ISTOPATHLOGICAL CHANGES CAUSED BY THE EXPOSURE OF SUPER BENZENE ON HAEMATOPOIETIC TISSUES (SPLEEN AND BONE MARROW)). IN EXPERIMENTAL RATS (*RATTUS NORVIGICUS*

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ABSTRACT

The present study was carried out to determine the effect of super benzene inhalathion and its metabolites on haemopoietic tissues(spleen and bone marrow) on experimental rats.Animals were exposed via inhalation to concentrations of (5 and 10) ppm super benzene for 2 hour/day, 5 days /week for 3 months.Histological studies revealed that spleen of the exposed animals to super benzene, were exposed to 5 ppm showed white pulp atrophy and absence of megakaryocytes in red pulp with blood vessels congestion.In 10 ppm showed of extramedulary haemopoiesis, acute splenitis and fibrosis as well as showed increased aggregation of lymphocytes in lymph node, thickness with centeral artery wall also inflammation of inflammatory cells inside centeral artery and vasodilatation in centeral artery in asplenic nodule.While the bone marrow related to the animals exposed to 5 and 10 ppm showed reduced in haemapoietic tissue which partly replaced by adipose tissue.

INTRODUCTION

Benzene, the simplest homologue of the aromatic hydrocarbons, is a planar, cyclic molecule with six carbon atoms arranged in a regular hexagon. The molecular formula for benzene is C6H6. It is a volatile, colourless liquid with a characteristic odour (2).Benzene is used in industry as a volatile solvent and as an intermediate in the production of many chemicals, including ethylbenzene/styrene (used in plastics), cumene, linear alkyl benzene, and maleic anhydride (3,10).Benzene is also present in gasoline as an octane enhancer and anti-knock agent, however, levels of benzene in gasoline have been reduced to below 1% by volume, and found naturally in the environment in very low concentrations (9).Benzene and crude oil and gasoline, including emissions resulting from fuel combustion. Vehicular

emissions constitute the main source of benzene in the environment. (1).The atmosphere and surface water are the major environmental sinks for benzene due to its relatively high vapour pressure, moderate water solubility, and low octanol/water partition coefficient. benzene released into the environment eventually distributes itself into the air (19). Since benzene is classified as a Group 1 carcinogen (carcinogenic to humans)(2).Epidemiological studies report similar toxic effects following exposure to benzene regardless of exposure pathway (inhalation or ingestion).The primary toxicological effects of chronic benzene exposure are on the hematopoeitic system, depression of the response of B cells and T cells) in rats of benzene inhalation(14). Reduced numbers of each of B cells in the spleen and bone marrow, T cells in the thymus and spleen (15).The hematologic lesions in the bone marrow can lead to peripheral lymphocytopenia and/or pancytopenia following chronic exposure, these lesions may lead to the development of hematologic damage(13).

MATERIALS AND METHODS

Thirty six male rats (*Rattus norvigicus)* weighning 180-200 gm. were randomly selected from the animal house.They were given standard pallet diet and housed in well ventilated room at control ambient temperature (25 ±5c◦) and natural day light cycle.

These rats were equally divied into 3 groups comprising 12 rats in each group. The first group was controle while the second and third groups were exposed in chamber (size $1m^3$) to 5 and 10 ppm of super benzene via inhalation for 2 hr/ day, 5 days/week, for 12 weeks respectively.

All the experimental rats after completing 12 weeks experimental peroid were anaesthetized and their spleen were fixed in 10% bufferd formalin for 48 hrs.

Other specimens were taken such as bone marrow isolated from femurs, fixed in acetic acid-zinc-formaline fixative (AZF) for 24hrs, washed in distilled water for 30 min, decalcified in 10% formic acid-5% formaldehyde for 24hrs, then all the specimens were processed to dehydrated, cleared, infiltration, embedded in paraffin wax, sectioned at 5um. Thickness stained with hematoxiline - eosin and examined by light microscope (12).

RESULTS

Histologic observations of the spleen related to control rats showed normal structure of white pulp and red pulp (Fig.1).While that of treated animals with super benzene via inhalation at 5 ppm showed atrophy in white pulp and absence of megakaryocytes in red pulp with blood vessels congestion (fig.2, 3).

Also the spleen of the animals exposed to 10 ppm showed atrophic of white pulp and increased of extramedulary haemopoiesis, acute splenitis and fibrosis (fig.4), the results also showed aggregation of lymphocytes in lymph node and increased thickness of centeral artery wall with infiltration of inflammatory cells inside it and vasodilation in centeral artery in asplenic nodule (fig. 5).

Observation on bone marrow related to controle animals showed active bone marrow in respect of haemopoiesis, showed mekakaryocytes and hemocytoblasts (fig. 6) While the bone marrow related to the animals exposed to 5 and 10 ppm showed reduced in haemapoietic tissue which partly replaced by adipose tissue comparative to controle animals (fig. 7).

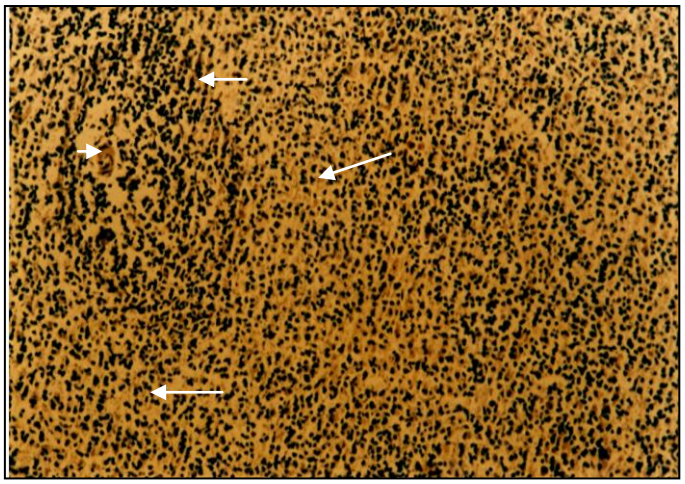

Fig.1: Microphotograph of T.S. of the spleen of the controle animals revealed white pulp → and red pulp → and central artery → (H&E 215 X).

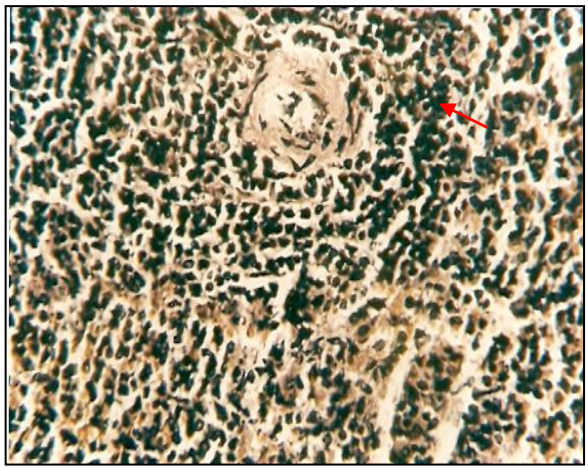

Fig.2: Microphotograph of T.S. of spleen of animals exposed to 5 ppm showing atrophic of white pulp (H&E 535 X)

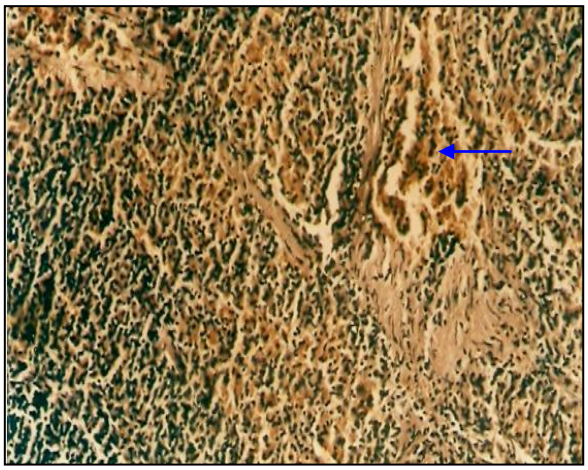

Fig. 3: Microphotograph of T.S. of spleen of animalse exposed to 5 ppm showing congestion in blood vessels → (H&E 215 X).

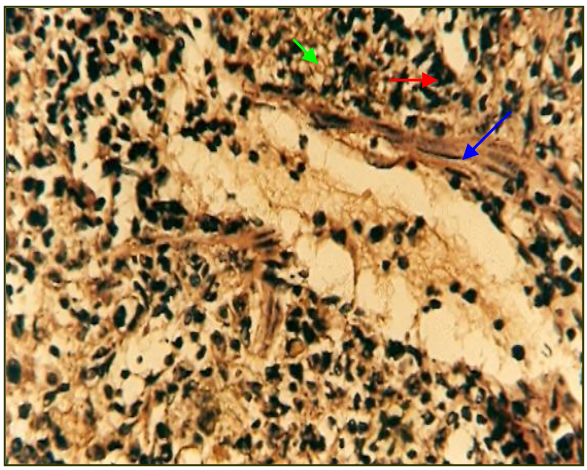

Fig.4: Microphotograph of T.S. of spleen of animals exposed to 10 ppm showing atrophic of white pulp and extramedulary haemopoiesis

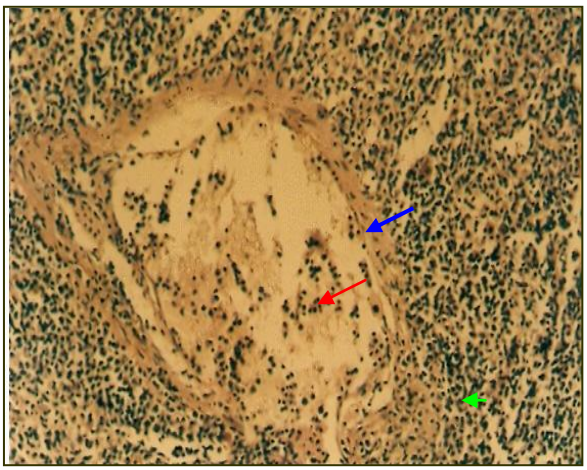

Fig.5: Microphotograph of T.S. of spleen of animals exposed to 10ppm showin aggregations of lymphocytes in lymph node and thichness in central artery wall with inflammatory cells inside in centeral artery (H&.E 215X).

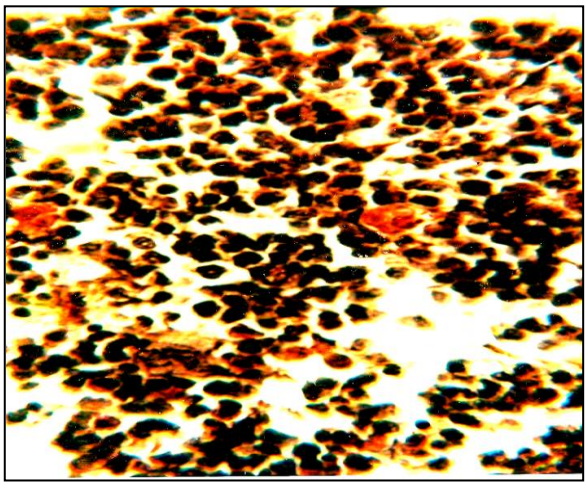

Fig. 6: Microphotograph showed bone marrow of controle animals showed megakaryocyte and hemocytoblast (H&.E. 775 X).

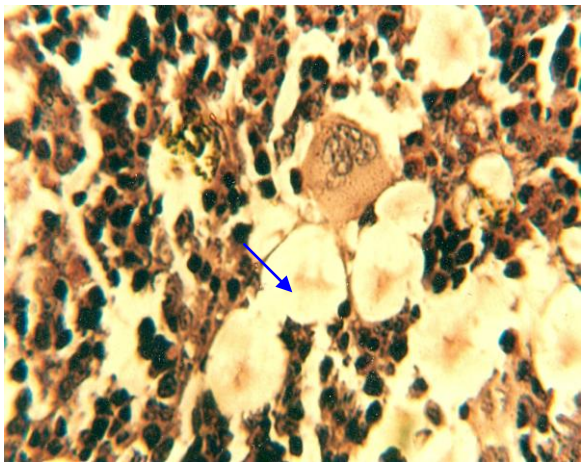

Fig. 7: Microphotograph of bone marrow related to animalse exposed to 5 and 10 ppm showing reduced haemopoiesis and bone marrow partly replaced by adipose

DISCUSSION

Results of this study showed many histopathological changes in hematopoietic tissues (spleen and bone marrow), related to animals exposed to super benzene. Benzene metabolized in the body by cytochromes P450 2E1 to phenol, catechol, and hydroquinone, these metabolites can accumulate in bone marrow and bioactivated by myleoperoxidases and other heme-proteinperoxidases, to reactive semiquinones and quinines, which can further lead to the formation of reactive oxygen spcies(ROS)(17).The role of ROS initiated toxicity in the hematopoietic tissues (8).In the present study, the aggregation of the lymphocytes inside the lymph node can be considered as a sign of an immune response, since these inflammatory cells play an important role to the toxic metabolites of benzene (5). The results indicated reduced in hemopoiesis of bone marrow this due to benzene and its metabolited very toxic to most cells.The major toxicity observed in experimental animals (mice, rats, and rabbits) has been on the blood- forming cells of the bone marrow, the hemopoietic system.(16).Cronkite et al (4) showed reduced bone marrow cellularity and adecreased number of pluripotent stem cells in bone marrow were exposed male and female mice by benzene inhalation.

In addition, hydroquinone and catechol have been shown to reduce the number of spleen and bone marrow progenitor B - lymphocytes and to inhibit polyclonal plaque - forming cells (21, 22).

Ward et al (20) observed histopathological findings included myeloid hypoplasia, depletion of the periarteriolar lymphoid sheaths in the spleen, lymphoid depletion in the mesenteric lymph nodes, and increased extramedullary haematopoiesis in the spleen, these changes observed in male and female rats and mice exposed to benzene vapour. Benzene and metabolites were concentrated in adipose tissues included bone marrow, sometimes bone marrow cells replaced with adipose tissues and reduction of erythrocytes in blood circulation cuasing aplastc anemia and effects of leucocytes cuasing leukemia (6).

التغيرات النسجية المرضية المتسببة عن التعرض للبنزين المحسن في النسيج المكون للدم (الطحال ونقي العظم) في الجرذان المختبرية*Rattus norvigicus* .

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الخلاصة

تمت دراسة تأثير أستنشاق بخار البنزين المحسن على الانسجة المكونة للدم (الطحال ونقي العظم) والمستخدم حاليا كوقود للسيارات ومولدات التوليد الكهربائية في الجرذان المختبرية. عرضت الحيوانات الى التراكيز (5 و10) جزء بالمليون من بخار مادة البنزين المحسن وبمعدل 2 ساعة / يوم و 5 يوم/ اسبوع ولمدة 12 اسبوع. اظهرت الدراسة تغيرات نسجية مرضية في الطحال تشتمل على ضمور اللب الابيض واحتقان للاوعية الدموية اضافة الى ظهور حالة التليف وزيادة في سمك جدار الشريان المركزي مع ارتشاح خلايا دفاعية داخل الشريان الطحالي. كما اظهرت الدراسة نقصان في خلايا الدم الناتج عن خلل وظيفي في نقي العظم مما ادى الى احلال النسيج الدهني بدل الخلايا . أكدت النتائج الحالية على التأثيرات السلبية لبخار هذه المادة من خلال التغيرات المرضية في تركيب نسيج الطحال ونقي العظم .

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