 5-thio nitrobenzoic acid (TNB) and GS-TNB. The level of TNB and GS-TNB is equivalent to the GSH preset in the tissue.

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The superoxide dismutase (SOD) activity was measured by the described method of Marklund and Marklund [22]. SOD inhibits auto-oxidation of pyrogallol. 50% inhibition of pyrogallol auto-oxidation is equivalent to one international enzyme Unit (IEU). Using standard pyrogallol, the level of auto-oxidation was measured in tissue at 420nm. The catalase...
activity was estimated by the method of Sinha [23]. Bone marrow sample and 0.2M H\textsubscript{2}O\textsubscript{2} solution mixed together for initiating reaction. The reaction was stopped by adding potassium dichromate-acetic acid reagent after incubation. Absorbance was measured at 620 nm.

**Micronucleus assay:** Bone marrow smear was prepared and stained according to the described protocol of Tinwell and Ashby [24]. A small drop of bone marrow cells spread onto a clean slide allows to air dried and then fix in methanol. After drying slides were dipped in phosphate buffer and stained by 2% Giemsa stain then rinsed in phosphate buffer and air dried. Slides were examined in oil immersion by light microscopy (Leica DM2500) at 100X magnification and scored micronucleus in bone marrow cells. 1000 bone marrow cells were counted for each animal. To assess the cytotoxicity of benzene, we counted total number of polychromatic erythrocyte (PCE) and normochromatic erythrocyte (NCE) for determination of PCE/NCE in rat bone marrow.

**Bone marrow chromosome aberration assay:** Three animals from each group were injected with 10 mg/Kg body weight of colchicines (500 µl) to arrest the cell division before 2.5 hrs of sacrificed. Metaphase chromosome plates were prepared by the method of Adler [25] and Savage [26] with required modification. Aspirated marrow in RPMI 1640 was centrifuged at 3000 rpm and pellet was resuspended in 0.6% KCl. Cell were incubated for 45 minutes at 45 °C and centrifuged at 3000 rpm for 10 min. Pellet was mixed with freshly prepared 3:1 methanol: acetic acid (Carnoy’s fixative). Aspirate for 5 minutes and centrifuged at 3000 rpm for 10 mins. The preparation was given two washing with Carnoy’s fixative. Pellet was resuspended in 500 µl fixative and cells were dropped down from the height on chilled slide then heated at 60 °C for few seconds and stained by 2% Giemsa for 10 minutes. Slides were examined under light microscope in oil immersion power (100X) for chromosomal abnormalities.

**Comet Assay:** The comet assay was performed in rat bone marrow cells, according to the *in vivo* comet assay guidelines of Tice et al. [27] as described by Saquib et al. [28]. In brief, bone marrow cells were flushed into RPMI 1640 medium and diluted as 50000–100000 cells/ml. The mixture of 10 µl of cells and 200 µl of 1% low melting agarose was layered onto precoated slides, containing 200 µl of 1% normal melting point agarose and then covered with a cover slip. The slides were placed in the chilled lysing solution (2.5 M NaCl, 0.1 M EDTA, 0.01 M Tris HCl-pH 10, 1% DMSO and 1% Triton X 100) for 12 h at 4 °C followed by alkaline buffer (pH > 12) for 20 min. Electrophoresis was carried out for 20 min, at 25 V and 300 mA. Slides were then washed three times with neutralized buffer. Each slide was stained with 20 µg/ml ethidium bromide solution for 2 min and visualized using fluorescence microscope (Leica DM2500) which was coupled with a charge coupled device (CCD) camera. Images were captured and a minimum of 50 comets per slide, in triplicates for a group were analyzed using the software “CometScore software” (TriTek Corporation, Virginia). Mean values of the Olive tail movement (OTM), tail length (µm) and tail intensity (%) were separately analyzed for statistical significance. Tail length (length of DNA migration) is related directly to the DNA fragment size and presented in micrometres.

**DNA ladder assay:** DNA fragmentation in the form of a ladder due to endonucleolytic attack is reportedly considered as a characteristic of apoptosis and fragmentation of DNA is considered as one of the postlytic steps in the apoptotic process. Bone marrow cell were lysed in lysis buffer with 10% SDS and 50µg/ml proteinase K for 2 hrs at 45 °C. The cell lysates were first extracted with Tris saturated phenol, phenol : chloroform : isoamyl alcohol (25:24:1, v/v/v), then with chloroform–isoamyl alcohol (24 : 1), precipitated overnight at -20°C in 2 volumes of absolute ethanol in the presence of 0.3 M Na acetate, and recovered by centrifugation. Pellets were air-dried, resuspended in TE buffer. Electrophoresis was carried out in a 1.4% agarose gel containing ethidium bromide. The gel was examined and photographed under GelDoc (GeNei Merck Doc ItLs) to visualize intra-nucleosomal DNA fragmentation (laddering), characteristic of apoptosis or smearing of DNA due to necrosis.

**Statistical Analysis:** Statistical analysis was performed using GraphPad Prism 6 software. All results are expressed as mean ± standard error (SE). The significance of the differences between mean values was evaluated through one-way analysis of variance (ANOVA) followed by Bonferroni post hoc analysis. Differences between groups were
considered significant when \( p < 0.05 \).

**RESULTS**

**Oxidative stress enzyme:** The administration of toxic dose of benzene caused a significant increase in LPO activity, as determined by the increase in TBARS level with reference to that of control group. Sub chronic oral intoxication of benzene significantly increased the formation of TBARS in low dose group \((P \leq 0.01)\) and high dose group \((P \leq 0.0001)\) of animals (Table 1). Increase level of TBARS in bone marrow indicating a marked production of oxidative stress.

Activity of antioxidant enzyme was measured which is the important parameter to analyzed the status of reactive oxygen species. Table 1 illustrates that the administration of benzene depleted the activity of SOD, catalase and GSH in bone marrow of rat. The level of SOD activity was lowered down significantly \((14.78\% \text{ less in low dose (pd } \leq 0.001)\) and \(24.56\% \text{ in high dose (pd } \leq 0.0001)\)) compare to that of reference group. There was significantly decreased \((p \leq 0.01)\) activity of SOD in high dose group compare to that of low dose intoxicated animals. Activity of catalase also decreased at the significant level of \( p < 0.001 \) \((8.74\% \text{ less than the control group})\) in 50mg/ml dose and \( p < 0.0001 \) \((20.65\% \text{ less than the control group})\) in 100mg/ml dose group of rats. Value was also found significant between the treated groups, \( pd'0.0001 \) with high dose group compare to that of low dose intoxicated benzene. Activity of catalase also decreased significantly as compare to that of untreated rats. Value was found highly significant in both the treatment groups \((p \leq 0.0001)\). Increased activity of GSH was assessed in corn oil gavaged group of rats in comparison with control but the value was not found statistically significant.

**Micronucleus assay:** The number of micronucleated PCE and NCE among 2500 bone marrow cells, were increased which indicating benzene as genotoxicant, is presented in Table 2. Benzene induced a significant \((p \leq 0.0001)\) increase in PCEMN number in both the treated group of rats. At the 100mg/ml dose of benzene the PCE with micronucleus number was 3.7 fold higher than that of the control PCEMN. Both the dosage group of the benzene, the number of micronucleated NCE was significantly increased. Value of significant was \( p \leq 0.05 \) and \( p \leq 0.01 \) for low and high dose group respectively. The number of NCEMN was increased 1.2 fold in low dose group and 1.5 fold in high dose group of benzene oral exposure. No major difference was found in the number of PCEMN and NCEMN in vehicle control as compare to that of reference group of rats.

The cytotoxic potential of benzene was evaluated through the ratio of PCE and NCE among total cell count of bone marrow. The decreased ratio of PCE/NCE is given in Table 2. The ratio of PCE/NCE was depleted 12.6% less \((p \leq 0.05)\) in 50mg/ml and 19% less \((p \leq 0.01)\) in 100mg/ml of benzene exposure compare to that of control group of animal. There was no observed changes in PCE/NCE ratio of vehicle control group of rats in comparison with reference group.

**Chromosome aberration assay:** Benzene induced structural and numerical aberration in bone marrow cells was enumerated in the present investigation, with special emphasis on gaps, breaks, translocation, fragmentation, centric association (dicentric and ring chromosome) and polyploidy. All these types of aberrations and their frequencies for all the groups are presented in Table 3. The frequency of chromatid type of aberration with reference to gap and break was significantly higher in both the benzene intoxicated group \((p \leq 0.0001)\). Benzene treatment also increased the frequency of chromosome type aberration significantly \((p \leq 0.001)\). Fragmentation of chromosome was found highest among all the type of chromosome aberration. Frequency of ring chromosome was significantly increased due to benzene intoxication (Fig. 1). Translocation and polyploidy were observed lowest in comparison to the other aberration of chromosome.

**Comet assay:** Using comet assay, genotoxicity of benzene on bone marrow cells were analyzed to observe the extent of DNA damage in individual cells. Results generated by comet score software are illustrated in Table 4. Single cell DNA damage was found significantly higher in rats exposed to benzene at low and high dose group \((p \leq 0.001)\). Significantly higher value of comet tail length was also detected in 100mg/ml dosage group of rats as compared to that of 50mg/ml benzene exposed rats \((p \leq 0.001)\). % DNA contain was also observed more in a tail of
A) Deletion, B) Telomeric fusion and ring chromosome, C) Fragmentation D) Deletion and dicentric chromosome

Fig. 1: Chromosomal aberration in bone marrow cells after 90 days of benzene treatment.
Table 1: Effect of benzene on oxidative stress parameters in rat bone marrow. Results are expressed as mean ± SE (N=5). * significantly difference at P<0.05, ** at P<0.01, * at P<0.001 and, b at P<0.0001.

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO (n M of MDA/gm of tissue)</th>
<th>SOD (Unit/gm of tissue)</th>
<th>Catalase (umole of H_2O_2/gm of tissue)</th>
<th>GSH (ug/gm of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.71±1.36</td>
<td>27.2±1.3</td>
<td>101.8±2.3</td>
<td>57.8±1.9</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>14.58±2.42</td>
<td>28.72±0.9</td>
<td>102.9±2.4</td>
<td>60.78±1.5</td>
</tr>
<tr>
<td>Low dose</td>
<td>28.9±1.67*</td>
<td>23.18±1.8*</td>
<td>92.9±2.8*</td>
<td>49.6±2.7*</td>
</tr>
<tr>
<td>High dose</td>
<td>39.6±2.13b</td>
<td>20.52±0.6b</td>
<td>80.78±2.4b</td>
<td>42.4±2.1b</td>
</tr>
</tbody>
</table>

Table 2: Induction of micronuclei by benzene in rat bone marrow. Results are expressed as mean ± SE (N=5). * significantly difference at P<0.05, ** at P<0.01 and b at P<0.0001.

<table>
<thead>
<tr>
<th>Group</th>
<th>Micronuclei/1000 cells</th>
<th>PCE/NCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PCE</td>
</tr>
<tr>
<td>Control</td>
<td>4.1±0.57</td>
<td>2.6±0.25</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>3.6±0.56</td>
<td>2.5±0.29</td>
</tr>
<tr>
<td>Low dose</td>
<td>12.8±1.0b</td>
<td>3.5±0.33*</td>
</tr>
<tr>
<td>High dose</td>
<td>15.2±0.7b</td>
<td>4.0±0.36*</td>
</tr>
</tbody>
</table>

Table 3: Frequency of chromosomal aberrations in rat bone marrow by benzene exposure. Results are expressed as mean ± SE (N=5). b significantly different at P<0.0001

<table>
<thead>
<tr>
<th>Type of aberration/100 metaphase plate</th>
<th>Control</th>
<th>Vehicle control</th>
<th>Low dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatid Aberration</td>
<td>1.19±0.021</td>
<td>1.12±0.019</td>
<td>2.32±0.043b</td>
<td>2.74±0.028b</td>
</tr>
<tr>
<td>Gap Break</td>
<td>0.82±0.015</td>
<td>0.88±0.021</td>
<td>1.41±0.024</td>
<td>1.58±0.029</td>
</tr>
<tr>
<td>Cromosome Aberration</td>
<td>0.37±0.013</td>
<td>0.24±0.012</td>
<td>0.92±0.014</td>
<td>1.16±0.017</td>
</tr>
<tr>
<td>Fragmentation</td>
<td>0.16±0.002</td>
<td>0.13±0.005</td>
<td>0.24±0.014</td>
<td>0.45±0.012</td>
</tr>
<tr>
<td>Translocation</td>
<td>0±0</td>
<td>0±0</td>
<td>0.03±0.002</td>
<td>0.06±0.007</td>
</tr>
<tr>
<td>Polyploidy</td>
<td>0±0</td>
<td>0±0</td>
<td>0.06±0.008</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Dicentric</td>
<td>0.04±0.001</td>
<td>0.04±0.004</td>
<td>0.03±0.011</td>
<td>0.04±0.018</td>
</tr>
<tr>
<td>Centromeric association</td>
<td>0±0</td>
<td>0±0</td>
<td>0.19±0.013</td>
<td>0.28±0.015</td>
</tr>
</tbody>
</table>

Table 4: Shows benzene induced DNA damage through Comet assay parameter. Results are expressed as mean ± SE (N=5). * 'significantly difference at P≤0.01, * at P≤0.001 and b at P≤0.0001. * significantly difference with low dose compare to high dose at P≤0.05, * at P≤0.01

<table>
<thead>
<tr>
<th>Group</th>
<th>Tail length(µm)</th>
<th>Min-Max</th>
<th>% DNA in Tail</th>
<th>Tail movement</th>
<th>Min-Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.9±1.2</td>
<td>35.8-38.7</td>
<td>73.7±0.6</td>
<td>27.91±1.1</td>
<td>25.09-29.7</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>36.1±0.89</td>
<td>35.6-38.1</td>
<td>72.0±0.6</td>
<td>25.98±0.7</td>
<td>25.05-26.5</td>
</tr>
<tr>
<td>Low dose</td>
<td>55.4±1.43c</td>
<td>50.6-61.8</td>
<td>82.3±0.9&lt;sup&gt;***&lt;/sup&gt;</td>
<td>57.5±3.1&lt;sup&gt;**&lt;/sup&gt;</td>
<td>46.15-69.5</td>
</tr>
<tr>
<td>High dose</td>
<td>91.7±1.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>87.5-95.7</td>
<td>86.5±1.6&lt;sup&gt;*&lt;/sup&gt;</td>
<td>79.3±2.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76.32-82.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

DNA ladder assay: The results indicate that in DNA isolated from control and vehicle control bone marrow cells of rats (Fig. 2, lane 1 and lane 2), no DNA fragmentation was detectable. However, isolated DNA from the low dose group and high dose group at the end of subchronic exposure, DNA fragmentation was detectable (Fig.2, lanes 4, 5 and 6). The extent of DNA fragmentation was potentiated in bone marrow cells treated by benzene and display of ladder on agarose gel that is associated with

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apoptosis mediated via caspase-3 activation. The intensity of DNA laddering in treated cells of bone marrow of 100mg/ml dose was more than the 50mg/ml of orally benzene exposed rats.

**DISCUSSION**

The enzymatic bioactivation of benzene leading to the formation of ROS and subsequent increased oxidative stress is thought to play a significant role in benzene-initiated toxicity. Antioxidant enzymes like catalase, GSH, GST, GPx and SOD inhibit DNA oxidation by depletion of oxidative stress to the bone marrow cells. Results from the present study demonstrate that the antioxidative enzymes activity was lowered down due to benzene treatment and activity of lipid peroxidation was increased. Bone marrow toxicity caused by benzene exposure is thought to occur due to the presence of mylperoxidase enzyme which metabolized phenolic compound of benzoquinone which is more potent toxicant to deplete antioxidant enzyme for proposed target organ in present investigation.

Elevated level of ROS induces oxidative stress which leads oxidative DNA damage and micronucleus formation, a probable mechanism of genotoxicity [29]. Higher expectation of DNA damage causes higher frequency of micronucleus in cells and so micronucleus assay is widely used to assess the genotoxic potency of test compound. Furthermore, it gives indication concerning the cytotoxic potential of the tested compound. Increased number of polychromatic erythrocyte with micronucleus was observed more in benzene exposed rats when compared with control group of rats. It seems likely that the genotoxic effects of benzene are mediated by its oxidation products, the 1,4-benzoemiquinone radical and/or 1,4-BQ [13]. The data obtained show clearly that benzene induced MN in bone marrow cells of rat in dose dependent manner. From this result we can deduce, that benzene may interact with DNA and leads DNA damage. Due to high reactivity of benzene and its metabolites, may deplete reduced glutathione and critical membrane thiols, resulting in toxicity without reaching the DNA and exerting a genotoxic effect which is actually responsible for formation of micronucleus compare to other metabolic product of benzene [30]. Increased oxidative stress and micronucleus frequency also observed by Moro et al. [31] and Goethel et al. [32] in subjects occupationally exposed to benzene.

The chromosome aberration test, recommended by regulatory authorities for the assessment of genotoxicity and mutagenicity of many chemicals (drugs, pesticides etc.) and natural compounds, is for testing genotoxic potency [33]. Benzene increased the number of chromosome aberration with high frequency as a dose dependent concentration in study. Structural aberration of chromosome was common and only with very low frequency the numerical aberration was observed. High frequency of DNA break, fragmentation and centric fusion were found compare to the other type of structural aberration. The mechanism by which benzene induces structural chromosomal aberrations is not as apparent, however, but may be related to its ability to produce oxygen radicals [34,35], or through covalent binding to DNA and adduct formation. Clastogenicity could also result, however, from inhibition of DNA and RNA polymerases [36,37], or other enzymes involved in DNA replication or transcription such as ligases or topoisomerases. Inhibition of the functionality of topo II and enhanced DNA cleavage was associated with exposure to benzene in vivo in mice [38] and exposure to HQ and BQ in vitro [39-43]. Bioactivation of HQ to BQ by peroxidase was found to enhance topo II inhibition [36] and benzoquinone was more potent than hydroquinone in a cell-free
assay system [39,44]. A quantitative structure activity relationship model of the interaction of benzene metabolites with human topo II alpha further supports the inhibition of topo II as a mechanism of benzene-induced genotoxicity [45-47]. The observation that the metabolites of benzene are able to induce both structural and numerical aberrations provides insights into potential mechanisms underlying benzene-induced leukemia. Sister chromatid exchanges [47] and chromosomal loss and breakage [48] were demonstrated in mice and humans, respectively, upon exposure to benzene. Trevisan et al. [49] have reported that sister chromatid exchange and chromosomal abnormalities were increased due to the exposure of benzene and its derivatives in worker of gas station. A growing body of molecular and cytogenetic evidence indicates that chromosomal aberrations play an important role in the neoplastic development of certain tumors [50,51]. Finding of Hirabayashi [52] suggested that DNA damage such as weak mutagenicity or chromosomal damage was retained and such damage induced consequent activation of proto-oncogenes and related genes, which led cells to undergo further neoplastic changes.

The total extent of DNA damage in bone marrow cell after benzene treatment was demonstrated by single cell gel electrophoresis. Tou [53] concluded that the ‘alkaline comet assay’ is a sensitive method to detect DNA damage induced by benzene. Our result clearly showed that the tail length at high dose group pronounced more DNA damage than low dose. The ROS parameter of this study also supportive to this result which pointing toward more DNA damage. Similar result also mentioned by Yang and Zhou [54] that significantly concentration dependent rise in comet cell parameters due to benzene exposure.

Overproduction of ROS damages the cell and leads apoptosis and so unhealthy cell with unrepaired DNA clear from the normal cell population. Our result of DNA ladder assay bring out clearly with forming bands on agarose gel that bone marrow cell death enhanced by exposure of benzene. The occurrence of apoptotic cell in bone marrow was dose dependent manner. Findings of Ibuki and Goto [55] suggested that benzene metabolites induced deregulation of apoptosis due to caspase-3 inhibition, which contributes to carcinogenesis. The ability of phenolic metabolites of benzene to induce apoptosis in human bone marrow progenitor cells may contribute to benzene myelotoxicity [56].

In summary, from the above finding that Benzene increased oxidative stress in bone marrow which increased the frequency of micronucleus and chromosome aberration in cell. We confirmed that bone marrow cell apoptosis was increased due to benzene intoxication which was confirmed by ladder pattern of DNA in agarose gel. High number of bone marrow cell apoptosis that may play significant role in the cancer pathogenesis of benzene induced toxicity. Finding of the present study make a clear idea that benzene has own identical potency to act as carcinogen and develop cancer pathogenesis in rat bone marrow.

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