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HISTOPATHOLOGICAL CHANGES IN THE LIVER AND KIDNEY TISSUES OF ALBINO WISTAR RATS FED VARIOUS PREPARATIONS OF DICHLORVOS-TREATED BEANS

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ABSTRACT

This study was aimed at evaluating the effects of differently prepared (parboiled and un-parboiled) beans treated with sniper (i.e. a dichlorvos based insecticide) on the histological architecture of liver and kidney of albino wistar rats. Thirty (30) male albino wistar rats of known body weights were assigned into six (6) groups of 5 rats each. The beans were treated with 0.7mil (high dose) and 0.3 mil (low dose) of dichlorvos per one kg of beans. Group 1 and group 2 of un-parboiled beans compounded with standard feed stock, group 3 and group 4 were fed with high dose and low dose of parboiled beans compounded with feed stock, group 5 received beans only while group 6 received standard feed for a period of 30 days. The experimental animals were euthanized at the end of the study period. Tissue sections of the liver and kidney were collected for histopathological studies. The histopathological results showed normalcy in the kidneys of all the rat groups but moderate vacuolar degeneration of hepatocytes and mild to moderate, multifocal and random hepatocellular necrosis with mild infiltration of phagocytic leucocytes were seen in the group fed with high concentration dichlorvos-treated beans for both the parboiled (group 1) and un-parboiled (group 3).

KEYWORDS: Dichlorvos, histopathology; liver and kidney.

INTRODUCTION

Organic insecticide poisoning remains one of the major issues in both developing and developed communities. A great proportion of acute poisoning cases are caused by exposure to pesticides, especially organophosphate (OP) compounds.[1] The mechanism for the toxicity

of organophosphorous compounds is mainly by blocking of acetyl cholinesterase (AchE). Once AchE has been inactivated, acetylcholine accumulates throughout the nervous system.[2]

Organic pesticides are still posing threats to man in both developing and developed countries. Although pesticides are very important tools used in the management agricultural products for a better yield, they can also cause harm when consumed.[3] One of the mostly used pesticides in agricultural management in Nigeria is dichlorvos – an organophosphorous (OP) pesticide which functions mainly by the inhibition of acetylcholinesterase. These pesticides are also biodegradable, cause minimum environmental pollution. But on contact and consumption, this group of synthetic pesticides can cause so much harm to the individuals as they are highly toxic to man causing toxicity to liver and muscles, as well as, nervous, immune, urinary, reproductive and hematological systems.[4, 5, 6] and research has recently postulated the toxicity emanates from the ability of organophosphates to generate free radicals due to their electrophilic nature[7] thereby causing "redox-cycling" activity where they readily accept an electron to form free radicals and then transfer them to oxygen to generate superoxide anions and then hydrogen peroxide through dismutation reactions, or to ROS generation.[8, 9, 10]

Being an important pesticide in agriculture, it is either subjected to abiotic degradation or biological degradation which is majorly done hydrolytically[11] where it yields dichloroethanol, dichloroacetaldehyde, dichloroacetic acid, dimethylphosphate and dimethylphosphoric acid as its metabolites.[12]

Toxicities of OP pesticides cause adverse effects on many organs and blood factors.[13] These compounds show strong insecticidal properties accompanied by low toxicity for vertebrates.[14] Although several researches have been carried out on the toxicological effects of dichlorvos especially as touching liver enzymes, the toxicological and hematological effects of consuming parboiled and un-parboiled beans treated with dichlorvos were evaluated. Therefore, this research work was conducted to study the histopathological effects of consuming parboiled and un-parboiled beans treated with dichlorvos on the male Wistar albino rats.

2. MATERIALS AND METHOD

2.1. Feed formulation

A. Beans preparation

Beans were bought in Ultra-modern market, Ubani, Umuahia, Abia State, Nigeria.

Dichlorvos based-pesticide, Sniper, was mixed with 1kg beans to achieve 1 ml/kg and air dried for one (1) week. The contaminated beans were washed and completely cooked without parboiling and no spices were added.

B. Feed formulation

Dichlorvos based-pesticide, sniper was mixed with beans with the ratio of 1mil: 1kg (i.e. dichlorvos: beans) and aired for one (1) week. The contaminated beans were grouped and prepared (cooked) separately where one group was parboiled and the other was not parboiled. Each group was sundried and ground into flour, mixed with feed at different ratios to determine the different concentrations of dichlorvos in each sample, while another sample which contained beans only was prepared.

2.2. Chemicals

Commercial grade dichlorvos (DDVP, 100% solution containing 100 g per liter of 2,3-dichlorovinyl dimethylphosphate) marketed as Sniper® was purchased from an agrochemical company in Umuahia, Nigeria. DDVP was reconstituted in soya oil (SO, Grand Cereals and Oil Mills Limited, Jos, Nigeria) to a 5% stock solution.

2.3. Experimental design

- Group 1 = High conc ddvp in parboiled beans + feed (ie. 70 : 30) + water.
- Group 2 = Low conc of ddvp in parboiled beans + feed (ie. 30 : 70) + water.
- Group 3 = High conc of ddvp in unparboiled beans + feed (ie. 70 : 30) + water
- Group 4 = Low conc. of ddvp in unparboiled beans + feed (ie. 30 : 70) + water
- Group 5 = Beans only (Negative control)
- Group 6 = Feed + water only (Positive control)

The rats were fed with the samples and closely monitored for four (4) weeks. The experimental animals were euthanized at the end of the study period.

2.4. Histopathologic examination

Tissue sections of the liver and kidney were collected for histopathological studies. The samples were fixed in 10% phosphate buffered formalin for a minimum of 48 hours prior to tissue preparation. The tissues were subsequently be trimmed, dehydrated in 4 grades of alcohol (70%, 80%, 90% and absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the tissue-containing wax blocks were cut into 5µm thick

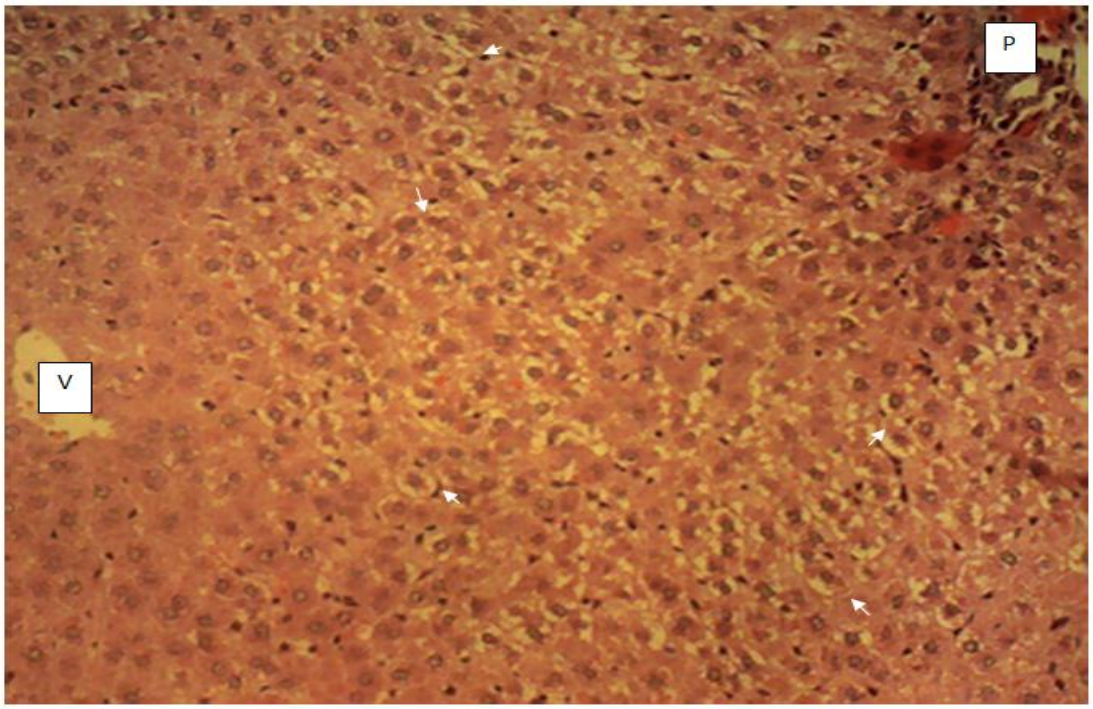
sections with a rotary microtome, floated in water bathe and incubated at 60˚C for 30 minutes. The 5µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90%, 80% and 70%). The sections were then stained with Hematoxylin for 15 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX. The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses.

3. RESULTS AND DISCUSSION

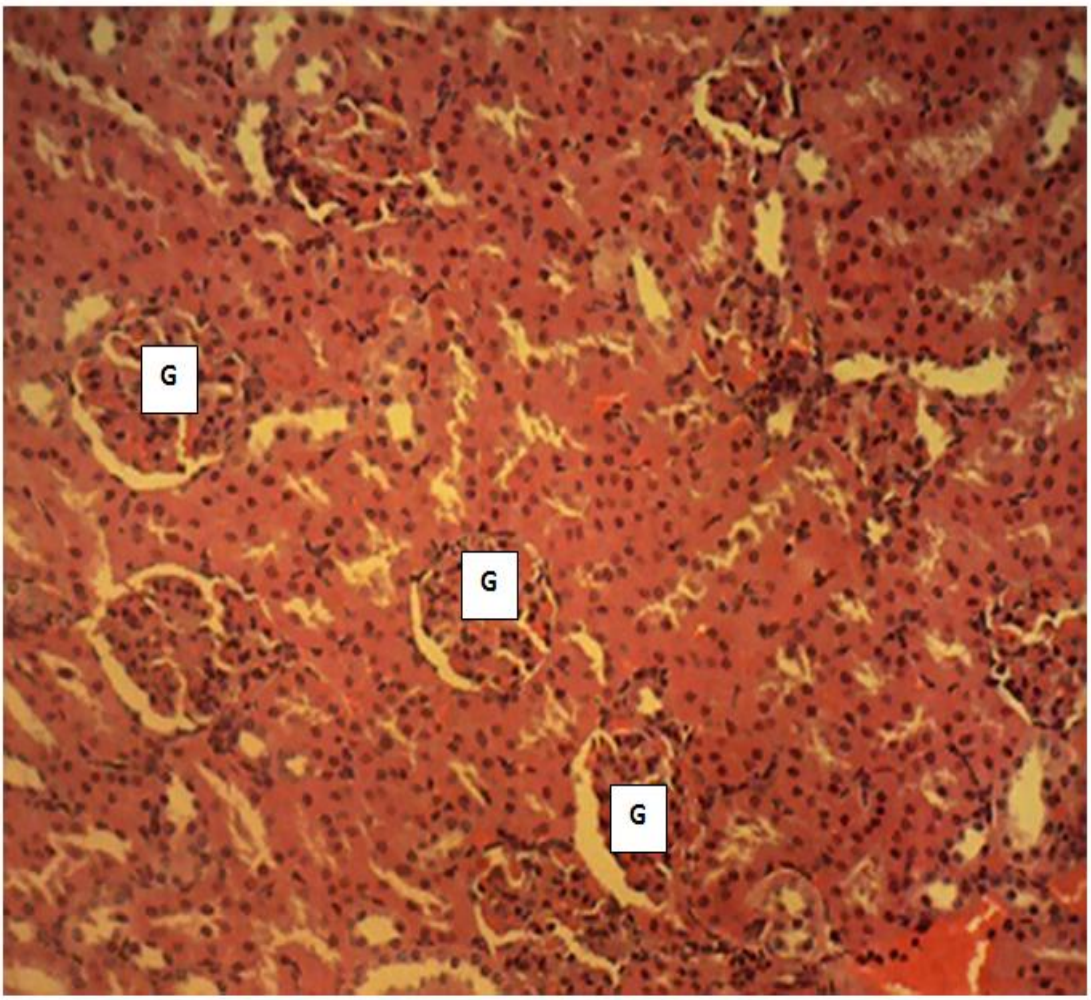
Dichlorvos has become one of the most commonly used organophosphate pesticides in agricultural preservation process especially for stored products.[15] Although being classified as a probable human carcinogen by the Environmental Protection Agency[16], the relevance of dichlorvos in agriculture has made it an indispensable pesticide in the storage of agricultural products.

GROUP 1

Sections of the liver presented on this group showed a moderate vacuolar degeneration of the hepatocytes, involving mostly those of the periportal and mid-zonal areas of the hepatic lobules. The affected hepatocytes are swollen, occluding the adjacent sinusoids and contain variably sized clear cytoplasmic vacuoles. Central vein (V); Portal area (P). H&E x160.

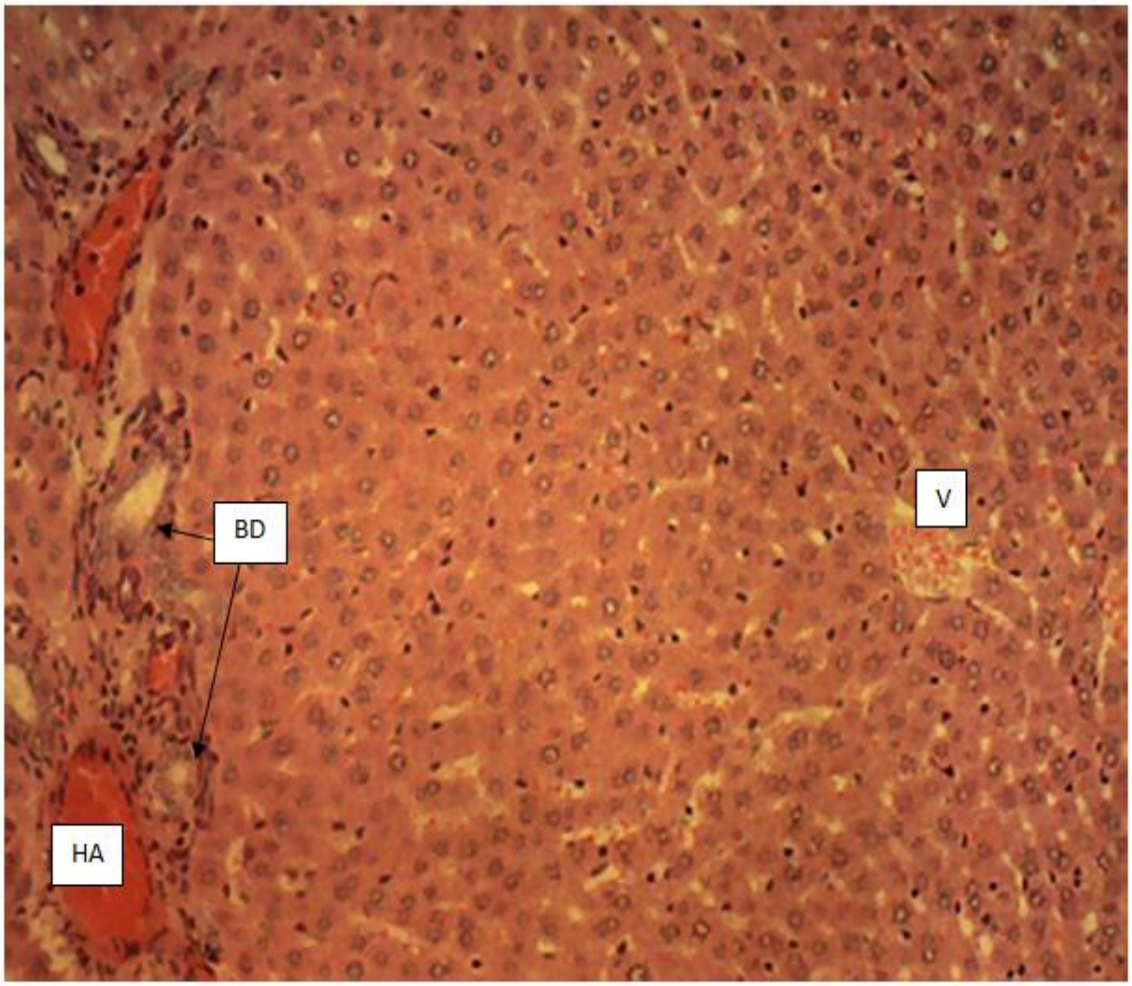


Sections of the kidney presented on this group showed the normal renal histo-architecture for laboratory rodents. Normal Glomeruli (G) in their respective Bowman's capsules were observed, surrounded by numerous normal renal tubules which are lined by a simple cuboidal epithelium, in a highly vascularised connective tissue meshwork. H&E x160

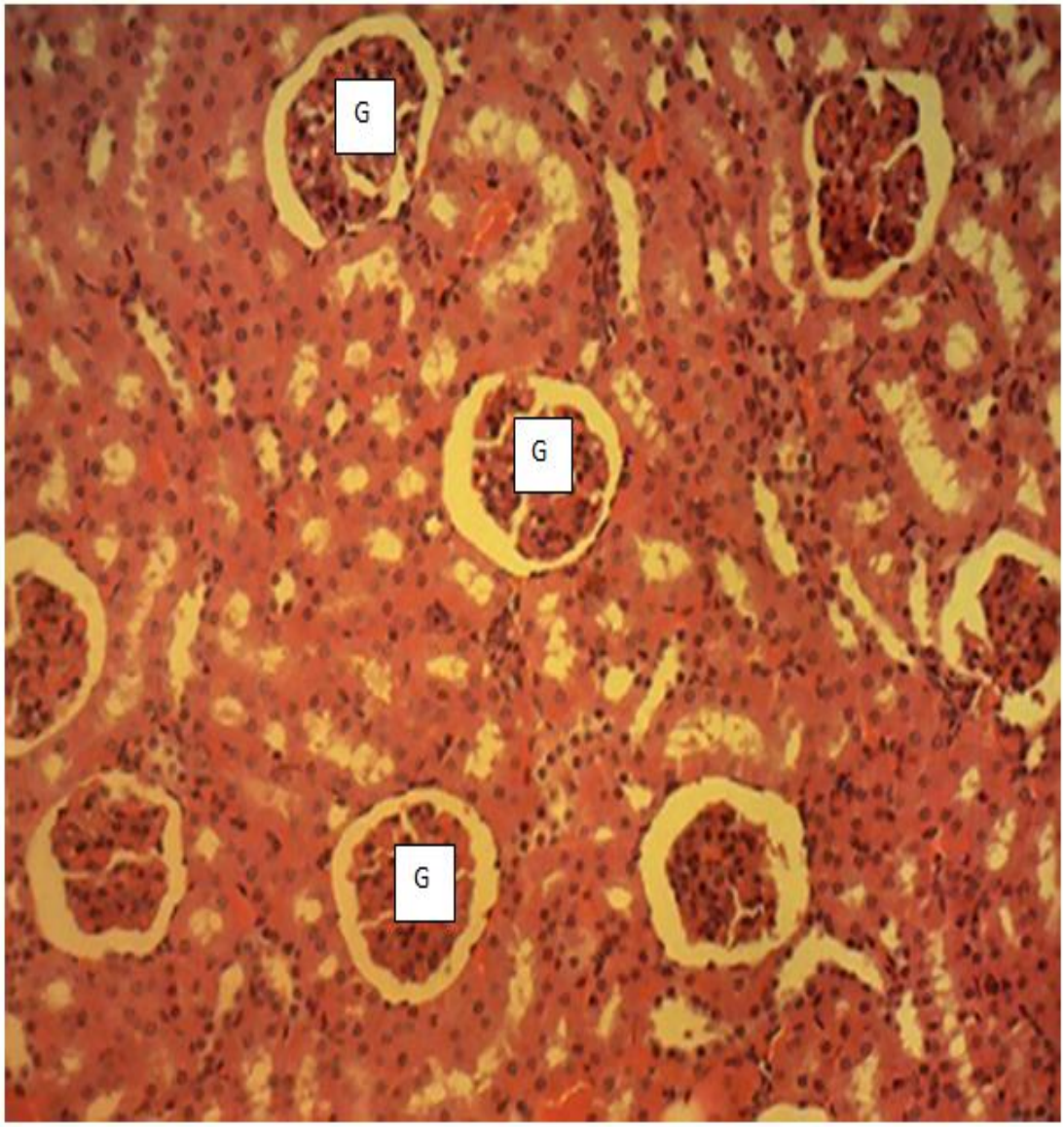


GROUP 2

Sections of the **liver** presented on this group showed the normal hepatic histo-architecture for laboratory rodents. The sections showed normal hepatic lobules consisting of normal hepatocytes arranged in interconnecting cords around the central veins. The cords are radially aligned, terminating at the portal areas of the hepatic lobules, where they meet with the hepatic vein, hepatic artery (HA) and bile duct (BD). H&E x160.

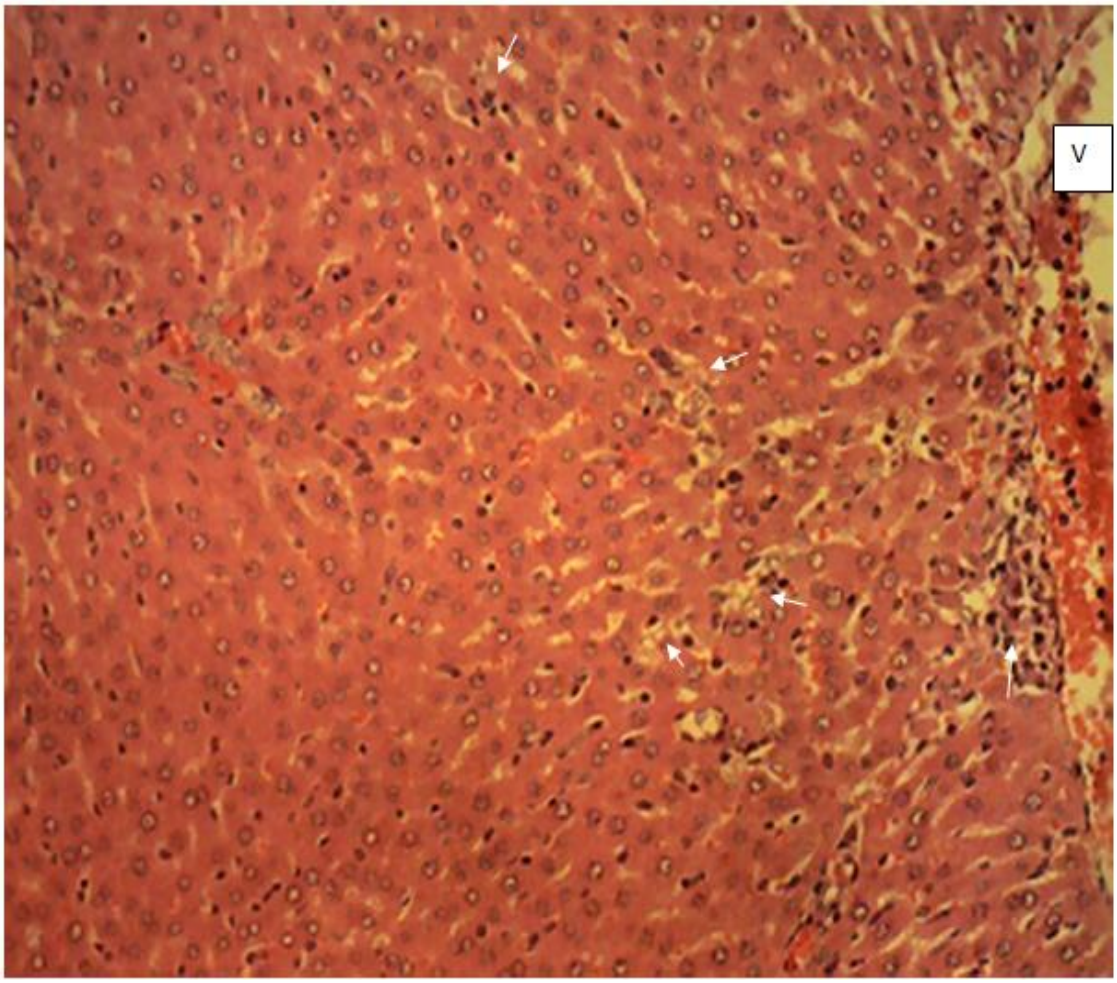


Sections of the **kidney** presented on this group showed the normal renal histo-architecture for laboratory rodents. Normal Glomeruli (G) in their respective Bowman's capsules were observed, surrounded by numerous normal renal tubules which are lined by a simple cuboidal epithelium, in a highly vascularised connective tissue meshwork. H&E x160.

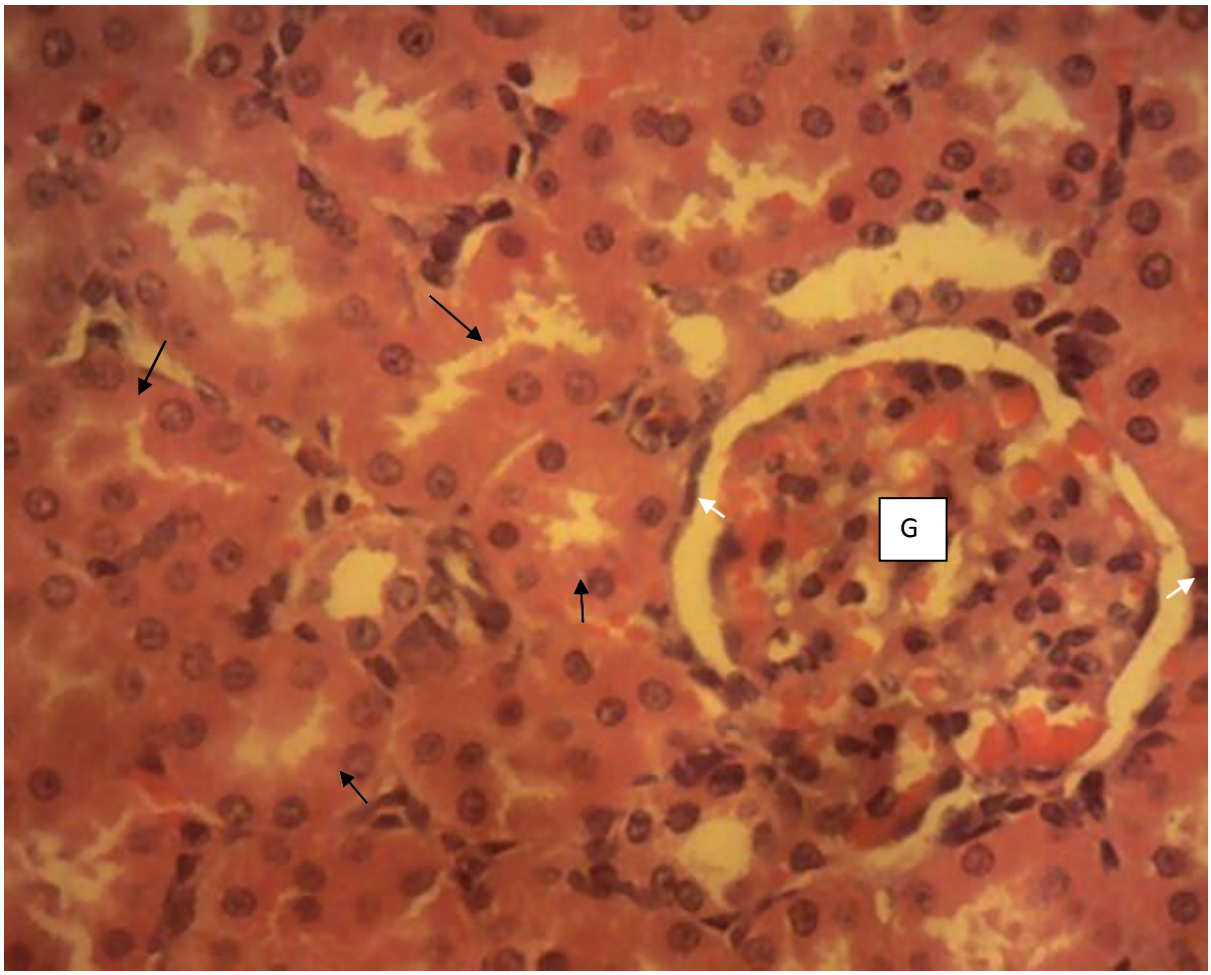


GROUP 3

Sections of the liver presented on this group showed mild to moderate, multifocal and random hepatocellular necrosis (arrow) with mild infiltration of phagocytic leucocytes. Central vein (V). H&E x160.

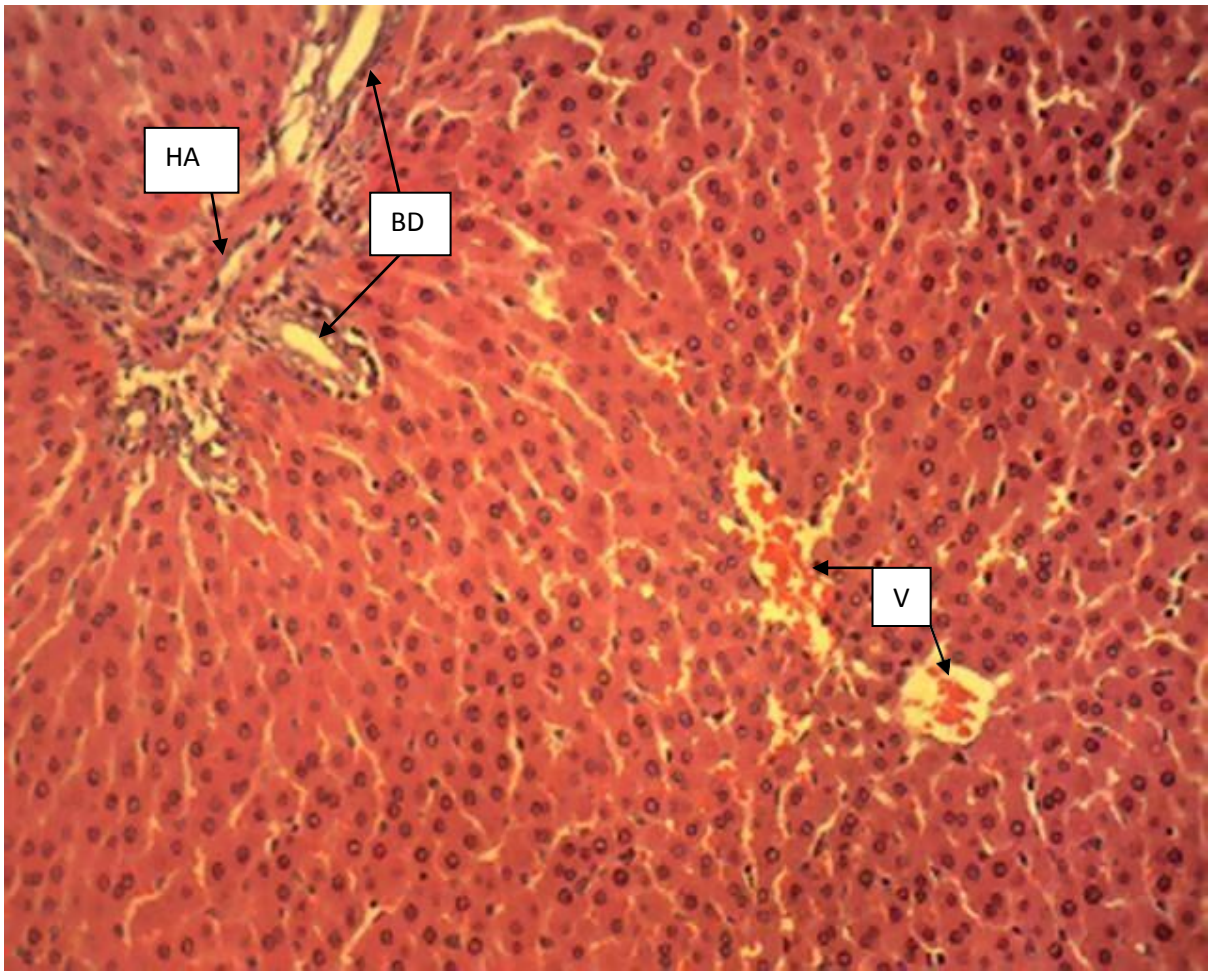


Sections of the **kidney** presented on this group showed the normal renal histo-architecture for laboratory rodents. Normal Glomeruli (G) in their respective thin Bowman's capsules (white arrow) were observed, surrounded by numerous normal renal tubules (black arrow) which are lined by a simple cuboidal epithelium, in a highly vascularised connective tissue meshwork. H&E x400.

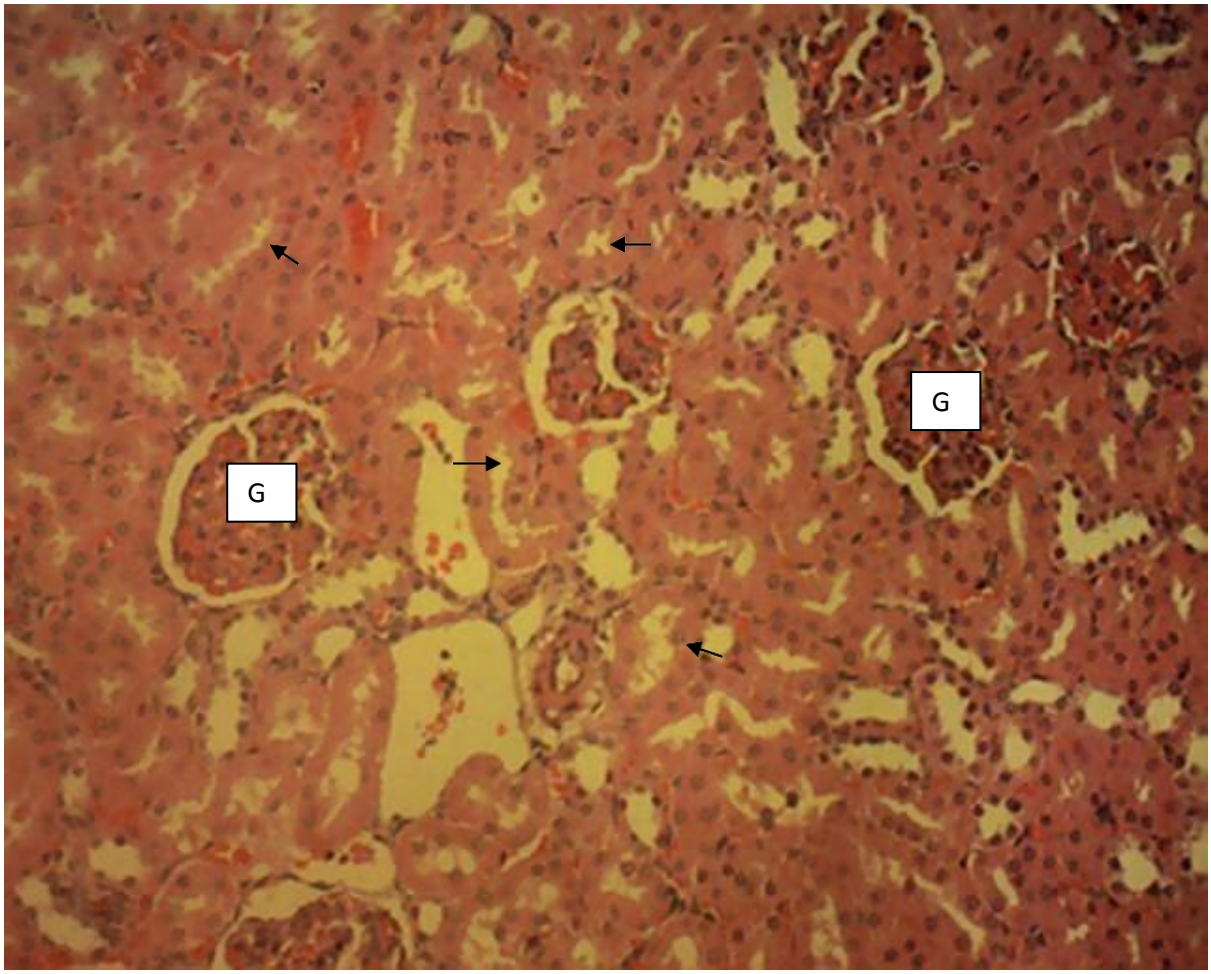


GROUP 4

Sections of the **liver** presented on this group showed the normal hepatic histo-architecture for laboratory rodents. The sections showed normal hepatic lobules consisting of normal hepatocytes arranged in interconnecting cords around the central veins. The cords are radially aligned, terminating at the portal areas of the hepatic lobules, where they meet with the hepatic vein, hepatic artery (HA) and bile duct (BD). H&E x160.

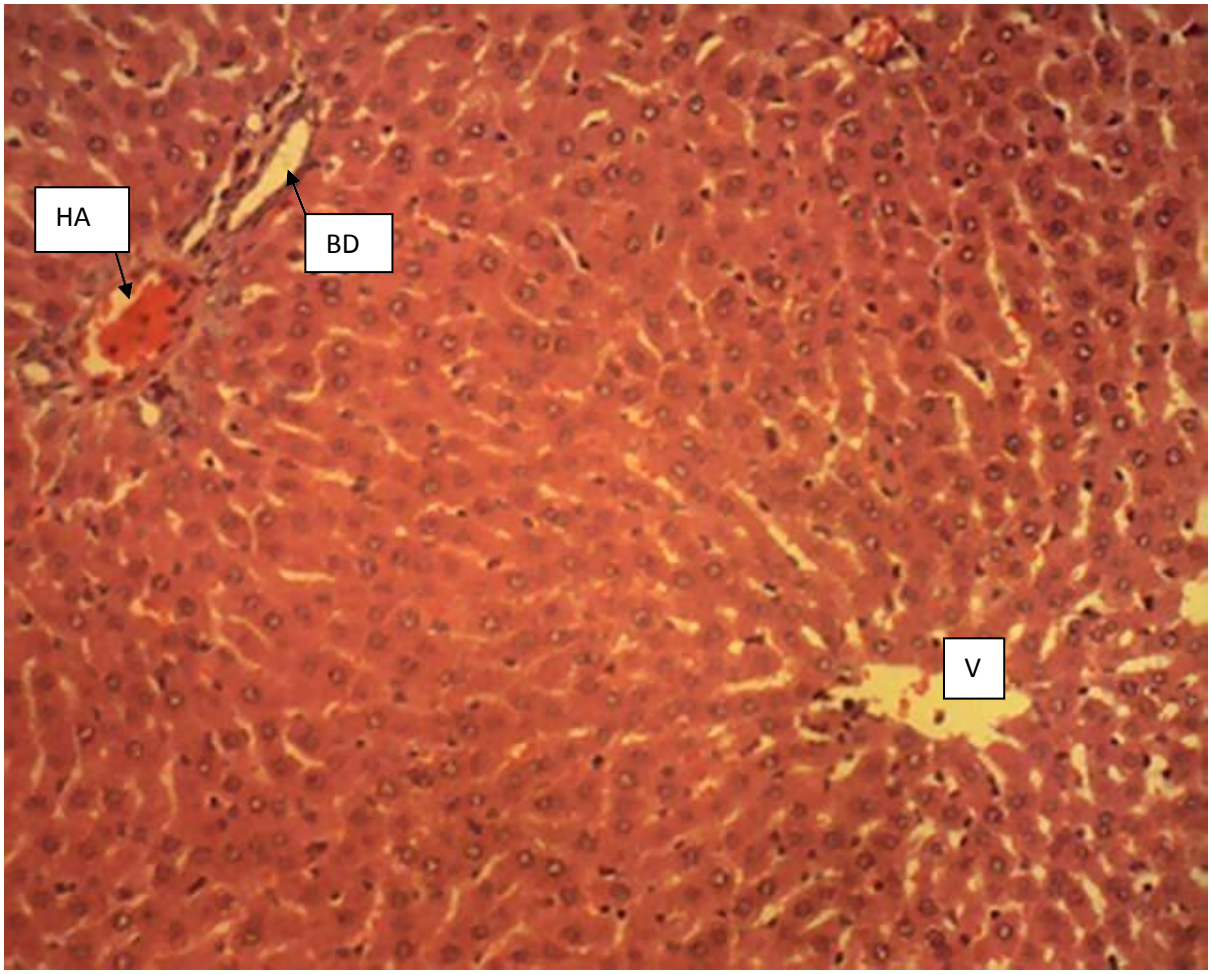


Sections of the **kidney** presented on this group showed the normal renal histo-architecture for laboratory rodents. Normal Glomeruli (G) in their respective thin Bowman's capsules were observed, surrounded by numerous normal renal tubules (arrow) which are lined by a simple cuboidal epithelium, in a highly vascularised connective tissue meshwork. H&E x160.

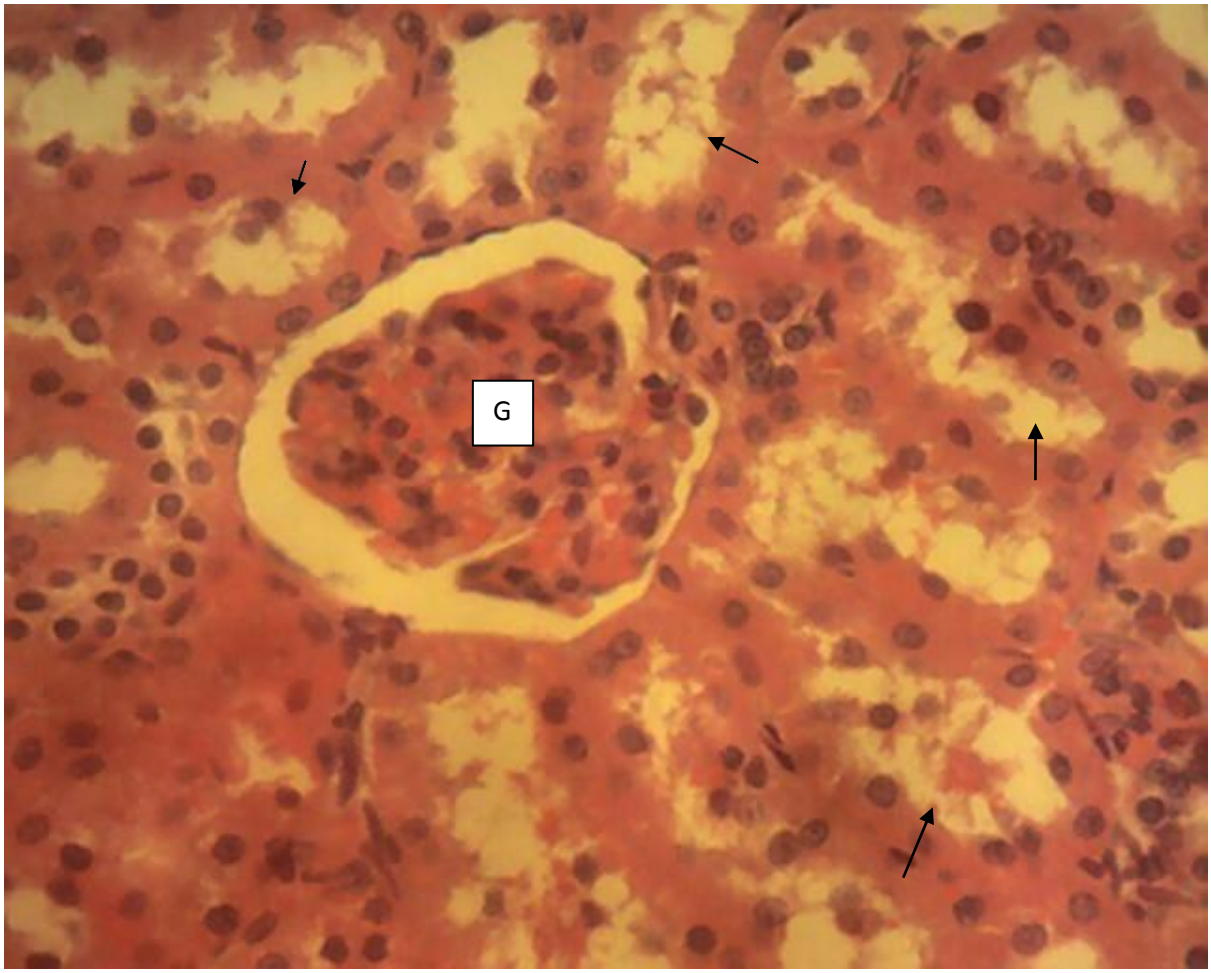


GROUP 5

Sections of the **liver** presented on this group showed the normal hepatic histo-architecture for laboratory rodents. The sections showed normal hepatic lobules consisting of normal hepatocytes arranged in interconnecting cords around the central veins. The cords are radially aligned, terminating at the portal areas of the hepatic lobules, where they meet with the hepatic vein, hepatic artery (HA) and bile duct (BD). H&E x160.

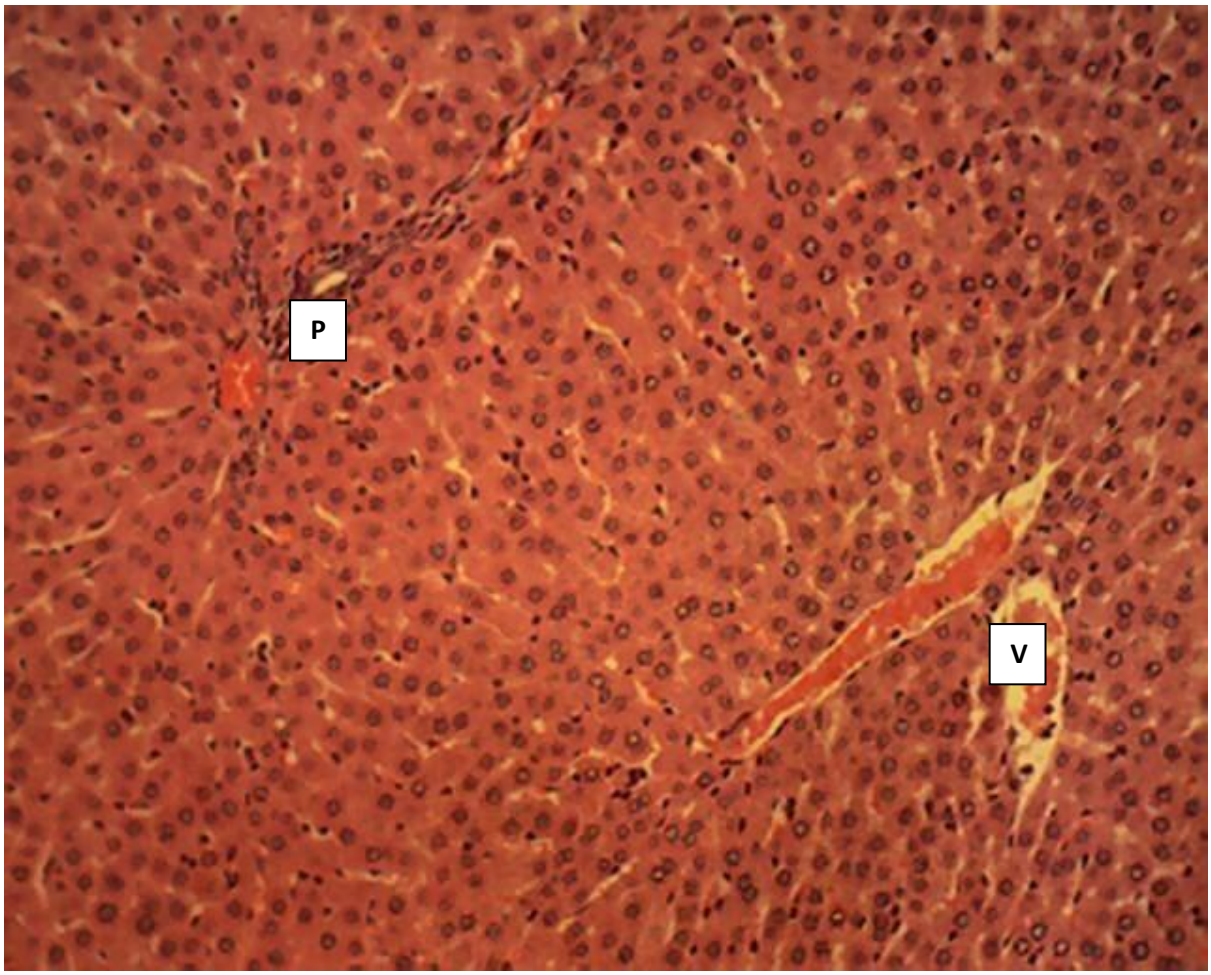


Sections of the **kidney** presented on this group showed the normal renal histo-architecture for laboratory rodents. Normal Glomeruli (G) in their respective thin Bowman's capsules were observed, surrounded by numerous normal renal tubules (arrow) which are lined by a simple cuboidal epithelium, in a highly vascularised connective tissue meshwork. H&E x400.

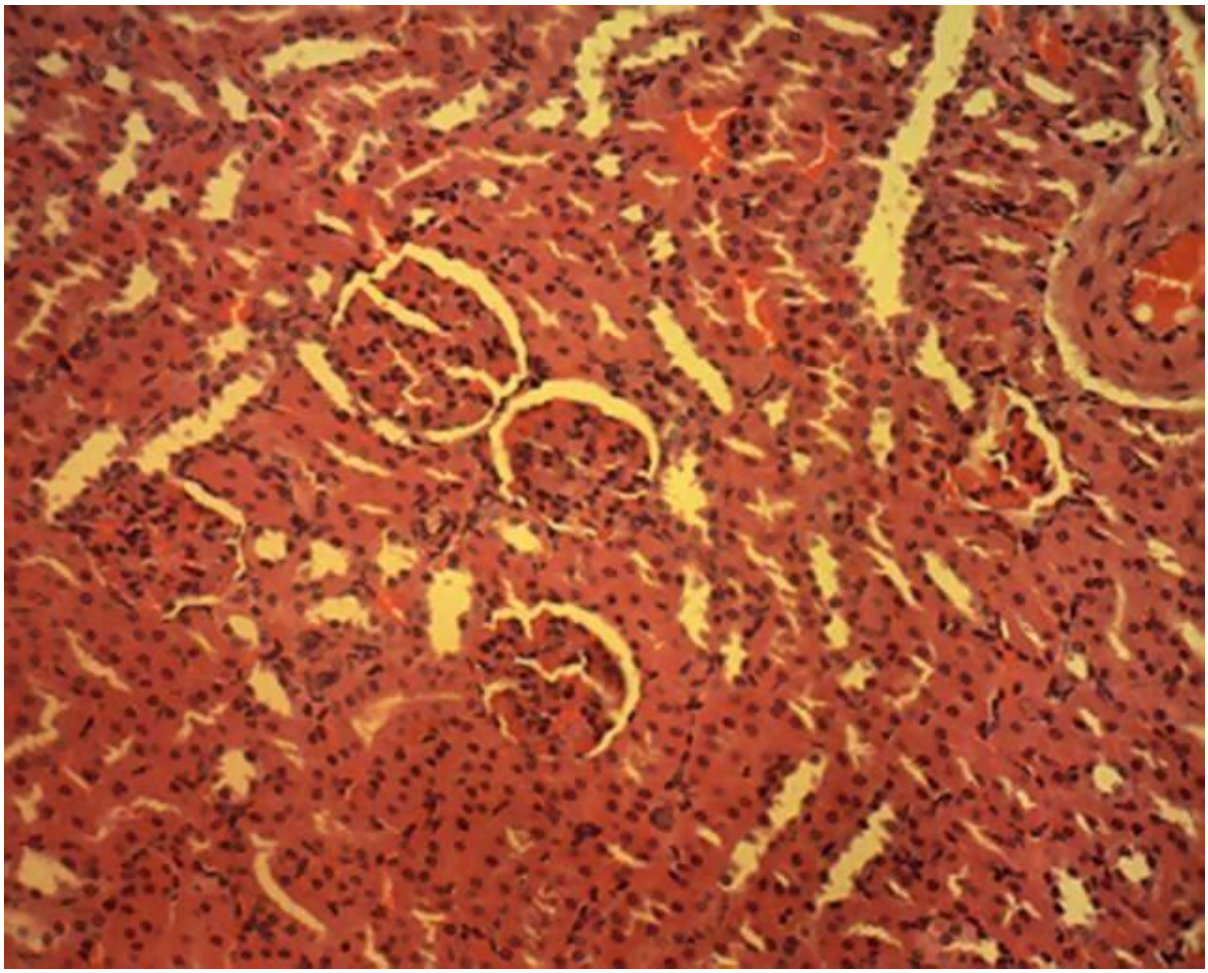


GROUP 6

Sections of the **liver** presented on this group showed the normal hepatic histo-architecture for laboratory rodents. The sections showed normal hepatic lobules consisting of normal hepatocytes arranged in interconnecting cords around the central veins (V). The cords are radially aligned, terminating at the portal areas (P) of the hepatic lobules, where they meet with the hepatic vein, hepatic artery and bile duct. H&E x160.



Sections of the **kidney** presented on this group showed the normal renal histo-architecture for laboratory rodents. Normal Glomeruli (G) in their respective thin Bowman's capsules were observed, surrounded by numerous normal renal tubules (arrow) which are lined by a simple cuboidal epithelium, in a highly vascularised connective tissue meshwork. H&E x160.



DISCUSSION

The histopathological results showed normalcy in the kidney of all the rat groups with normal glomeruli in bowman's capsules surrounded by numerous normal renal tubules lined by a simple cuboidal epithelium which could imply that the xenobiotic (dichlorvos) compounded in the beans had no effect(s) on the kidney which might be as a result of the role of the liver in the biotransformation of xenobiotics. Nevertheless, deviations were seen in the liver of group 1 and group 3 rats fed with parboiled and un-parboiled beans treated with high concentration of dichlorvos respectively; group 1 rats showing moderate vacuolar degeneration of hepatocytes where affected hepatocytes were swollen and group 3 rats showing a necrotic cell growth are all indications of oxidative damage of the liver cells rising from the high concentration of dichlorvos. This oxidative damage occurs majorly in the

mitochondria because electrons will continually leak from the respiratory chain to form reactive oxygen species which has been shown to have the ability to modify mitochondrial proteins, DNA and lipids which may lead to the failure of the mitochondrial bioenergetics hence resulting to necrotic cell death.[17] The liver is a well-known target organ of the toxic impact regarding its function in biotransformation and excretion of xenobiotics. After entering uptake, liver is the first organ to be exposed by portal circulation.[18] Hepatotoxicity is toxicity to the liver, bile duct, and gall bladder. The liver is particularly susceptible to xenobiotics due to a large blood supply and its role in metabolism.[19]

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