



Chronic Exposure to Carbon Tetrachloride (CCl₄) Disrupted the Functional Integrity of the Bone Marrow of Splenectomized Wistar Rats

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ABSTRACT

Splenectomy is a surgical intervention recommended for a variety of diseases and neoplastic conditions. Understanding the vulnerabilities of splenectomized individuals to various forms of toxicities will inform the lifestyle changes and management of such individuals. In this study, bone marrow integrity following chronic carbon tetrachloride (CCl₄) toxicity in splenectomized female Wistar rats was investigated. Fifteen (15) female Wistar rats, with a mean body weight of 172.36 ± 2.48g and randomly allocated into three groups of five rats each were used. Group I were untreated and served as the control. Group II were unsplenectomized (intact) and CCl₄ treated. Group III were splenectomized and CCl₄ treated. For the treatment, CCl₄ was diluted in paraffin oil to a concentration of 10% CCl₄ and administered at the dose of 3 ml/kg each, intraperitoneally. Bone marrow activities for all groups were evaluated using haematopoietic indices relating to proliferation and maturation of myeloid, erythroid and lymphoid cell lines. The result showed that while chronic CCl₄ toxicity in rats moderately depressed the maturation of myeloid and erythroid cell lines without severely affecting the overall haematopoietic capabilities of the marrow, these changes were exacerbated in the bone marrow of splenectomized rats.

Keywords: Splenectomy, Carbon Tetrachloride, Bone Marrow, Toxicity.

INTRODUCTION

The debate for the merits and demerits of splenectomy has continued over the years. Under certain disease conditions where the prognosis for the procedure absolutely outweighs any nonsurgical interventions, partial or complete

splenectomy has been recommended as the intervention of choice (Weledji, 2014). Some of these conditions include splenic rupture, splenic cysts and some types of neoplasms (Morris and Clark, 1994; Lewis and Swirsk, 1996). For other disease conditions such as blood and

reticuloendothelial diseases, splenic infections/inflammations and metabolic storage disorders, splenectomy was only proposed as optional (Coon, 1985). But splenectomy has been reported to improve liver fibrosis, hepatocyte regeneration (Unamba-Oparah *et al.*, 2020; Akahoshi *et al.*, 2002) and liver function (Ushitara *et al.*, 2011) in conditions of liver toxicities.

In the absence of overwhelming post-splenectomy infection (OPSI) commonly associated with splenectomy for which medical solutions exist (Weledji, 2014), splenectomized individuals are reportedly able to lead a normal life. However, these individuals could still be routinely exposed to different forms of chemical and environmental toxicants like their intact counterparts. Their physiologic response under these conditions will inform any lifestyle changes that may be required. This factor has been an ongoing debate as indication for splenectomy.

The toxicity of carbon tetrachloride (CCl₄) or its metabolites has been widely studied over the years and used as a model for the study of the effects of hepatic (Gregory, 2006) and renal (Mebius and Kraal, 2005) toxicants. However, there are little or no studies that have investigated the direct or indirect toxic effects of CCl₄ on the bone marrow and splenectomized animals.

This study thus investigated the effect of chronic exposure to carbon tetrachloride on the integrity of the bone marrow of splenectomized Wistar rats.

MATERIALS AND METHODS

Study site: The study was carried out in the teaching and research laboratory of the Department of Veterinary Surgery and Radiology,

College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State.

Experimental animals

Fifteen (15) female Wistar rats of about 6 months, with a mean body weight of $172.36 \pm 2.48\text{g}$ were used for this study. They were obtained from the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed at the Animal House Unit of the Department of Veterinary Surgery and Radiology, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State under ambient weather conditions. They were provided standard rat chow (Vital ® Growers feed, GCOML, Jos, Nigeria) and clean water *ad libitum* throughout the duration of the experiment. The rats were allowed to acclimatize for 2 weeks before the commencement of the experiment.

This study was approved by the College of Veterinary Medicine Research Ethics Committee, Michael Okpara University of Agriculture, Umudike (MOUAU/CVM/REC/2018011).

Experimental design

The rats were randomly allocated into three (3) groups of five (5) rats each. Group I served as the control, group II were CCl₄ – treated and intact (unsplenectomized), while group III were CCl₄ – treated and splenectomized.

Parameters related to the cellularity of the bone marrow were calculated and recorded.

Splenectomy procedure

The rats in group III were sedated with Xylazine at the dose of 5 mg/kg , intra-muscularly (IM),

followed by induction of anaesthesia 5 minutes later with ketamine hydrochloride at the dose of 35 mg/kg, IM.

Following anaesthesia, each rat was placed on right lateral recumbency and the left lateral part of the body was clipped generously and scrubbed with chlorhexidine solution.

The dorsal approach was adopted for this work. A skin incision of approximately 1 cm was made parallel to the 13th rib from just below the spinal muscle. The abdominal muscle was incised to expose the spleen beneath, which was then gently exteriorized. The splenic blood vessels were ligated with 5/0 vicryl suture material and transected caudal to the ligatures to remove the spleen. The abdominal muscles were then closed with 3/0 chromic catgut with a simple continuous suture pattern and the skin was routinely closed.

The rats were allowed three weeks to recover under optimum post-operative care before the commencement of carbon tetrachloride treatment.

CCl₄ treatment

Carbon tetrachloride (CCl₄) was diluted in paraffin oil to a concentration of 10% CCl₄ and administered to both group II (intact) and group III (splenectomized) rats at the dose of 3 ml/kg each, intraperitoneally (i.p.), every 5 days for 90 days according to the method by Shetty and Anika (1982). All rats were humanely sacrificed using chloroform inhalation in a chamber after 90 days each in accordance with the prescribed guidelines of the Institutional Animal Ethics Committee.

Bone marrow cytology and haematology

Following euthanasia, the femoral bones were carefully exposed and neatly cut, transversely. A

compressing instrument (pair of pliers) was used to squeeze one end of the cut bone firmly, allowing the marrow to squirm out. A little portion of the marrow was spotted onto one end of a clean, grease-free slide, squashed with another grease-free slide and a thin smear made from it. The smear was air-dried and stained with Wright-Giemsa stain.

The slides were viewed under a light microscope at x1000 magnification and the longitudinal counting method used to count 300 cells each of the erythroid and myeloid series with each cell type identified and scored using a differential cell counter.

Data analyses

The parameters evaluated included the myeloid:erythroid (M:E) ratio and the proliferation indices relating to leucopoiesis and erythropoiesis (immature:mature leucocytes [I:M] and immature:mature erythrocytes [I:Me] respectively). The myeloid maturation index (MMI) and the erythroid maturation index (EMI) were also calculated.

The data were recorded and presented as mean \pm standard error of the mean (SEM) and analysed using one-way analysis of variance (ANOVA). The variant means were separated using the least significant difference (LSD) post hoc test at $p < 0.05$, using the statistical package for the social sciences (SPSS), version 20.

RESULTS

Haematopoietic indices

The myeloid: erythroid (M:E) ratio was obtained by dividing the number of the myeloid cells by the number of nucleated erythroid cells, and did not

include the lymphocytes. Proliferation indices related to myelopoiesis (I:Mm) and erythropoiesis (I:Me) were also calculated. The maturation indices (MMI for the myeloid series and EMI for the erythroid series) were calculated as the inverse of their respective proliferation indices. Thus the proliferation and maturation indices for both the erythroid and myeloid series were presented and used as a means of evaluating the haematopoiesis of the bone marrow in both the CCl₄ - treated intact and splenectomized rats.

Myeloid to Erythroid ratio

The values for the M:E ratio (Fig. 1) for both group II (intact CCl₄ - treated) and group III (splenectomized CCl₄ - treated) increased. The increment was higher for group III than for group II, but neither increase was statistically significant (p>0.05), when compared with the control.

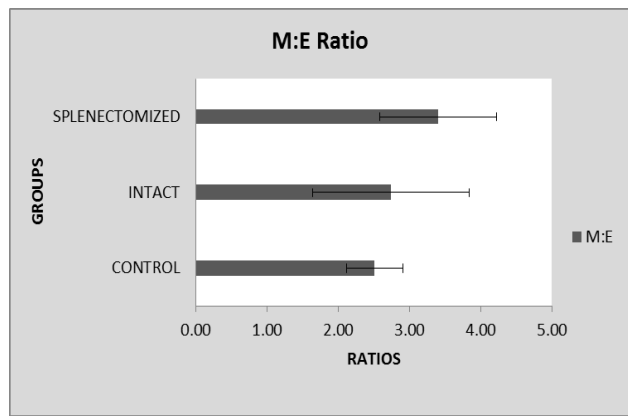
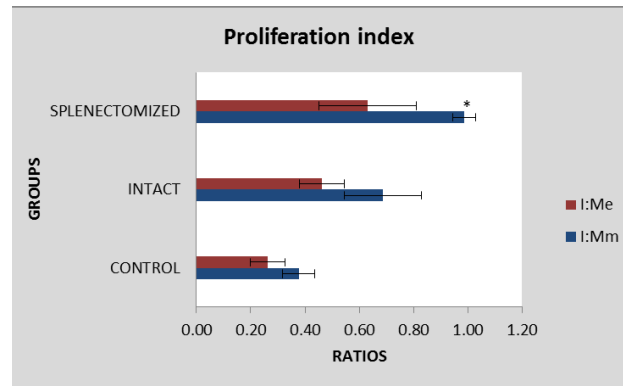


Fig. 1: The myeloid: erythroid ratio (M:E); showing the relative proportion of the myeloid cells to the erythroid cells in chronic carbon tetrachloride - treated intact and splenectomized female rats.

Proliferation indices

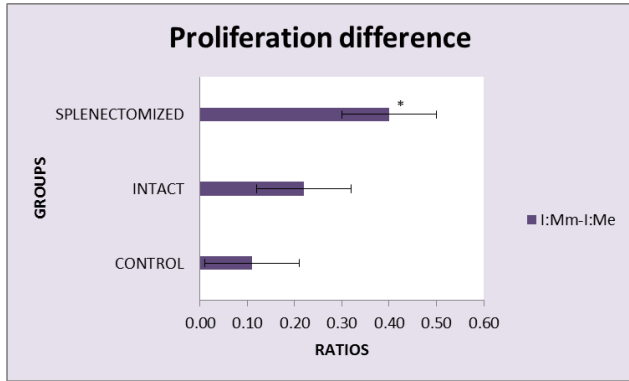
The myeloid (I:Mm) and the erythroid (I:Me) (Fig. 2) indices of the bone marrow increased when compared to the corresponding values for the control. However, significant (p<0.05) increases were only recorded for proliferation of the myeloid series.



*Values significantly (p < 0.05) different from those of the control

Fig. 2: The haematopoietic proliferation indices; the ratio of immature: mature myelocyte (I:Mm) and the ratio of immature: mature erythrocyte (I:Me) in chronic carbon tetrachloride - treated intact and splenectomized female rats.

Fig. 3 shows that the difference in the degree of proliferation of the myeloid to erythroid series (I:Mm - I:Me) in both CCl₄ groups were higher than recorded for the control group, with the splenectomized group showing the highest elevation which was significantly (p<0.05) higher than the control.

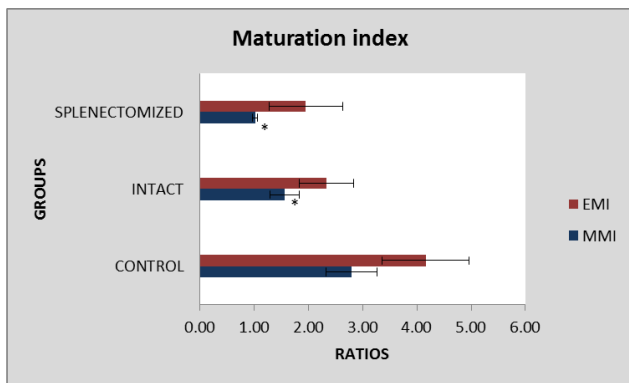


*Values significantly ($p < 0.05$) different from those of the control

Fig. 3: The difference in proliferation ratios of myelocyte (I:Mm) and erythrocyte (I:Me) cell series in chronic carbon tetrachloride - treated intact and splenectomized female rats.

Maturation indices

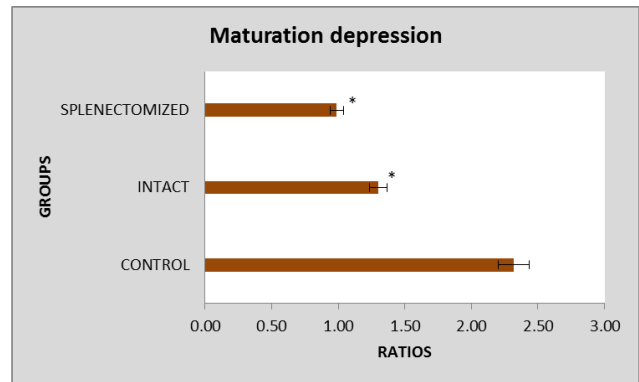
For the maturation indices (Fig. 4), both the myeloid (MMI) and the erythroid (EMI) maturation indices decreased to lower than that of the control, with a significant ($p < 0.05$) decrease recorded for the myeloid series for both the intact and the splenectomized groups.



*Values significantly ($p < 0.05$) different from those of the control

Fig. 4: The haematopoietic maturation indices; the ratio of mature: immature myelocyte (MMI) and the ratio of mature: immature erythrocyte (EMI) in chronic carbon tetrachloride - treated intact and splenectomized female rats.

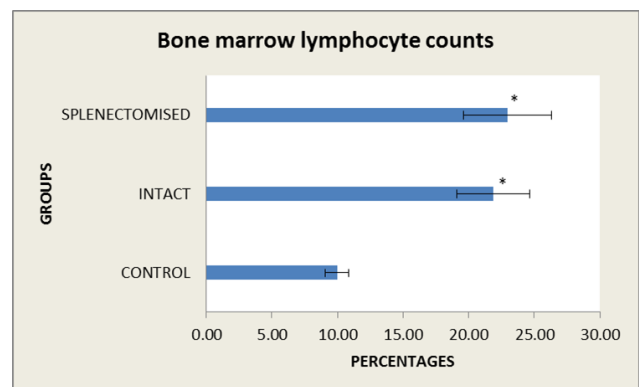
A comparison of the depression in maturation that occurred in both groups II and III when compared to the control was highlighted in Fig. 5. This was determined by the total haematopoietic maturation index (myeloid and erythroid) for the respective groups as a fraction of the mean number of cells counted.



*Values significantly ($p < 0.05$) different from those of the control

Fig. 5: Shows the depression at the maturation stage of haematopoiesis in the bone marrow of intact and splenectomized female rats with chronic carbon tetrachloride treatment.

Fig. 6 shows the percentage lymphocyte population in the bone marrow for all the groups. Both groups recorded significant ($p < 0.05$) increases in bone marrow lymphocyte populations when compared with the control.



*Values significantly ($p < 0.05$) different from those of the control

Fig. 6: Percentages of lymphocyte counts in

bone marrow of chronic carbon tetrachloride - treated intact and splenectomized female rats.

In the peripheral circulation (Fig. 7), the lymphocyte populations increased for both groups when compared with the control with a significant ($p < 0.05$) increase recorded for the intact group (group II).

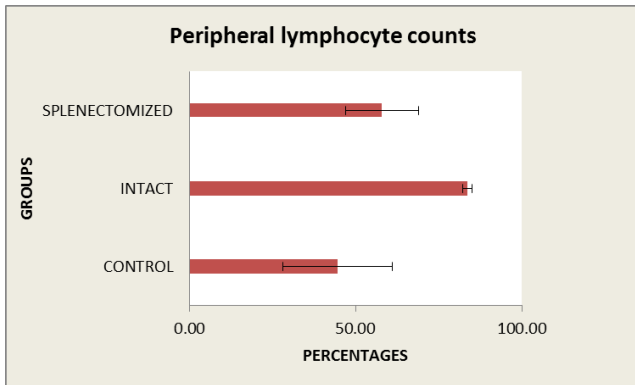


Fig. 7: Percentages of lymphocyte counts in the peripheral circulation of chronic carbon tetrachloride - treated intact and splenectomized female rats.

DISCUSSION

The M:E ratio is a measure of the balance of haematopoiesis between the myeloid and the erythroid cell series (Bain *et al.*, 2011). An increase in the M:E ratio reflects either an increased myelopoiesis relative to erythropoiesis or a decrease in erythropoiesis relative to myelopoiesis. The higher M:E ratios observed in both group II (intact CCl₄- treated) and group III (splenectomized CCl₄- treated) compared to the control group (Fig. 1) was likely due to an increased myelopoiesis in both groups relative to erythropoiesis. This was further confirmed by the higher increase in the myeloid proliferation indices for these groups when compared to the control group as shown in Fig. 2 and the greater turnover of the myeloid cell lines against that of

the erythroid series (I:Mm - I:Me) as shown in Fig 3. Notwithstanding, erythropoietic proliferation was also higher in both groups II and III compared to the control with the highest increase also occurring in group III. These highest values recorded for group III may be attributed to compensatory bone marrow activity in this group in the absence of the spleen. The spleen acts as a temporary blood storage organ in intact individuals and its absence would have elicited a compensatory reaction from the bone marrow. The increased myeloid to erythroid turnover for both CCl₄ – treated groups is also an indication that the CCl₄ in these animals did not severely affect the marrows’ ability for myelopoiesis. Thus their ability to mount an immune response when required was likely not affected.

The proliferation and maturation indices (Figs. 2 and 4) show the respective levels of activity at the proliferating and maturing stages of haematopoiesis. Fig. 2 and 4 also show that during haematopoiesis, as recorded for both group II and III, there was a higher proliferation but a decreased maturation of both haematopoietic cell lines when compared to the control (group I). Again, at both stages of the haematopoietic process, group III was more affected than group II. This implies that the toxicity of CCl₄ in groups II and III seemed to have depressed the rate of maturation of the haematopoietic cells and this depression was worsened (Figs. 4 and 5) by the absence of the spleen in group III. The depression in maturation may be as a result of direct toxicity to the marrow, but may also not be unconnected to the damage done by CCl₄ to the liver (Weber *et al.*, 2003) and the kidney (Khan *et al.*, 2010; El Denshary *et al.*, 2012). Both the liver and the kidneys are involved in the production of elements/components required at different stages

of myelopoiesis (Jones *et al.*, 2010) and erythropoiesis (Schoener and Borger, 2022).

With both groups also showing higher lymphocytosis than the control group in both the marrow and peripheral circulations (Figs. 6 and 7), it further confirmed a retained capacity for immune response in the presence of CCl₄ toxicity. However, for the splenectomized group III, the peripheral lymphocytosis was lower than what was observed in the intact group II. In higher animals, the spleen is a secondary haematopoietic organ. It only assumes haematopoietic activities where there is failure of the bone marrow except in lymphocytosis where it continues to contribute from its white pulp along with other lymphoid tissues (William, 1983). However, in rodents the spleen retains its normal haematopoietic activity in consonance with the bone marrow (William, 1983). Thus, the lower peripheral lymphocytosis of the splenectomised group compared to the intact group was likely due to the absence of the lymphocytic support act otherwise provided by the spleen in these species. Also the slightly higher but insignificant marrow lymphocytosis observed in the splenectomized group compared to the intact group may also be due to the compensatory functional activity of the bone marrow in the splenectomized.

CONCLUSION

In conclusion, chronic CCl₄ toxicity in rats caused moderate disruption of the functional integrity of the bone marrow of female Wistar rats by depressing the maturation of myeloid and erythroid cell lines. These depressions in the maturation of the haemopoietic cell lines were exacerbated in splenectomized rats. Thus continuous exposure to CCl₄ and other toxic chemicals must be evaluated in the

recommendation and management of splenectomized individuals.

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