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## ARTICLE

**Safety of collagen and reticulin fibres in the liver and kidney of broiler chicken fed with *Aspergillus niger* -hydrolyzed cassava peel meal as carbohydrate source**      5  
Muhammed Abdurrasheed Ola, Adeyemo Isiah Adeyemi, Olutunde Oluwumi Abiola, Afodun Moyosore Adam and Ajayi Ayooye Samuel

Full Length Research Paper

## Safety of collagen and reticulin fibres in the liver and kidney of broiler chicken fed with *Aspergillus niger* - hydrolyzed cassava peel meal as carbohydrate source

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Cassava peels which is available in large quantity as waste is being considered as a possible replacement for maize in chicken feeds due to the world shortage of cereals. This study examined the safety of collagen and reticulin fibres in the liver and kidney of broiler chicken, when the *Aspergillus niger*-hydrolyzed cassava peel meal (HCPM) was used as diet carbohydrate. HCPM was used as replacement for maize in chicken feeds at 0, 25, 50, 75, and 100%, respectively and were fed for 42 days. The animals were sacrificed and the liver and kidney were excised and fixed immediately in 10% formal-saline for 48 h. The tissues were processed for paraffin embedding. Sections were cut at 3 microns, dried for 30 min at 60°C and were stained using both Gordon & Sweet and Masson's Trichrome methods. The result showed the preservation of the reticulin and collagen fibres up to the 50% inclusion of HCPM as replacement for maize. The implication is that; the use of HCPM will make available for human consumption, half of the world maize cereals been consumed by the poultry birds.

**Key words:** *Aspergillus niger*, cassava peels, cellulolytic enzymes, collagen fibre, reticulin.

### INTRODUCTION

Cassava, yam, sweet potatoes, cocoyam, and sorghum are important staple foods and they are sources of food for about 200 to 300 million people in tropical areas

(FAO, 1986). The first step in the processing of these tubers is the removal of the peels which are the two coverings of the tubers. These peels usually end up as

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waste or sometimes as feeds for ruminants. However, their nutritional contents are low and as such cannot be regarded as high quality feed for non-ruminants. Sorghum chaff is obtained as a residue after the sorghum is wet-milled and filtered. Cassava is a major source of calories in developing tropical countries. 35% of the 137.4 million ton world production of agricultural waste came from African countries (FAO, 1986; Sabiiti, 2011). The peels (wastes) from cassava and other tubers when not properly disposed off, serve as reservoir for disease-causing organisms and also during rainfall, surface runoff takes the pathogens from the wastes to nearby streams which serve as source of drinking water to many people, leading to disease outbreaks (Adeyemo, 2003; Sabiiti, 2011).

Fungi have been shown to have cellulase enzyme which can degrade the fibre in cassava peel thereby making available more energy to monogastrics from hydrolysis of the fibrous materials (Belewu and Banjo, 1999; Raji et al., 1988; Sani et al., 1992). This ultimately promotes weight gained and overall improved performance (Atteh, 2000; Abdulrashid et al., 2007).

Addition of carbohydrate-degrading enzymes to monogastric feed allowed improved digestion and absorption in the intestine, likely due to a reduced viscosity of the material compared to the initial fibrous material. Similarly, dietary supplementation with microbial enzyme preparations capable of hydrolyzing endosperm cell walls has previously increased performance of broiler chickens receiving cereal based diets (Abdulrashid et al., 2007; Atteh, 2000; Kayode, 2009). The effect of carbon source replacement of maize in broiler chicken feed is well documented (Abdulrashid et al., 2007; Atteh, 2000; Kayode, 2009; Muhammad and Oloyede, 2009).

The safety of fibres, which give mechanical support to the vital organs, should be an object of concern whenever new feed is being formulated. Collagen is the main structural protein of the various connective tissues in animals (Di Lullo et al., 2002). It is one of the long, fibrous structural proteins whose functions are quite different from those of globular proteins, such as enzymes. It is the most abundant protein in mammals (Di Lullo et al., 2002) making up from 25 to 35% of the whole-body protein content. Tough bundles of collagen called collagen fibers are a major component of the extracellular matrix that supports most tissues and gives cells structure from the outside, but collagen is also found inside certain cells (BCE, 2007). Collagen has great tensile strength and is the main component of fascia, cartilage, ligaments, tendons, bone and skin (Fratzl, 2008; Buehler, 2006). Along with soft keratin, it is responsible for skin strength and elasticity, and its degradation leads to wrinkles that accompany aging (Marcia, 2013). It strengthens blood vessels and plays a role in tissue development. It is present in the cornea and lens of the eye in crystalline form. Collagen constitutes

one to two percent of muscle tissue, and accounts for 6% of the weight of strong tendinous muscles. The fibroblast is the most common cell that creates collagen (Sikorski, 2001). Reticulin is a type of fiber in connective tissue (Strum et al., 2007) composed of type III collagen secreted by reticular cells. Reticular fibers usually crosslink to form fine meshwork (reticulin) (Strum et al., 2007). This network acts as a supporting mesh in soft tissues such as liver, bone marrow, and the tissues and organs of the lymphatic system. The liver is one of the organs in which the cells are supported by a network of reticular fibres (Burkitt et al., 1993). They appear as fine black lines in silver stained preparation. The fibres surround the individual sheets of liver cells (hepatocytes) and are the only fibrous connective tissue components supporting the cells. While providing support, the fine, open meshwork of reticular fibres facilitates the exchange of substances between the hepatocytes and the blood, which circulates in the irregularly shaped blood vessels (sinusoids) between the hepatocytes. Reticular fibres are also present in the connective tissue surrounding the larger vessels, which penetrate the parenchyma of the liver (Burkitt et al., 1993).

However, information on the safety of collagen and reticulin fibres in the liver and kidney of broiler chicken with *Aspergillus niger*-hydrolyzed cassava peel meal (HCPM) as carbohydrate source, are very scanty in literatures. The current study therefore investigated the effect of fungal hydrolyzed cassava peel as main energy source in broiler chicken feeds on the collagen and reticulin fibres in the liver and kidney of broiler chickens after 42 days of feeding trials.

## MATERIALS AND METHODS

### Preparation of HCPM

*A. niger* was isolated from cassava peel collected from cassava peel dumpsite. A modified method of Ali et al. (1991) was used to delignify the cassava peel which was autoclaved for 1 h at 121°C with 5% (w/v) NaOH. The autoclaved materials were filtered through muslin cloth, neutralized with dilute acids (0.1 M H<sub>2</sub>SO<sub>4</sub>), and then washed with water. They were finally washed in distilled water and dried at 70°C in a regulated oven (Gallenkamp). Each was ground with domestic blender (Nakai, Japan Mx- 736) for increased surface area. Mineral salts medium (MSM) was prepared for cultivation of fungal isolate using the compositions as subsequently shown (g/L).

KH<sub>2</sub>PO<sub>4</sub>, 10 g; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10.5 g; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.3 g; CaCl<sub>2</sub>, 0.5 g; FeSO<sub>4</sub>, 0.013 g; MnSO<sub>4</sub>.H<sub>2</sub>O, 0.04 g; ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.04 g; Yeast extract, 0.5 g; Cassava peel, 40 g. *A. niger* was grown in this composed medium under a pH of 5.0, concentration of 3% and 35°C for five days after which the culture filtrate was filtered with Whatman under suction. 100 ppm of the cellulase enzyme was incorporated into cassava peel so as to hydrolyze it. The cassava peel was then air dried and then used to replace maize in broiler chicken feeds for groups A to E (0, 25, 50, 75, and 100% respectively). Also, unhydrolyzed cassava peel was used to replace

maize 100% for group F. Starter and finisher diets at 0, 25, 50, 75, and 100% replacement value for maize were composed to meet the nutrient requirement of broiler chickens (NRC, 1984). The chickens were fed starter diet for the first three weeks of the feeding trials and then fed finisher diets from the fourth to the sixth week. Soya bean oil was added to obtain equal metabolizable energy.

### Chickens grouping and feeding

Thirty six (36) chickens with average initial body weight of  $0.685 \pm 0.0027$  g were randomly allocated to six dietary treatments A to F using a completely randomized design. Each treatment group contained two replicates of three broiler chickens each. Group A chickens ( $A_1$  and  $A_2$ ) were fed with the control diet (0% hydrolyzed cassava peel as main carbon source). Groups B to E (in replicates 1 and 2) were administered with experimental diets containing 25, 50, 75, and 100% of hydrolyzed cassava peels, respectively replacing maize, while group F ( $F_1$  and  $F_2$ ) were fed with diet containing 100% unhydrolyzed cassava peels replacing maize as the main carbon source. Feed and water were supplied *ad libitum* for the six weeks feeding trial period. Vaccine and drugs were administered as at when due.

### Animal sacrifice and tissue processing

The birds were weighed individually, sacrificed and the liver and kidney tissues were carefully excised and fixed immediately in 10% formal-saline for 48 h. The tissues were processed for paraffin embedding using Leica TP 1020 Automatic tissue processor and embedded using Leica EG 1160 Embedding system. Sections were cut at 3 microns on a Leica rotary microtome and dried for 30 min at 60°C. They were stained using Gordon & Sweet and Masson's Trichrome methods, respectively. The slides were viewed using (Olympus) light microscope and photomicrographs were taken.

## RESULTS

Group A to E (both replicates) recorded 0% mortality, while Group F ( $F_1$  and  $F_2$ ) that were fed with diet containing 100% unhydrolyzed cassava peels recorded 100% mortality within the first six days of commencement of the feeding trials. No loss in weight was recorded across board, as the average final weight ( $n=30$ ) was  $0.818 \pm 0.0019$  g (Figures 1 to 4).

## DISCUSSION

The earlier researchers, who have worked on poultry feed formulation were probably not interested in the effects of such new formulation on the connective tissues of the experimental birds. This is because of the paucity of both published and unpublished information on this subject. The test groups (B to E) were then compared with the control group (A).

Reticulin fibres were seen to be well preserved with distinct inter-hepatic and arterial wall distribution in Groups A, B and C of the liver tissues. This indifference in the staining and reticulin distributional appearance

confirmed the safety of the feed to the reticulin fibres of the liver tissue, at least up to 50% inclusion of HCPM in the poultry feed mill. Groups D and E however, showed areas of compressed and collapsed reticulin fibres, these correspond to areas of reticulin degeneration and cell loss, respectively. According to Saxena (2010), reticulin provides the stromal support for the parenchyma and the reticulin stain provides important information about the architecture of the organ. When hepatocytes are damaged and undergo necrosis, the reticulin fibers surrounding them collapse in the empty space left behind. Areas of reticulin crowding thus indicate focal hepatocyte loss. Large areas of cell necrosis appear as reticulin collapsed.

It can therefore be inferred that 75 and 100% HCPM inclusion can pose a danger to the functions of hepatocellular tissue, and may lead to hepatic necrosis and subsequent cells death (Saxena, 2010).

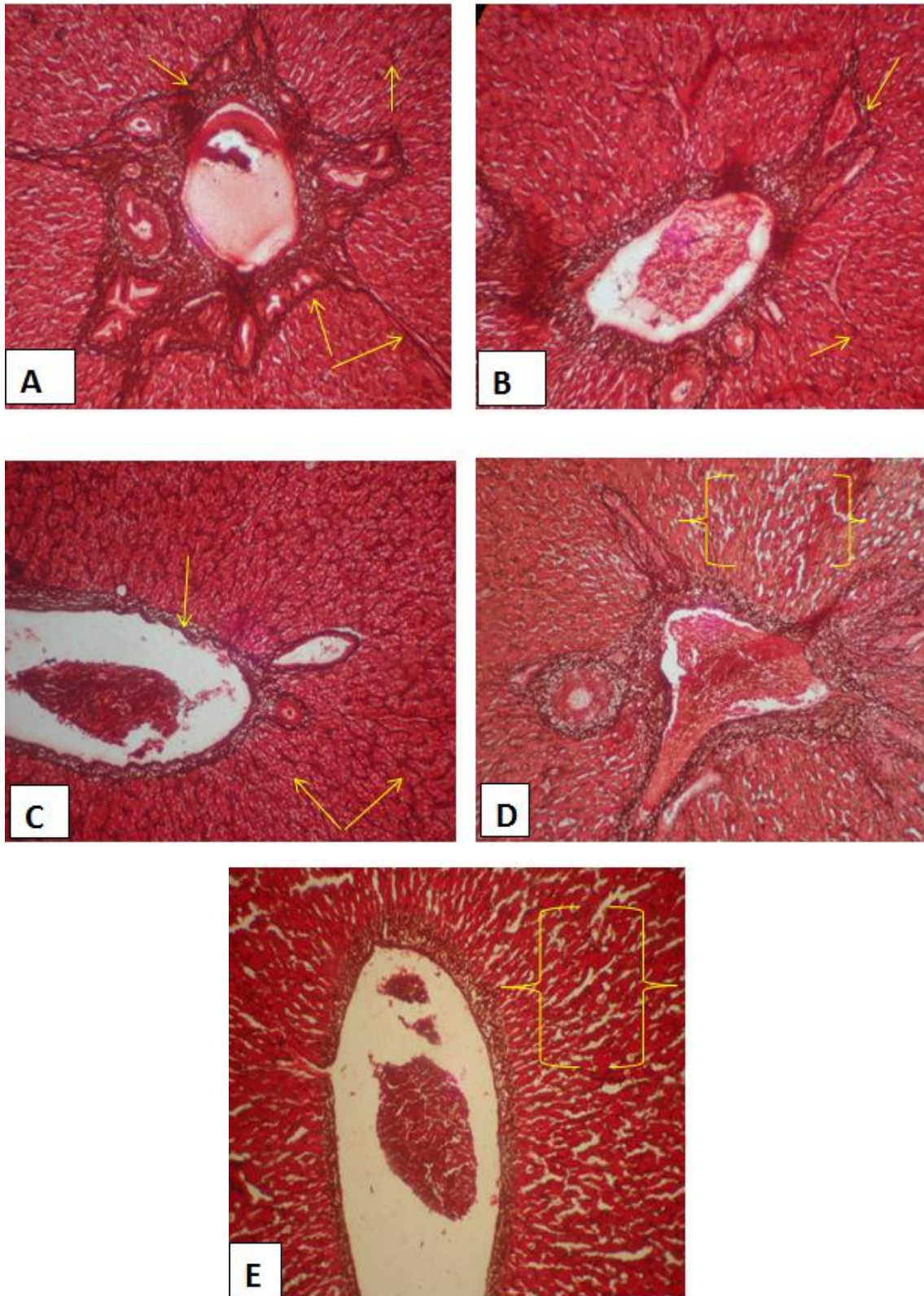
The staining intensity of the renal tissues showed the arterial reticulin fibres, reducing progressively from group A through E. This may correspond to the reduction in the rigidity and mechanical strength functions of the fibres (Burkitt et al., 1993). It is the characteristics of the ground substance formation defect, which determine the permeability of the connective tissue layer to solutes and proteins. This further buttress the fact that more than 50% inclusion of HCPM could be detrimental to the kidney functions.

The masson's trichrome technique showed perfect preservation and distribution of collagen fibres up to 50% inclusion (Groups B and C). While reduced collagen preservation was noted in the hepatic vessels of group E (100% inclusion), zero-preservation was recorded in its renal counterparts (Group E Kidney). This result of collagen in renal and hepatic tissue is not at variance with that of reticulin.

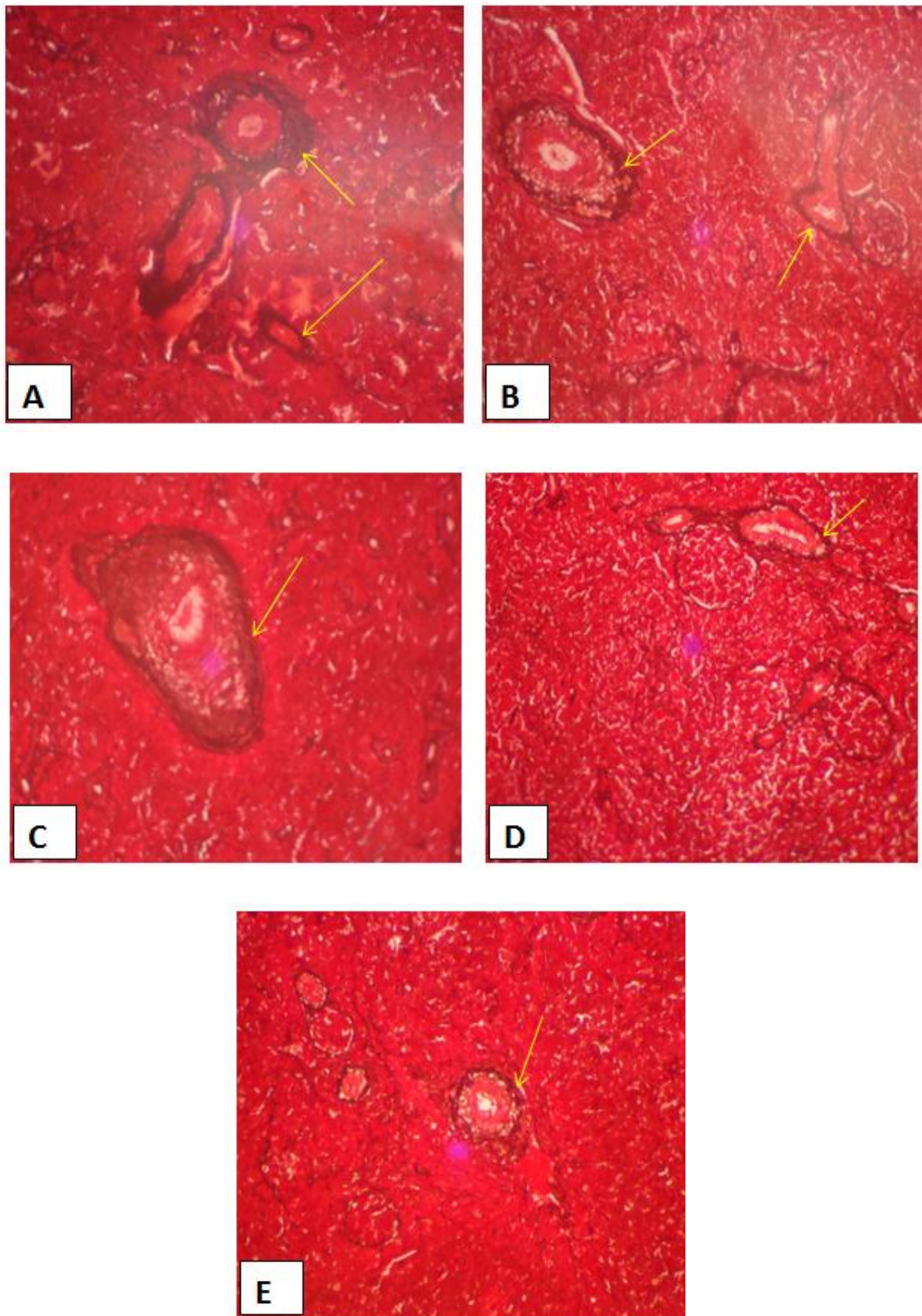
The death (within six days) of Group F animals that were fed with diet containing 100% HCPM was as a result of acute cyanide poisoning (Ansel and Lewis, 1970; Banerjee et al., 1997; Carlsson et al., 1999; Priya et al., 2011). According to Cereda and Mattos (1996), cassava roots, and leaves cannot be consumed as they contain two cyanogenic glycosides, linamarin and lotaustralin. They are decomposed by linamarase, a naturally occurring enzyme in cassava, liberating hydrogen cyanide (HCN). Hydrogen cyanide is the chemical responsible for tissue hypoxia. Chronic exposure to HCN or acute exposure to very high dose may cause neurological, respiratory, cardiovascular and thyroid defects (Carlsson et al., 1999; Priya et al., 2011).

Cyanide poisoning is a form of histotoxic hypoxia, because the cells of an organism are unable to use oxygen, primarily through the inhibition of cytochrome c oxidase. If cyanide is inhaled, it causes a coma with seizures, apnea, and cardiac arrest, with death following in a matter of seconds (Blanc et al., 1985; Okafor et

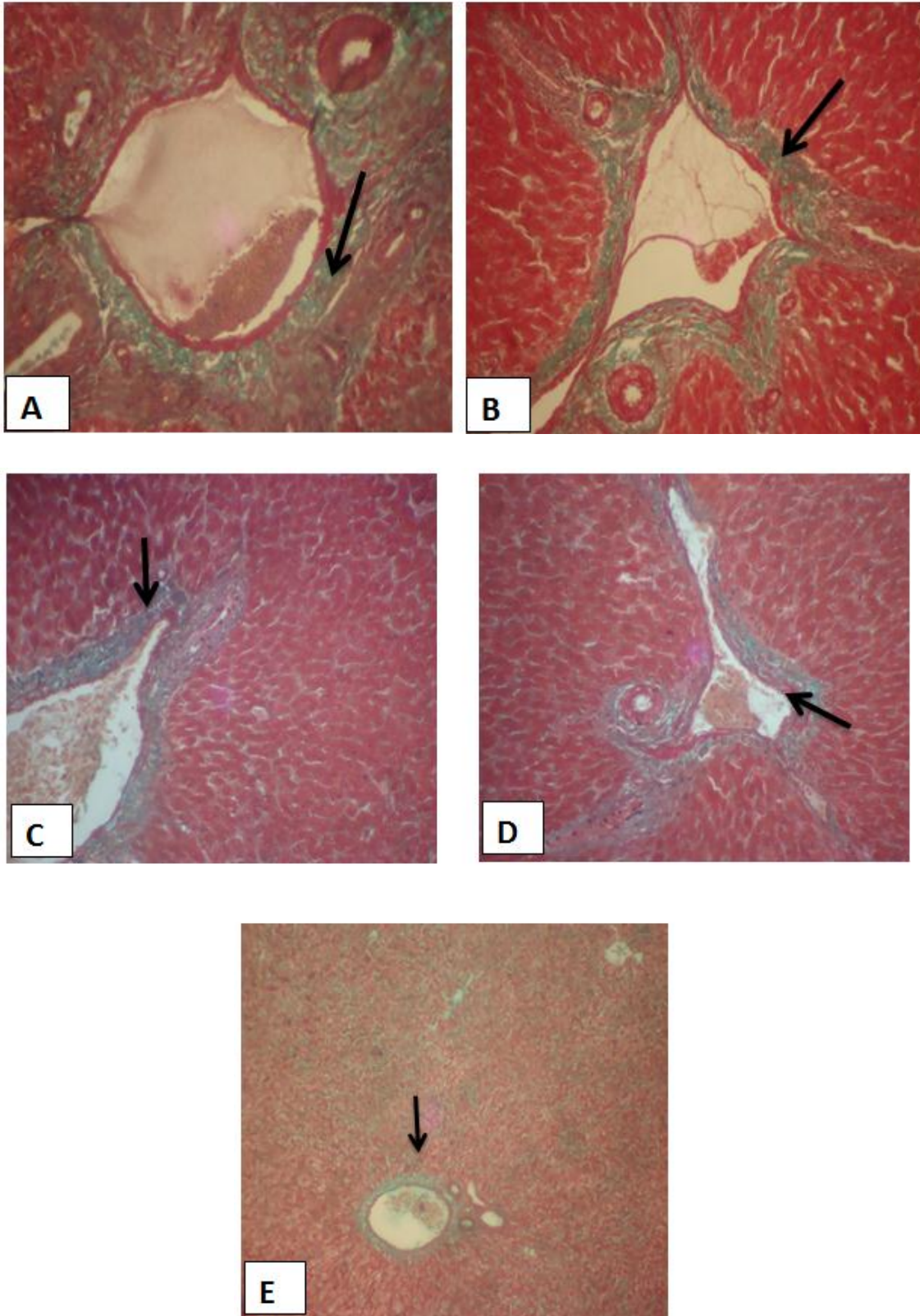




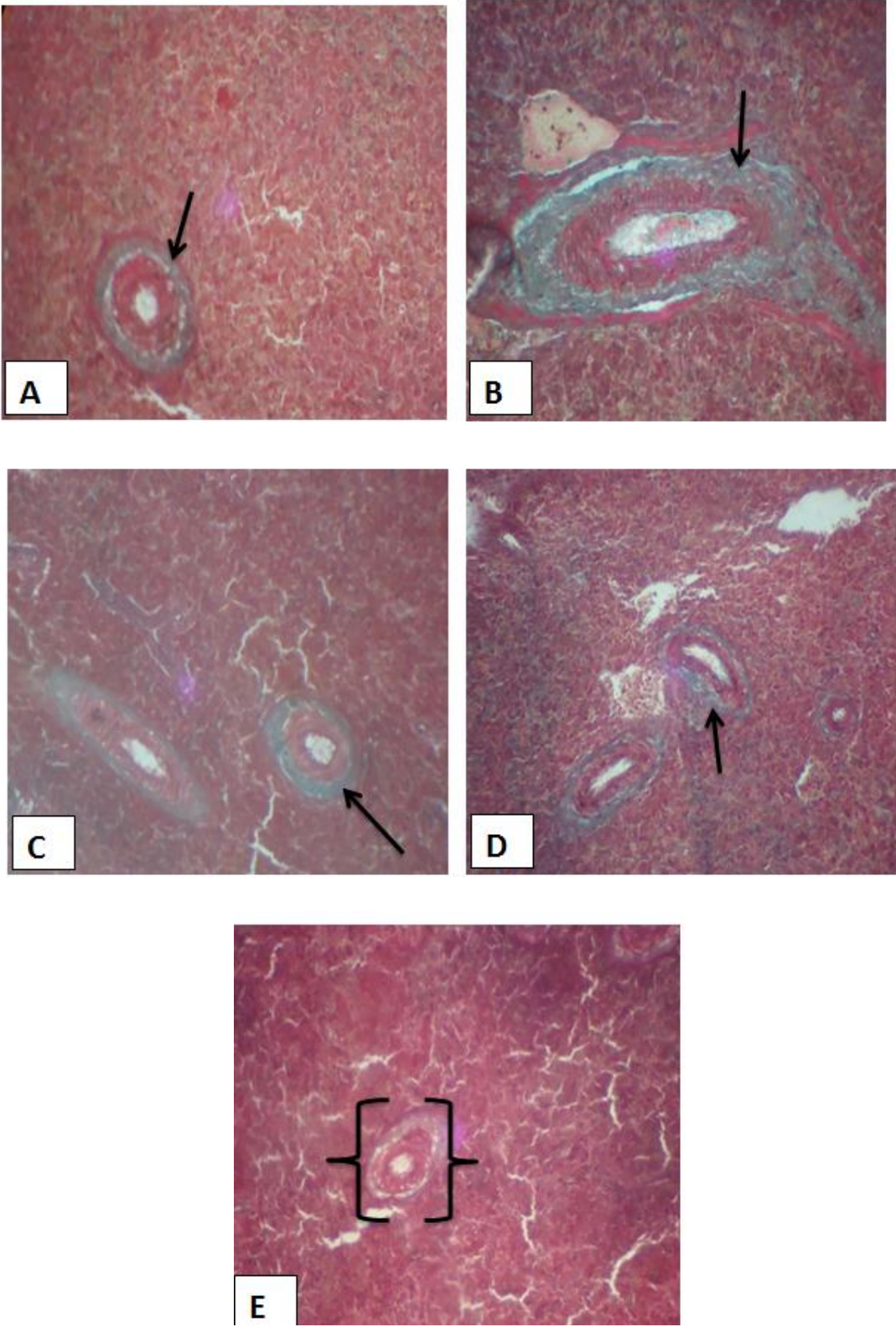
**Figure 1.** Photomicrographs of the liver A-E (GORDON & SWEET METHOD X400). Reticulin fibres were well preserved, with distinct inter-hepatic and arterial wall distribution (Yellow Arrow) in Groups A, B and C. In Groups D and E however, the stain showed areas of collapse of reticulin fibres (Yellow brackets), with disperse reticulin distribution.



**Figure 2.** Photomicrographs of the kidney (A-E) (GORDON & SWEET METHOD X400). The Arterial reticulin fibres were demonstrated across all the groups (Yellow arrows). The staining intensity was noted to be reducing progressively from group A to E.



**Figure 3.** Photomicrographs of the liver A-E (MASSON'S TRICHROME METHOD X400). The collagen fibres (Black arrows) were well preserved with normal pattern of distribution across all the groups, though reduced preservation was noted in group E.



**Figure 4.** Photomicrographs of the kidney A-E (MASSON'S TRICHOME METHOD X400). The collagen fibres in the wall of the blood vessels (Black Arrows) were preserved with moderate pattern of distribution. The only exception is in Group E, where Zero-collagen preservation was observed in the entire microscopic fields (Black Bracket).

al., 2002; Priya et al., 2011).

It must therefore be noted that inclusion of HCPM, as the only source of carbohydrate, should not exceed 50%. This is to maintain the rigidity and mechanical strength of the vital organs of the animals.

### Conflict of interest

The authors hereby declare that no conflict of interest

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