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RESEARCH ARTICLE

EVALUATION OF BENZENE INDUCED HISTOPATHOLOGICAL ALTERATION IN RAT.

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Abstract

Human exposure to benzene is associated with multiple adverse health effects. Its exposure is known to affect many critical organs including the hematological, hepatic, renal, lung, and cardiac functions. The purpose of this study is to examine the potential of benzene as hepatotoxicant. We used Wistar rat as a model and benzene was administrated orally at the doses of 50mg/Kg body weight/day and 100mg/Kg body weight/day for subchronic study of 90 days. Using diagnostic kit, liver function test which are biomarker for early detection, antioxidant parameters were estimated and compared with unexposed rats. Liver enzymes (ALP, GGT, AST and ALT) in treated rats were significant higher compared to control. The benzene exposures also led to significant reductions in antioxidant enzyme activities and significantly increased malondialdehyde (MDA) levels. Also, on microscopic examination, the liver tissues of experimental groups exhibited severe damage like sinusoidal dilation and necrosis. Subchronic exposure to benzene on rat has potential to develop hepatotoxicity by altering liver enzymes activity.

Introduction:-

Benzene is an industrial solvent and widely distributed environmental pollutant that has been linked to adverse health effect in humans and animals (Nazia et al., 2008; Uzma et al., 2008). A large section of population is occupationally exposed to benzene through work environment (Krewski et al., 2000; Ragia et al., 2014). Hematotoxicity is the most noted damage due to the chronic benzene exposure which leads to aplastic anemia, leukaemia and leucopenia. Chronic exposure of benzene resulted into consistent structural and numerical chromosomal aberrations in lymphocytes and bone marrow cells (Glass et al., 2003; Uzma et al., 2008)

Majority of benzene metabolism occurs in liver by cytochrome P450 and then in bone marrow (Uzma et al., 2008). Liver damages due to benzene exposure, ranging from hepatitis, cirrhosis, and carcinoma have been proved to associate with the redox imbalance and oxidative stress (Costa et al., 2006; Ragia et al., 2014). The most common chemicals known to cause liver injury are the organic solvents such as benzene, acetone, ether, and so on. There is some evidence that organic solvents especially benzene may express their toxicity by the way of ROS that was found to induce cell damage (AbdEllah et al., 2007; Ragia et al., 2014). Elevated level of ROS induces hepatocyte damage which results into altered liver function test (Loganathan et al., 2005). Therefore, the present study is conducted to evaluate the toxic effect of benzene on albino rat as a mammalian model.

Material and Methods:-

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. All the protocols for experiments were approved by IAEC of the Department of Zoology according to CPCSEA, India. They had access to 12 hrs of darkness and 12 hrs of daylight and were provided with standard rat feed (PranavAgro

Limited, India) and water *ad libitum.* Animals were divided into following four groups: Group 1, Control; Group 2, Low dose (50mg/Kg Body weight/Day); Group3, High dose (100mg//Kg Body weight/Day); Group 4, Vehicle control (corn oil) with 15 rats in each group. The animals were sacrificed 24 hrs after the 90 days of benzene administration. Blood was removed from orbital sinus before sacrificing and serum was separated for liver function test. Liver tissue was homogenized in PBS, centrifuged and supernatant was separated to measure oxidative stress parameters.

Biochemical Analysis:-

Serum alanine transaminase (ALT, Bergmeyer and Horder, 1980), aspartate transaminase (AST, Bergmeyer and Horder 1978), alkaline phosphatase (ALP, Tietz et al., 1983) and gamma glutamyl transpeptidase (GGT, Szasz, 1969) were measured using standard kits (Reckon diagnostics) on PerkinElmer spectrophotometer. Total protein concentration was measured at 660nm according to the method of Lowry et al. (1951). Glucose level was estimated using GOD/POD (Trinder, 1969) method and absorbance was measured at 505nm.

Oxidative stress parameters:-

The lipid peroxidation product present in the tissues was estimated by thiobarbituric acid (TBA) method (Janero, 1998). Marklund and Marklund (1974) method was used to estimate superoxide dismutase (SOD) activity. Catalase activity was assayed by the method of Sinha et al. (1972). The activity of glutathione peroxidase (GPx), in sample was determined by the method of Rotruck et al. (1973). The GSH content remaining after the reaction was measured by the method of Ellman et al. (1961). The level of reduced glutathione, a non enzymatic antioxidant, was determined by the method of Beutler et al. (1963).

Statistical analysis:-

Data generated from the experiment were subjected to statistical analysis and presented as mean and standard error of the mean. The statistical significance of the differences between the mean values of control and experimental groups was evaluated through one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. Statistical analysis was performed using GraphPad prism (version 6) software.

Result:-

The effects of benzene on certain serum biochemical parameters are summarized in Table 1. After subchronic exposure of benzene to rat, significant increased activity of ALT was observed. Values of ALT were significantly higher in both low dose ($p<0.05$) and high dose benzene ($p<0.01$) groups. Values were observed significantly higher in GGT activity in both the treated group of rats ($p\leq0.05$). Benzene intoxication at 100mg/Kg body weight also resulted into significant higher activity of AST ($p\leq0.05$). ALP activity was significantly higher in low dose ($p<0.05$) and high dose ($p<0.01$) benzene groups. There was no observed change in vehicle control group compared to control.

Table 1:- Effect of benzene on liver function in serum after 90 days of oral exposure to rat.

Group	ALT IU/L	AST IU/L	ALP µM pNP released /mg tissue	GGT IU/L
Control	20.7±1.2@	11.4±0.6	62.3±2.9	8.6±0. 5
Vehicle control	20.6±1.2	11.2±0.6	32.1±2.2	8.4±0.7
Low dose	24.2±1.6↑*	12.4±1.1	67.8±1.7↑*	10.1±0.7↑*
High dose	26.2±1.8↑**	13.6±1.2↑*	71.3±3.6↑**	10.2±0.9↑*

@Values are expressed as Mean±SE; n=5 for each group; * $p\leq0.05$;** $p\leq0.01$

Total protein level was noted significantly higher in both the treated group ($p\leq0.05$, Table-2). The level of serum glucose was depleted significantly in both the treated groups as compared to control ($P<0.05$).

Table 2:- Effect of benzene on serum biomolecules after 90 days of treatment.

Biomolecules	Control	Vehicle Control	Low dose	High dose
Protein (gm/dl)	10.3±0.9@	10.8±0.9	12.6±1.2↑*	13.1±1.5↑*
Glucose (mg/dl)	25.2±1.4	26.3±1.4	22.3±1.6↓*	21.2±1.9↓*

@Values are expressed as Mean±SE; n=5 for each group; * $p \leq 0.05$

The administration of toxic dose of benzene caused a significant increase in MDA level, as determined by the increase in TBARS level with reference to that of control group. Sub chronic oral intoxication of benzene significantly increased the level of MDA in low dose group (P≤0.05) and high dose group (P≤0.001) of rats (Table 3). Increased level of MDA in liver tissue indicates a marked production of oxidative stress.

Table 3:- Effect of benzene on the activities of LPO, antioxidant enzyme and non enzymatic antioxidant after subchronic oral exposure in rat liver

Group	**LPO** (nmol/min/mg tissue)	**Catalase** µmole H_2O_2 liberated/ minute/ mg protein	**SOD** (% inhibition /min/mg tissue)	**GPx** (mM of GSH consumed/ mg tissue	**GSH** (µg/ gm tissue)
Control	25.9±1.3@	85.1±3.5	18.3±0.9	8.9±0.7	26.2±1.4
Vehicle Control	25.2±1.6.	85.9±4.1	19.6±0.9	9.5±1.2	27.5±1.2
Low dose	29.3±1.6↑ *	75.3±4.2 ↓**©	16.2±1.2 ↓*	7.2±0.9 ↓*	22.8±1.6 ↓*
High dose	32.9 ±2.1↑***	66.3±5.2↓***	14.2±1.3↓**	6.3±1.1 ↓**	20.7±1.5↓**

@Values are expressed as Mean ± SE; n=5 for each group; * p≤0.05; ** p≤0.01; ***p≤0.001; © significance difference with high dose: p≤0.05

Activity of antioxidant enzyme was measured which is the important parameter to analyze the status of reactive oxygen species. Table 3 illustrates that the administration of benzene depleted the activity of SOD, catalase, GPx and GSH in liver homogenate. The level of SOD activity lowered down significantly at low dose (p≤0.05) and high dose (p≤0.01) compared to that of the reference group. Activity of catalase also decreased at the significant level of p≤0.01 in 50mg/ Kg body weight and p≤0.001 in 100mg/Kg body weight of dose group. Value was also found significant between the treated groups, p≤0.05 with high dose group compared to that of low dose intoxication of benzene. GSH activity decreased significantly as compared to that of untreated rats and found highly significant in both the treatment groups. Activity of GPx also decreased at the significant level of p≤0.05 in 50mg/Kg body weight and p≤0.01 in 100mg/Kg body weight of dose group. Increased activity of stress marker enzyme was found in corn oil gavaged group of rats in comparison with control but the value was not found statistically significant.

Microscopic examination of liver of control rats showed normal structure of the central vein, radially arranged hepatocytes around the central vein and blood sinusoids (Figure-1 A & B). Structural irregularity of hepatocytes and cellular congestion were noticed. Benzene exposure increased vacuoles formations as well as it was also noticed that position of the nucleus was shifted from central part. Benzene treatment distortion of liver cord, vacuolation and necrosis were noticed which accomplish with hemorrhage and sinusoidal congestion (Figure-1 C, D, E and F).

Discussion:-

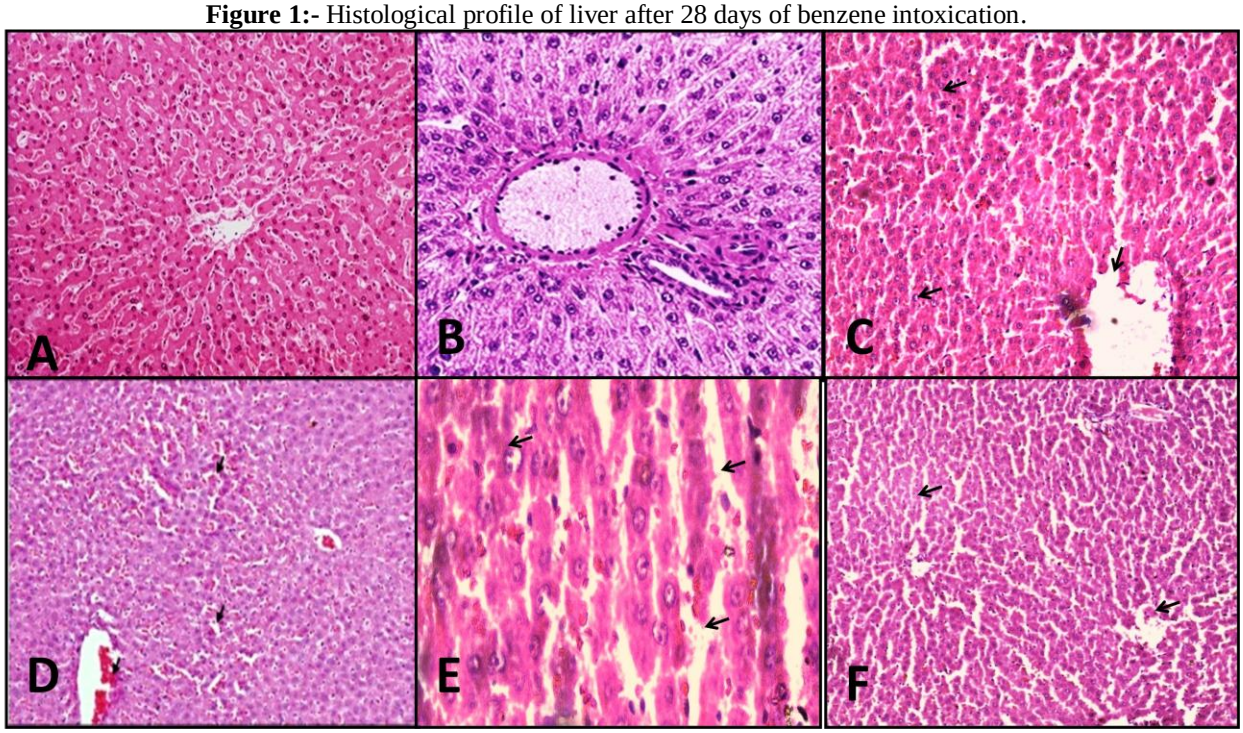
Human exposure to benzene is associated with multiple toxicities affecting the hematological, hepatic, immunologic, and chromosomal functions and an increased risk of carcinogenesis (Mark et al., 2014). However, the precise mechanism of benzene induced toxic effects is not fully understood. The findings of the present study indicate that benzene exposure induces significant alterations in hepatic functions after 90 days of exposure. In current work, we assessed liver function by examining the serum levels of ALP, GGT, AST and ALT in rats those underwent benzene treatment. The results demonstrated that the serum levels of ALP, GGT, AST and ALT were found to be elevated in the benzene exposed rats compared to the untreated reference group. Several other investigators also reported elevated liver enzymes among subjects exposed to benzene or petroleum products and organic solvents (Uzma et al., 2008; Mohammadi et al., 2010; Chang et al., 2013; Mark et al., 2014). The increased serum levels of these enzymes could be due to the overproduction or release of enzymes from the liver cells in response to stimuli of hepatocellular injury or cell death (Mark et al., 2014). Significantly higher concentration of total serum protein content was observed in the treated rats after 90 days of benzene exposure. Elevation in total protein content may be due to the hepatic detoxification, which results in the inhibitory effect on the activities of enzymes involved in detoxification. Significantly decreased serum glucose level was observed for the treated rats. Hypothetically, the low glucose level in the treated rats can be correlated to the low food intake or it may be possible that benzene affects the process of glucose absorption.

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. Present findings showed high statistical significant difference among benzene exposed group compared to control group regarding the level of MDA or lipid

peroxidation. This is in agreement with other studies and also with our previous finding of genotoxic effect which illustrated that benzene exposure has been associated with elevated level of MDA (Georgieva et al., 2002; Ragia et al., 2014; Roy and Pillai, 2015). Results from the present study demonstrate that the antioxidative enzymes like SOD, catalase, GSH and GPx activity was lowered down due to benzene treatment which is supported by the findings of Ragia et al. (2014) and Khadiga et al. (2011). Nonetheless, the findings of this study reveal that exposure to benzene is associated with significant adverse effects on liver. These effects may lead to the impairment in the function of hepatic and increased ROS production.

Significantly higher levels of liver marker enzymes in serum were supported by histopathological alteration of hepatic tissue. Necrosis was observed in treated tissue of rat liver which is one of the possible causes of increased enzyme activity. Benzene and its metabolic molecules lead to hepatocarcinoma via alteration in histoarchitecture of liver which has been noticed by many authors (Kari et al., 1992).

Figure 1:- Histological profile of liver after 28 days of benzene intoxication.



(A) Control showing normal histological profile, 10X; (B) Control section showing Central Vein and regularly arranged hepatic cord, 20X; (C) Low dose treatment with mild sinusoid dilation; Centrilobular destruction and vacuolation (D) High dose treatment with hemorrhages (E) Treated section showing sinusoidal dilation, necrosiswith gross hepatocellular damage (F) High dose treatment with centrilobular damage and sinusoidal dilation

Conclusion:-

The findings of this study revealed that benzene exposure has a potential to induce hepatic alterations. Increased levels of ALP, GGT, AST and ALT in the serum indicate histological alteration of liver via oral intoxication of benzene. In addition benzene exposure to animal increases ROS levels which depleted the activity of antioxidant system.

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References:-

1. AbdEllah, M., Okada, K. and Yasuda, J. (2007): Oxidative stress and bovine liver diseases: Role of glutathione peroxidase and glucose 6 phosphate dehydrogenase. J. Vet. Res., 54: 163-173.
2. Bergmeyer, H.U. and Horder, M. (1980): IFCC methods for the measurement of catalytic concentrations of enzymes: Part 3. IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2). Clinca Chimca Acta., 105: 145-172.
3. Bergmeyer, H.U., Horder, M. and Moss, D.W., (1978): Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes: Revised IFCC Method for aspartate aminotransferase. Clinca Chimca Acta,. 24: 720-722.
4. Beutler, E., Duron, O. and Kelly, B.M., (1963): Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.
5. Chang, W.J., Joe, K.T., Park H.Y., Jeong J.D. and Lee D.H. (2013): The relationship of liver function tests to mixed exposure to lead and organic solvents. Ann. Occup. Environ. Med., 25: 5.
6. Costa, C., Pasquale, R.D., Silvari, V., Barbaro, M. and. Catania S. (2006): In vitro evaluation of oxidative damage from organic solvent vapors on human skin. Toxicol., 20: 324-331.
7. Ellman, G.L., Courtney, K.D., Andres, V. and Feather-Stone, R.M. (1961): A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.
8. Georgieva, T., Michailova, A., Panev, T. and Popov, T. (2002): Possibilities to control the health risk of petrochemical workers. Int. Arch. Occup. Environ. Health., 75: 21–26.
9. Glass, D.C, Gray, C.N. and Jolley, D.J. (2003): Leukemia risk associated with low-level benzene exposure. Epidemiology, 14: 569–577.
10. Janero, D. (1998): Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. cidaR eerF. loiB. deM. ,9: 515-540.
11. Kari, F., Bucher, J., Eustis, S. L., Haseman, J. K., and Huff, J. (1992): Toxicity and carcinogenicity of hydroquinone in F3444/N rats and B6C3F1 mice. Food. Chem. Toxicol., 30: 737.
12. Khadiga, S., Ibrahim, Z., Saleh, A., Abdel-Razik, H., Farrag, E. and Shaban, E. (2011): Protective effects of zinc and selenium against benzene toxicity in rats. Toxicol. Ind. Health., 27: 537-545.
13. Krewski, D., Snyder, R., Beatty, P., Granville, G. and Meek, B., Sonawane, B. (2000): Assessing the health risks of benzene: a report on the benzene state of the science Workshops. J. Toxicol. Environ.Health., 61: 307–308.
14. Loganathan, G., George, R., Eapen, C.E., Jasper, P., Seshdri, L., Shankar, V., Paul, S., Joseph, G., Balasubramaniam K.A. and Chady, G.M. (2005): Liver function tests in normal pregnancy: A study from Southern India. Indian J. Gastroentero.24: 68-69.
15. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
16. Mark, A., D'Andrea G. and Kesava R. (2014): Hematological and hepatic alterations in nonsmoking residents exposed to benzene following a flaring incident at the British petroleum plant in Texas City. Environ.Health., 13:115.
17. Marklund, S. and Marklund, G. (1974): Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.
18. Mohammadi, S., Mehrparvar, A., Labbafinejad, Y. and Attarchi, M.S. (2010): The effect of exposure to a mixture of organic solvents on liver enzymes in an auto manufacturing plant. J. Public Health., 18: 553-557.
19. Nazia, U., Salar, B.M., Santhosh, K., Nusrat, A. and Anthony, D. (2008): Impact of organic solvents and environmental pollutants on the physiological function in petrol filling workers. IJERPH, 5: 139-146.
20. Ragia, M., Hegazy, H.F. and Kame, M. (2014): Oxidant Hepatic & /or Haem. Injury on Fuel-Station Workers Exposed to Benzene Vapor, Possible Protection of Antioxidants. AJMS., 4: 35-46.
21. Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.C. and Hoekstra, W.G. (1973): Selenium: biochemical roles as a component of glutathione peroxidase. Science, 179: 588-590.
22. Roy, H. and Pillai, A. (2015): Evaluation of ROS induced genetic aberration in benzene intoxicated rat bone marrow. J. cell Tissue Res., 15: 4957-4965.
23. Sinha, A.K. (1972): Colorimetric assay of catalase. Anal. Biochem., 47: 389-394.
24. Szasz, G. (1969): A kinetic photometric method for serum γ-Glutamyl transpeptidase. Clinca Chemica. 15: 124-136.
25. Tietz, N.W., Rinker, A.D. and Shaw, L.M., (1983): IFCC methods for the measurement of catalytic concentration of enzymes. Part 5. IFCC method for alkaline phosphatase (orthophosphoric- monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1). J Clin. Chem. Clin. Biochem., 21: 731-748.
26. Trinder, P. (1969): Determination of glucose in blood using glucose oxidase with alternative oxygen acceptor. Annals. Clinca Biochem., 6: 24-27.
27. Uzma, N., Kumar, B., Salar, K., Madhuri, A. and Reddy, V. (2008): In vitro and in vivo evaluation of toxic effect of benzene on lymphocytes and hepatocytes. Inter. J. Toxicol, 6: 2.