



Pathomorphology of Liver and Kidney in Chronic Carbon Tetrachloride (CCl₄) Toxicity in Splenectomised Female Albino Rats

Unamba-Oparah, I.C.¹, Unamba-Oparah, C^{2*}, Okonkwo, J.C¹, Njoku, N.U²,
Nwagbara, N.D¹.

¹ Department Of Veterinary Pathology, Michael Okpara University Of Agriculture, Umudike, ² Department Of Veterinary Surgery and Radiology, Michael Okpara University Of Agriculture, Umudike, *Corresponding author: Email: unambaoparahc@gmail.com, Tel No: +2348030942147

SUMMARY

Splenic abnormalities including splenomegaly and hypersplenism are common findings in animals with liver fibrosis/cirrhosis. This work sought to investigate the effect splenectomy will have on the pathomorphology of the liver and kidneys of rats with carbon tetrachloride (CCl₄) – induced chronic liver damage. Eighteen eight month old female albino rats were randomly assigned into three groups of six rats each. Group I was the untreated and served as the control. Group II was intact (not splenectomised), while Group III was splenectomised. Groups II and III were injected with 10 % CCl₄ at the dose of 3 ml/kg body weight intraperitoneally, every five days. After 90 days, both groups II and III liver and kidneys showed pathologic changes consistent with chronic liver damage. Grossly there were nodular lesions in the livers while microscopically fibrosis and regeneration were observed. In the kidneys, interstitial congestion and generalised nephrosis were observed microscopically. However, it was also observed that these gross and the microscopic changes progressed in the liver of the splenectomised (Group III) than that from the unsplenectomised (Group II), while the pathology seen in the kidneys of Group II was more severe than in Group III. This suggested advanced fibrotic and regenerative changes in the pathomorphology of both the liver and kidney of splenectomised rats with chronic liver damage.

Key words: splenectomy, chronic, pathomorphology, liver, kidney, regeneration.

INTRODUCTION

Many metabolic changes occur in the liver allowing for uptake of nutrients from food and for the metabolism of xenobiotics (Moore and Dalley, 2006). In the adult animal, the liver is the largest organ, the

largest gland and one of the most vital organs sustaining the life of the animal. It lies mainly in the right upper quadrant of the abdomen where it is conveniently protected by the thoracic cage and the

diaphragm (Ozougwu, 2017). The liver functions primarily to control or modulate the safety of all ingested substances, including drugs and food absorbed from the digestive system before the eventual distribution of these substances or their metabolites to the systemic circulation (Allen, 2002). Essentially therefore, the functions of the liver in adult species are to metabolise carbohydrates, proteins and fat, the detoxification of drugs and toxins, storage of glycogen, vitamins and minerals, as reservoir of blood, filtration of microbes, excretion of bile and urea, as well as immunological functions via the resident phagocytic kupffer cells (Guyton and Hal, 2006). The liver is thus continuously exposed to a lot of metabolic insults and metabolic stress and in spite of its functional and structural resilience, liver diseases still occur regularly in man and animal species.

The kidneys as found in all vertebrates primarily function to maintain homeostasis by regulating body fluid levels, electrolyte balance, and other factors that keep the internal environment of the body consistent and comfortable (William, 2000). They also serve to remove waste products from the body, regulate blood pressure (Pennabecker, 2004) in addition to the secretion of active compounds (Brattin, 1985). Because of its role in the filtration, metabolism, and excretion of compounds, the kidney remains one of the most important organs examined in toxicological studies and is often the site of test-article-induced lesions (Mebius and Kraal, 2005). Patients with liver cirrhosis are prone to acute kidney injury (Jiang, 2011). Hepato-renal syndrome as initiated by progressive portal hypertension, may be prematurely triggered by bacterial and non-bacterial systemic inflammatory reactions, excessive diuresis, gastrointestinal haemorrhage, diarrhoea or nephrotoxic agents (William, 2000). One of the last events in the natural history of chronic liver disease is acute kidney injury (Brattin, 1985).

The spleen is a secondary lymphoid organ that has been known to influence the progression of multiple diseases, notably liver cirrhosis (Li *et al.*, 2017). Splenomegaly, usually revealed by ultrasonography, is a common finding in cases of chronic liver diseases involving cirrhosis and portal hypertension, as well as those with cystic fibrosis (Orlando *et al.*, 2011). Hypersplenism, a clinical syndrome in which the spleen is overactive is characterized by splenomegaly, and is associated with the destruction of one or more cell lines in the peripheral blood (Ferri, 2007). It correlates with, and has been postulated to facilitate the progression of liver fibrosis to cirrhosis, although the precise mechanisms for this remain poorly understood (Li *et al.*, 2017). In severe cases of splenomegaly and hypersplenism, splenectomy is performed to correct the effects of low blood cell and platelet counts (Yoshida *et al.*, 2012) and can effectively improve liver function (Ushitora *et al.*, 2011).

Carbon tetrachloride (CCl₄), commonly used to induce hepatotoxicity in experimental models for studies in acute and chronic liver failure (Gregory, 2006), is a colourless and non-inflammable liquid chemical, and is an environmental pollutant known to cause tissue necrosis or cell damage causing leakage of the enzyme content into the general circulation where it is transported to other organs (Vidona *et al.*, 2018). Carbon tetrachloride (CCl₄) undergoes biotransformation in the liver leading to the production of toxic metabolites; the highly reactive trichlormethyl free radical and the peroxytrichlormethyl radical both of which are strongly hepatotoxic (Vidona *et al.*, 2018).

This work seeks to throw more light on what is known of the pathomorphologies of the liver and kidney in chronic carbon tetrachloride (CCl₄) toxicity in splenectomised and non-splenectomised female albino rats.

MATERIALS AND METHODS

Study area: The study was carried out in the teaching and research laboratory of the Department of Veterinary Pathology, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State.

Experimental animals

Eighteen (18) female albino rats of about 8 months of age, with mean bodyweight of 180 g were obtained from the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were paired in stainless steel cages at room temperature in clean well ventilated, fly-proof animal houses in the Department of Veterinary Pathology, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State, and provided commercial feed (Vital® Growers feed, GCOML, Jos, Nigeria) and clean water ad libitum throughout the period of the study. Before the commencement of any procedures, the rats were acclimatized for 2 weeks in accordance with the permission and prescribed guidelines of the Institutional Animal Ethics Committee. They were then randomly allocated into three (3) groups of Six (6) rats each. Group I was the control, group II was the intact unsplenectomised, while group III was splenectomised.

Splenectomy

The rats in group III were premedicated with Xylazine (5 mg/kg body weight intramuscularly), followed by induction 5 minutes later with ketamine hydrochloride (35 mg/kg body weight intramuscularly). Each rat was placed on right lateral recumbency and the left lateral part of the body was liberally clipped and scrubbed with chlorhexidine solution.

The dorsal approach was adopted for this work. A skin incision (approx. 1 cm) was made parallel to the 13th rib beginning just below the spinal muscle. The abdominal muscle was incised to reveal the spleen

beneath, which was exteriorized. The splenic blood vessels were ligated with 5/0 vicryl and transected caudal to the ligatures to remove the spleen. The abdominal muscles were closed with 3/0 chromic catgut in a simple continuous suture pattern and the skin was routinely closed.

The rats were allowed three weeks to recover from the surgery before the commencement of carbon tetrachloride treatment.

Experimental design

Carbon tetrachloride (CCl₄) was diluted in paraffin oil to a concentration of 10% CCl₄ and administered to group II (unsplenectomised) and group III (splenectomised) rats at the dose of 3 ml / kg body weight, intraperitoneally, every 5 days for 90 days using the modified method of Shetty and Anika, (2005). All groups of rats were humanely sacrificed after 90 days each.

Gross examination

The livers of all the rats in all the groups were carefully harvested and assessed grossly for any morphologic changes suggestive of chronic hepatic damage.

Histopathology

Portions of the liver and kidneys from all groups of rats were cut and fixed in 10% phosphate-buffered formal saline for 48 hours. They were then dehydrated in ascending concentrations of alcohol, cleared in xylene for 1 h 30 min, and embedded in paraffin wax. Sections 5 µm thick were cut and mounted on slides. The slides were stained with haematoxylin, counterstained with eosin (H&E stains), and viewed under light microscope. The method used was that described by Drury and Wellington (1967).

Fibrinogen test

The fibrinogen test was used to assess the functionality of the liver of the animals. One milliliter of blood was collected from

each rat and centrifuged to obtain the plasma. The plasma was then used to assess for blood fibrinogen levels for each group using the heat precipitation method (Stockham and Scott, 2008). Data obtained were analysed using one-way analysis of variance (ANOVA).

RESULTS

Liver pathomorphology

On gross examination of the liver of all the groups, the group II rats (unsplenectomised) showed generalised to multifocal areas of moderately raised / nodular lesions (Plate 1a). These nodular lesions were also present in the liver of group III rats, but were clearly more severe or distinct than in the group II livers (Plate 1b). Microscopically in the liver of

both groups of rats, these nodular lesions were seen to represent multifocal areas of variably-sized pseudolobulation showing generalised hepatocyte degeneration and necrosis, interspersed with areas of hepatocytes regeneration and fibrous tissue formation (Fig 1). Fibrosis and hepatocyte regeneration are common findings in chronic liver damage. What was noteworthy was that the degree of pseudolobulation was more distinct and prominent in the livers of the group III rats than for group II rats. The fibrosis and regeneration in group III was more advanced with the hyperplasia of hepatocytes being more prominent in this group (Fig 1). Both sets of livers also showed clear evidence of bile duct proliferation around the peri-portal areas.



(Plate 1. Gross morphology of liver of experimental groups; (a) Group II (unsplenectomised): Mild to moderately raised areas (micronodules) were observed on the surface of the liver. (b) Group III liver (splenectomised): More severe raised/nodular areas (macronodules) can be observed on the surface of the

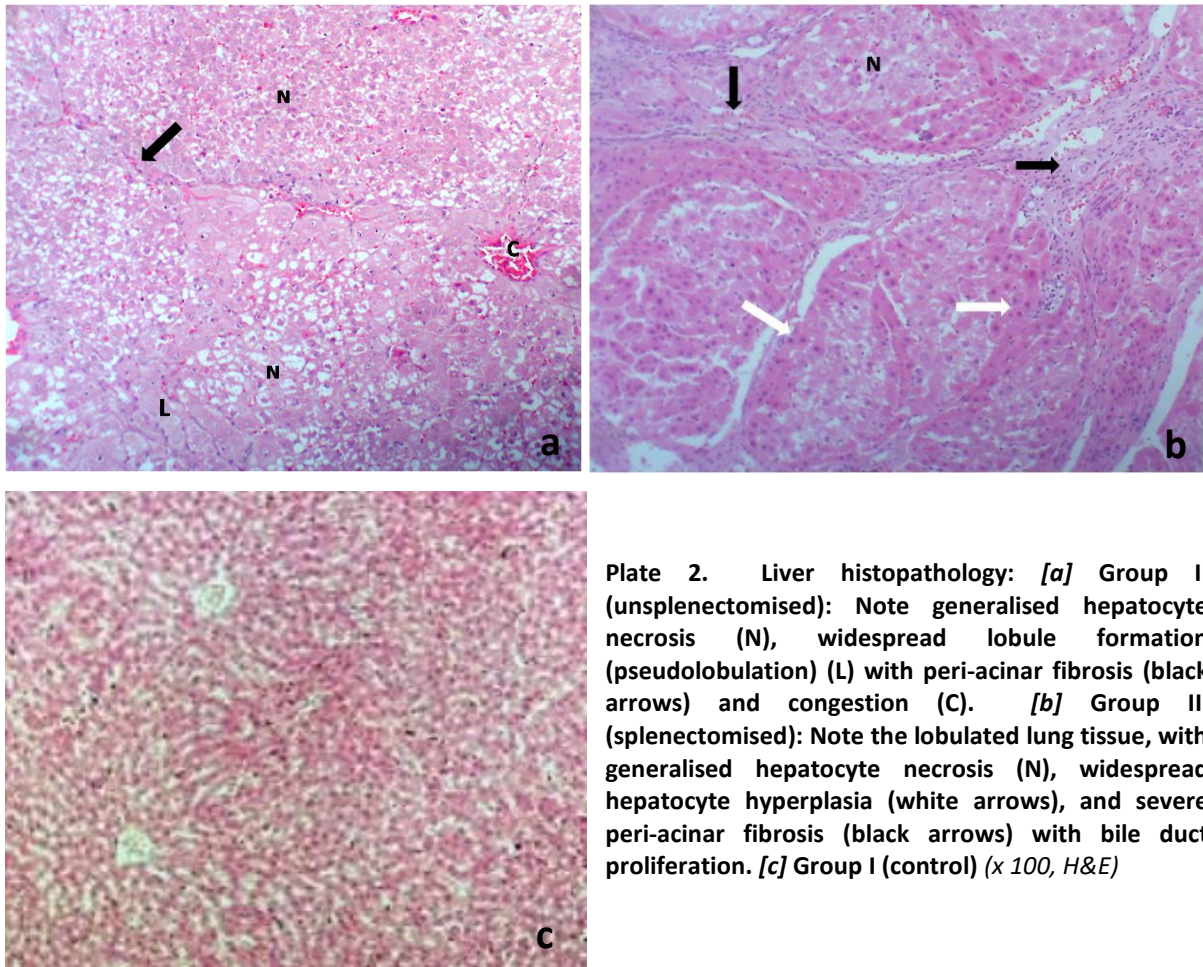


Plate 2. Liver histopathology: [a] Group II (unsplenectomised): Note generalised hepatocyte necrosis (N), widespread lobule formation (pseudolobulation) (L) with peri-acinar fibrosis (black arrows) and congestion (C). [b] Group III (splenectomised): Note the lobulated lung tissue, with generalised hepatocyte necrosis (N), widespread hepatocyte hyperplasia (white arrows), and severe peri-acinar fibrosis (black arrows) with bile duct proliferation. [c] Group I (control) (x 100, H&E)

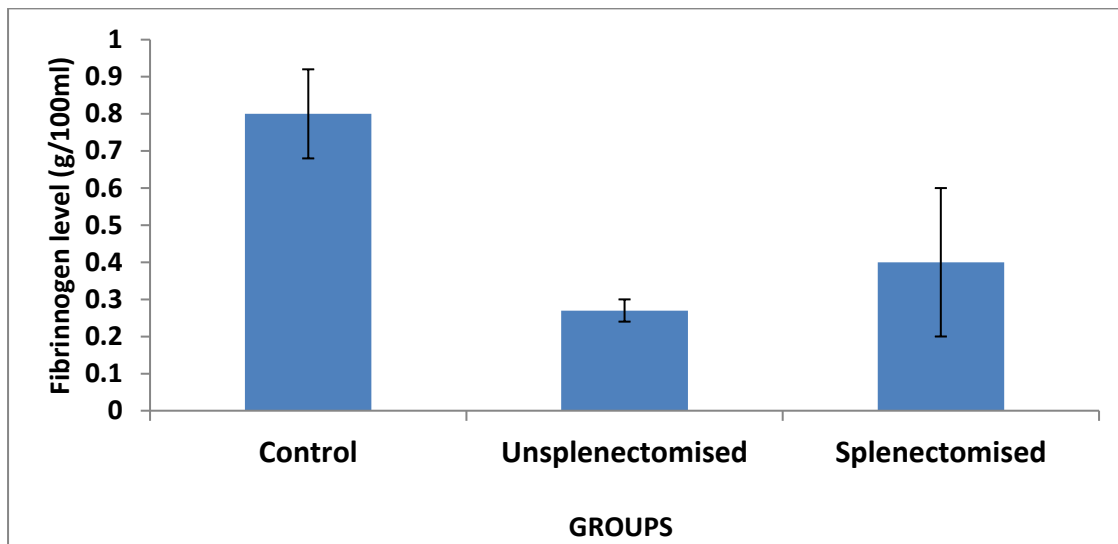


Fig 1: Fibrinogen test; both treatment groups were still able to produce fibrinogen. However, the unsplenectomised group produced significantly less fibrinogen than the splenectomised.

Fibrinogen

The fibrinogen test showed that although both groups of rats were still able to

synthesize some fibrinogen, the levels of fibrinogen synthesis in the livers of both test groups dropped when compared to that

of the control group. This drop in fibrinogen levels was more significant ($p < 0.05$) in group II than in group, III (Fig 1).

Kidney pathomorphology

The histopathology of group II kidney (unsplenectomised) showed there was generalised interstitial congestion and haemorrhage and generalised nephrosis with the glomeruli fully affected (Plate 3) whereas the group III kidney (splenectomised) also showed generalised congestion and haemorrhage and mild nephrosis but with the glomeruli fairly intact (Plate 3).

DISCUSSION

Carbon tetrachloride (CCl_4) hepatotoxicity in animals is characterised by hepatocyte necrosis with fat degeneration (Weber *et al.*, 2003). Free radicals, resulting from the breakdown of CCl_4 , and the parent molecule itself, damage the endoplasmic reticulum of hepatocytes leading to build up of lipids, reduced protein synthesis and mixed function oxidases activity (Weber *et*

al., 2003). The conditions of fibrosis, hyperplasia and regeneration seen in the liver in this study are more or less typical of the liver under chronic conditions (Zimmerman and Ishak, 2002). What is interesting is that the degree of fibrosis and regeneration was marked in the splenectomised group (Gp III) than the unsplenectomised (Gp II) (Plate 2). Previous studies have reported splenic Transformation Growth Factor ($\text{TGF-}\beta 1$) production in the context of liver cirrhosis and hypersplenism, and emphasized its critical role in the development of hepatic fibrogenesis (Liang *et al.*, 2017). Splenic macrophages were proposed as a major source of $\text{TGF-}\beta 1$ in that study. Splenectomy was reported to significantly decrease serum $\text{TGF-}\beta 1$ levels whilst improving liver fibrosis and regeneration parameters (Akahoshi *et al.*, 2002). The mechanism of CCl_4 nephrotoxicity is thought to be similar to the pathophysiology of its liver toxicity: i.e. bio-activation to the CCl_3 radicals, with resulting oxidative injury (Abraham *et al.*, 1999; Ozturk *et al.*, 2003).

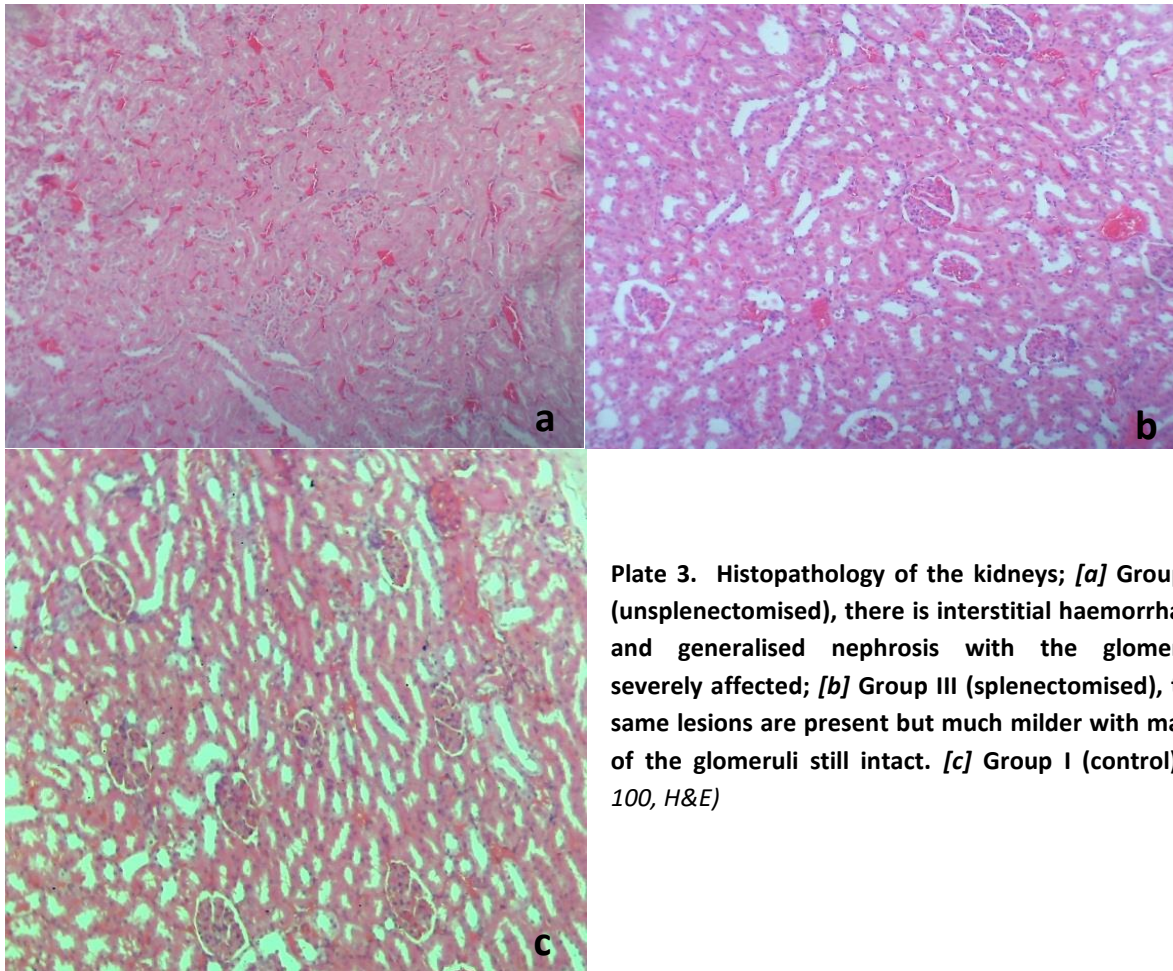


Plate 3. Histopathology of the kidneys; [a] Group II (unsplenectomised), there is interstitial haemorrhage and generalised nephrosis with the glomeruli severely affected; [b] Group III (splenectomised), the same lesions are present but much milder with many of the glomeruli still intact. [c] Group I (control) ($\times 100$, H&E)

The intracellular and cell membrane damage seen as proximal tubule cell oedema and vacuolization, glomerular necrosis, and interstitial haemorrhage as observed, has been attributed to oxidative injury (Khan *et al.*, 2010; El Denshary *et al.*, 2012). Like with the liver, it is again interesting to note that the damage to the kidneys of the unsplenectomised (Grp II) was more severe than in the splenectomised (Grp III). It could be that the same factors that contributed to enhanced hepatocytes regeneration also prevented severe nephritis in the kidneys of the same animals. It could also be that the liver of the Grp III rats having showed better regeneration, was probably able to perform more of its detoxifying functions hence the lesser toxicity seen on the kidney downstream. As was evident by the fibrinogen test (Fig 1), it was obvious that at this level of chronic toxicity and

widespread fibrosis, while both groups of livers still retained some of their functionality, the splenectomised group III animals probably showed more functional competence, by their ability to synthesize more fibrinogen, due to the higher degree of hepatocytes hyperplasia/regeneration. It is known that the liver is still considered to be functionally competent in the midst of cell proliferations induced by chronic lesions (Callea *et al.*, 1991). Also, Ushitora *et al.*, (2011) reported improved liver function following splenectomy (Ushitora *et al.*, 2011).

CONCLUSION

In conclusion, the pathomorphology of the liver and kidneys of splenectomised rats in chronic carbon tetrachloride (CCl_4) toxicity is characterised by increased fibrosis and advanced hepatocytes regeneration in the liver; and mild to

moderate forms of tubular degenerations and glomerular necrosis in the kidney than in unsplenectomised rats.

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