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Research Article

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ORGAN TOXICITY STUDY OF HEATED PALM KERNEL OIL AND SOYA OIL ON ALBINO RATS

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ABSTRACT

Background: Heating of oil repeatedly changes the physical and chemical appearances of the oil and this has been linked to the aetiopathogenesis of rising pandemic of non-communicable/chronic diseases. **Objective**: This study evaluated the effect of heated palm kernel and soya oil on the liver, kidney and heart of albino rat. **Methods:** Sixty(Male and Female) albino rats weighing between (75-160g) were purchased and housed in cages at room temperature at university of Nigeria Enugu campus animal house where the experiment was carried out. After two weeks of acclimatization, they were randomly distributed into five groups each containing 12 rats and were fed with growers' mash– and water was given to them *ad-libitum.* The principles of laboratory animal care were followed. One liter of each sample was heated in an electric oven at temperature of 180^0C for 15 minutes. The rats were grouped into five different groups of twelve (12) each: Group 1: Control Group, received feed +water only, group 2 received feed + water + unheated PKO, group 3 received feed + water + heated PKO, group 4 received feed + water + unheated SO, group 5 received feed + water + heated SO. To each group, 10mls of respective oil samples were added to 100g of feed +water *(ad libitum*). Baseline weights were measured before exposing them to the oil meals. After six weeks' exposure, they were fasted overnight and reweighed and further exposure for another six weeks was observed. They were reweighed and from each group the least, mid and highest weighted rats were sacrificed and the liver, kidneys and heart were harvested and fixed in 10 percent formalin for histopathological analysis. **Result**: Histopathology study of the liver, showed that all the test samples caused hepatic inflammation. UHPKO, HPKO, and HSO caused distortion in hepatic architecture and UHPKO, UHSO, and HSO caused cytoplamic ground glass appearance. However, the HPKO caused intrahepatic heamorrhage while UHSO caused formation of giant cells. All the test samples caused renal tissue loss, and intrarenal haemorrhage. UHPKO, HPKO and UHSO caused renal fatty changes and distortion in renal architectures. HSO caused renal tissue necrosis and tubular clumping. On the heart, all the test samples caused loss of myocardial tissues. The UHPKO and HPKO caused intramyocardial hemorrhage, and the HSO caused inflammation (myocarditis), disorganization of cardiac architecture and reduction in muscle bulk. **Conclusion:** Histological evidence of inflammation by all the test samples on liver, kidney and heart of albino rat is an indication that these products may be toxic to the body organs studied. Therefore, consumption of these products especially when heated, may not be completely safe.

KEYWORDS: Heating, toxicity, Palm kernel oil, Soya oil, albino Rats.

INTRODUCTION

Heating of oil repeatedly changes the physical and chemical appearances of the oils. Some of the chemical reactions that occurred during the heating of oils are hydrolysis, oxidation, and polymerization. [1,2] A joint WHO/FAO expert consultation on diet, nutrition and prevention of chronic disease recognized that the growing epidemic of chronic diseases affecting both developed and developing countries was related to dietary and life style changes. Hydrolysis reactions following heating of oil usually result in the increment of free fatty acids, reactive oxygen species (ROS), and trans fatty acids which have the propensity to induce organ failure and histopathology changes on different organs like the heart, intestinal mucosa, liver, and kidney.[3,4] Oxidation of oils during frying alters the nature of enzymes and the status of antioxidants and causes the formation of lipid peroxidation and trans fatty acids.[5-7]

Furthermore, rapid changes in diet and life style that have occurred with industrialization, urbanization, economic development and market globalization have accelerated over the past decade. This is having significant impact on health and nutritional status of populations, especially heating of oil particularly in developing countries and countries in transition.[8] While standard of living improved, food availability has expanded and becomes more diversified, and access to services has increase, there have also been significant negative consequence in terms of inappropriate dietary patterns, decreased physical activities, increase tobacco use, and a corresponding increase in diet related chronic disease, especially among poor people. Because of these changes in life style patterns, chronic non communicable diseases (NCDs) – including obesity, diabetic mellitus, cardiovascular diseases, hypertension and stroke, and some types of cancer are becoming increasingly significant cause of disability and premature death in both developing and newly developed countries

The proportion of the burden of NCDs is expected to increase to 57% by 2020, chronic disease will account for almost three quarters of all death worldwide, and that 71% of deaths due to stroke and 70% of death due to diabetes will occur in developing countries.

This increasing pandemic of non-communicable/chronic diseases mandates urgent research into possible aetiopathogenic influences. Epidemiological studies show association to entity called metabolic syndrome and this suggests that chronic diseases may to an extent, share common genetic and/or environmental predisposing factors.

Saturated fatty acids (SFA) and trans fats increase low density lipoprotein (LDL) cholesterol in blood, which lead to plaque formation. Polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) reduce LDL cholesterol and increase high density lipoprotein (HDL) cholesterol.

MUFAs are beneficial in that they increase cholesterol esterification in the liver, thereby reducing the free cholesterol pool and increasing receptor mediated uptake of LDL cholesterol, resulting in a decrease in blood cholesterol level as reported by the dietary Guidelines Advisory Committee (DGAC) on the dietary Guidelines for Americans 2010. Evidence from controlled clinical studies have shown that MUFA favourably affect a number of risk factors for coronary heart diseases (CHDs). Heating of the oil cause increase in liver function enzymes, hepatocyte death, and chronic liver disease, [9,10] as well as glomerular injury [11]. In the context of oxidative stress, pathogenic mechanisms such as the accumulation of protein adducts aberrant lipid metabolism as well as deranged liver functions set the stage for non-communicable diseases (NCDs). [12-14] During oxidative stress, 4 hydroxynonenal (4-HNE) can form stable protein adducts which accumulate in metabolic diseases, [12,15] and are also a key component of the pathogenesis in a spectrum of hepatic diseases. [13]

MATERIALS AND METHODS MATERIALS

Reagents/ instruments/equipment.
The materials used during the research work are: experimental animals (albino rats) Solive oil (batch number 25530), Grand Soya oil (batch number 160518), weighing balance, oven, Refrigerator, dissecting set, microtome, light microscope, and growers' mash (Top Feed), hand gloves, commercial kits Randox reagents.

EXPERIMENTAL ANIMALS

Sixty (Male and Female) albino rats weighing between (75-160g) were purchased and housed in cages at room temperature at university of Nigeria Enugu campus animal house where the experiment was carried out. After two weeks of acclimatization, they were randomly distributed into five groups each containing 12 rats and were fed with growers' mash– and water was given to them *ad-libitum.* The principles of laboratory animal care were followed.

EXPERIMENTAL DESIGN

Solive oil (PKO) and Grand Soya oil (SO) were purchased at Ogbete main market, Enugu state Nigeria. One liter of each sample was heated in an electric oven at temperature of 180^0C for 15 minutes. Sixty albino rats were grouped into five different groups of twelve (12) each:
Group 1: Control Group; they received feed +water only.
Group 2: They received feed + water + unheated PKO
Group 3: They received feed + water + heated PKO
Group 4: They received feed + water + unheated SO
Group 5: They received feed + water + heated SO

To each group, 10mls of respective oil samples were added to 100g of feed +water *(ad libitum*). Baseline weights were measured before exposing them to the oil

meals. After six weeks' exposure, they were fasted overnight and reweighed and further exposure for another six weeks was observed. They were reweighed and blood samples were collected into plain bottles for assay of the liver function and renal function tests. From each group the least, mid and highest weighted rats were sacrificed and the liver, kidneys and heart were harvested and fixed in 10 percent formalin for histopathological analysis. The histopathology analysis was carried at International Center for Cancer Research Abakaliki respectively.

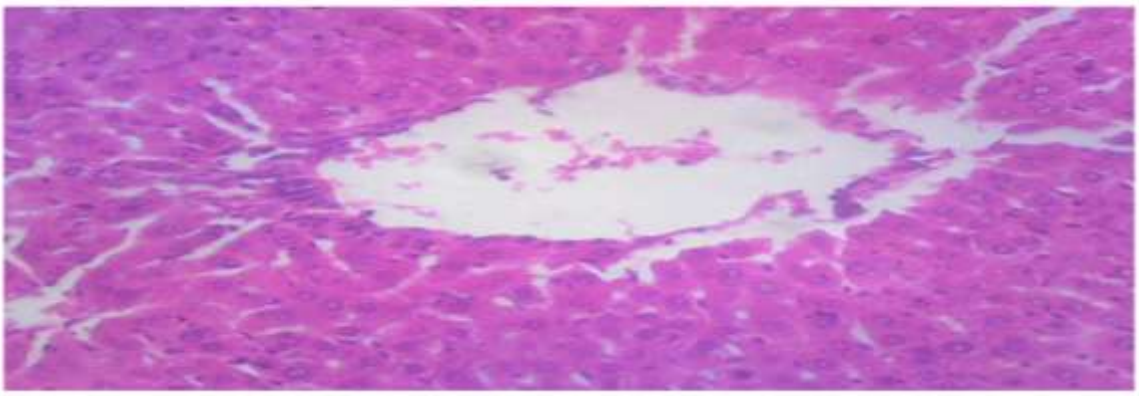
HISTOLOGY

The rats were sacrificed humanly by allowing them in a container with swab of ether solution which they inhaled. The excised liver, kidney and heart tissues were fixed in 10% formalin and processed for light microscopy study. These include dehydration through graded ethanol, clearing in xylene, infiltration in paraffin wax for 2 hours at 56 C and embedding of tissue in paraffin wax for 48 hours. 6mm section were stained with haematoxylene and eosin stain (H and E).

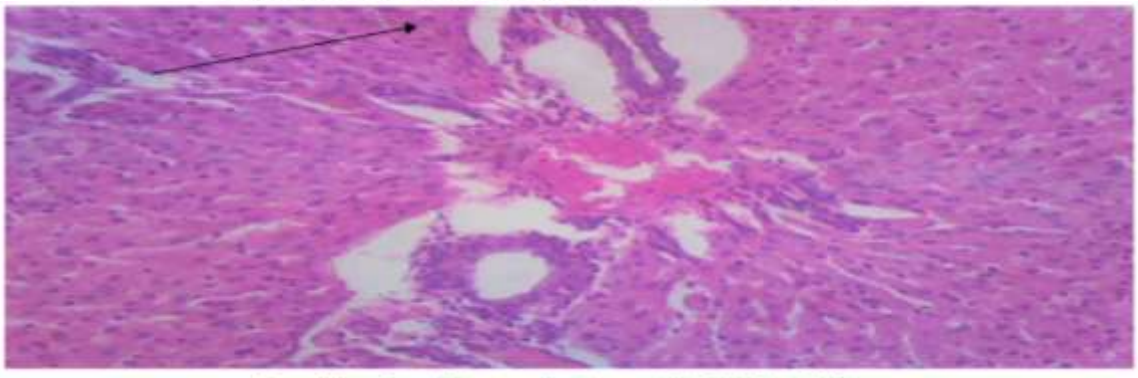
RESULTS

KEYS

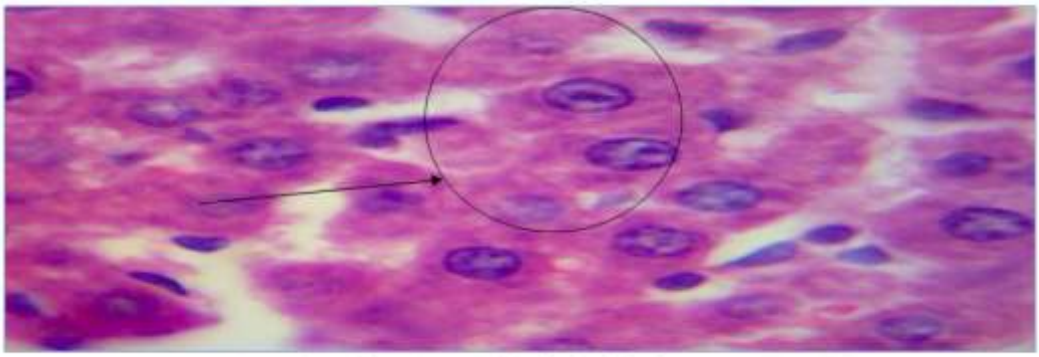
ALL: Group A least weight liver ALK: Group A least weight kidney ALH: Group A least heart
BLL: Group B least weight liver BLK: Group B least weight kidney BLH: Group B least heart
CLL: Group C least weight liver CLL: Group C least weight kidney CLH: Group C least heart
DLL: Group D least weight liver DLK: Group D least weight kidney DLH: Group D least heart
ELL: Group E least weight liver ELK: Group E least weight kidney ELH: Group E least heart
AML: Grp A medium weight liver Grp AMK medium weight kidney AMH: Grp A med. heart
BML: Grp B medium weight liver Grp BMK medium weight kidney BMH: Grp B med. heart
CML: Grp C medium weight liver Grp CMK medium weight kidney CMH: Grp C med. heart
DML: Grp D medium weight liver Grp DMK medium weight kidney DMH: Grp Dmed. heart
EML: Grp E medium weight liver Grp EMK medium weight kidney EMH: Grp E med. heart
AHL: Grp A highest weight liver Grp AHK medium weight kidney AHH: Grp A highest heart
BHL: Grp A highest weight liver Grp BHK medium weight kidney BHH: Grp A highest heart
CHL: Grp A highest weight liver Grp CHK medium weight kidney CHH: Grp A highest heart
DHL: Grp A highest weight liver Grp DHK medium weight kidney DHH: Grp A highest heart
EHL: Grp A highest weight liver Grp EHK medium weight kidney E HH: Grp A highest heart



Section Shows Normal Hepatic Architecture with Central Vain X150 ALL.

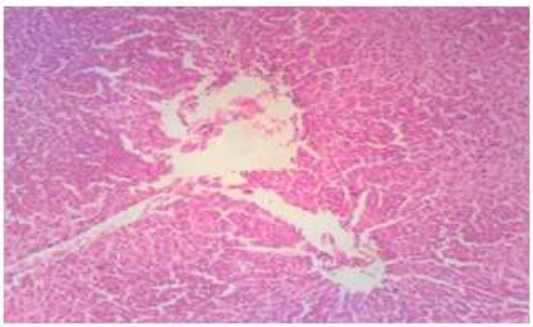


Normal hepatic architecture showing portal traid X 150 AML.

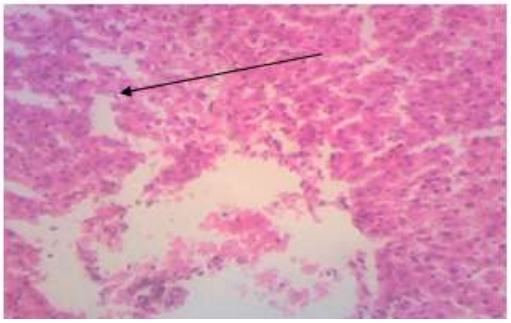


Normal hepatic architecture showing healthy hepatocytes X 600 AHL.

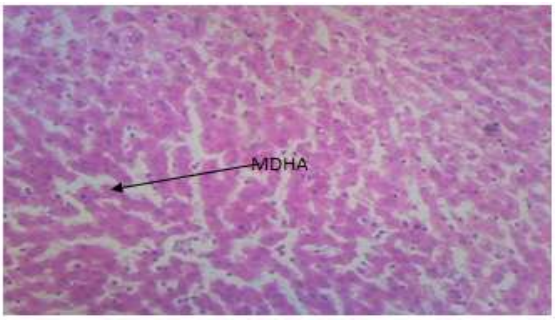
Plate 1: Liver: Control, Photomicograph of All, Aml, And Ahl of Liver Section (X150, /600)H/E Section Showing Normal And Healthy Hepatocytes.



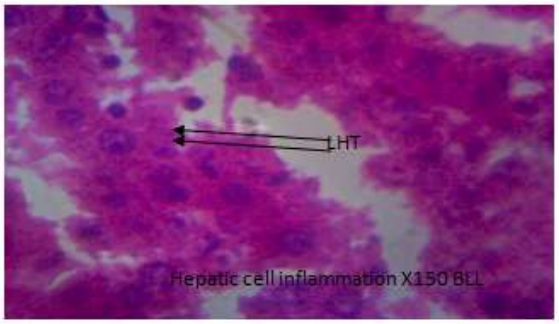
Moderate infiltrate of inflammatory cell X 600 BML.



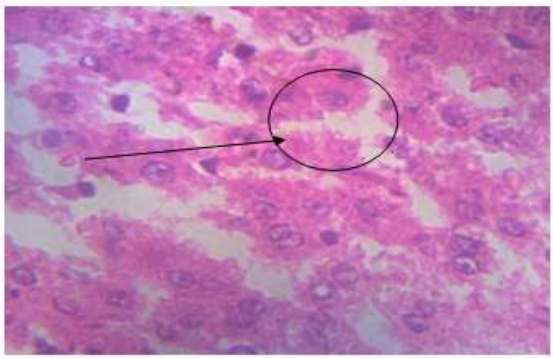
BLLx60



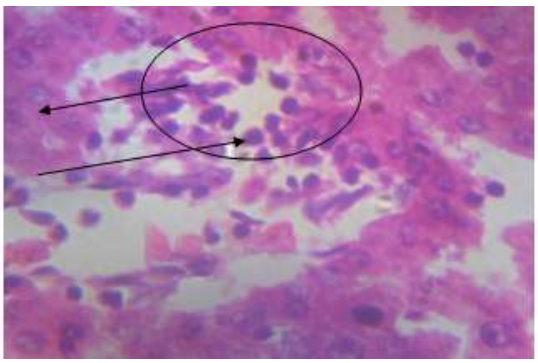
Mild distortion of hepatic architecture X 150 BML.



Focal loss of hepatic tissue (FLHT) X 60 BLL.

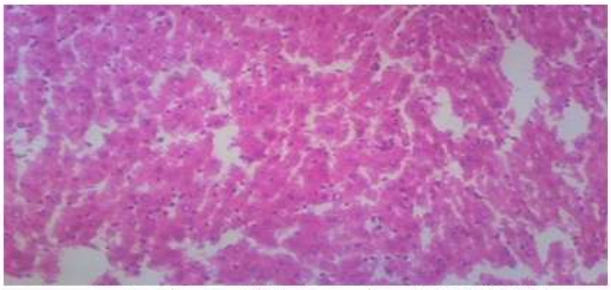


Cytoplasmic ground glass appearance and moderate distortion of hepatic architecture X 600 BML

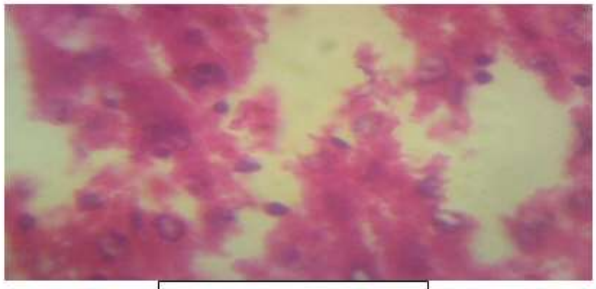


Focal aggregate of inflammatory cell X 600 BML.

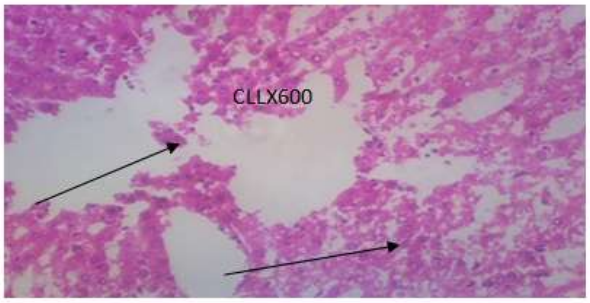
Plate 2: Liver: Uhpko, Photmicography of Bll, BML and BHL of THE Liver Section (X 60/150/600). H/E Showing deranged liver architecture.



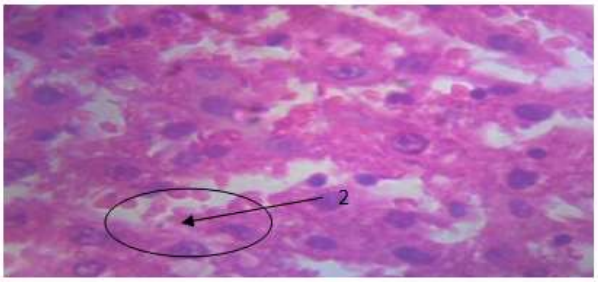
Section shows mild inflammation (MI)X150 CLL



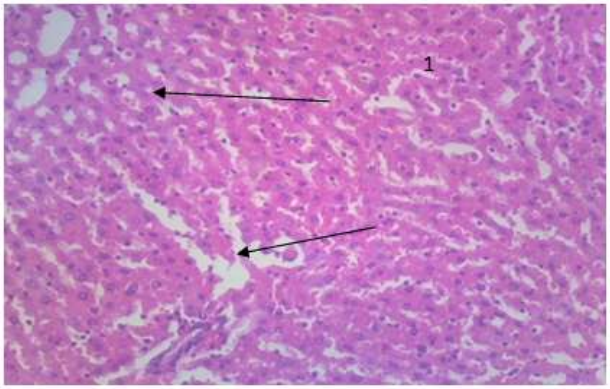
BLL



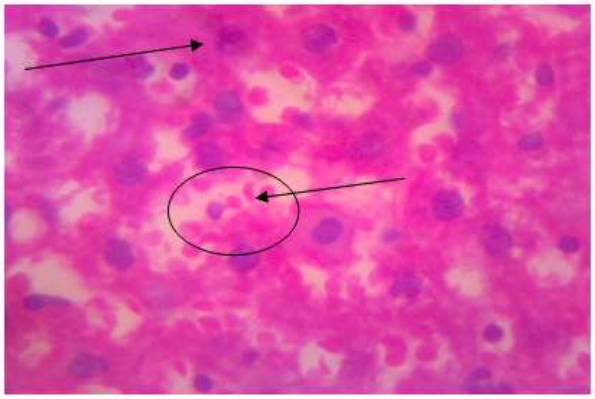
Section shows moderate distortion of hepatic architecture(MDHA) X150 CML



Section shows: 1 mild inflammation (MI) and 2 intra hepatic heamorrhage(IHH) X600 CML

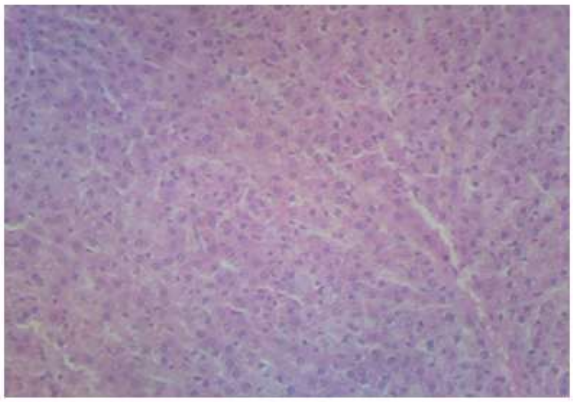


Section shows mild distortion of hepatic architecture (MDHA) X150 CML

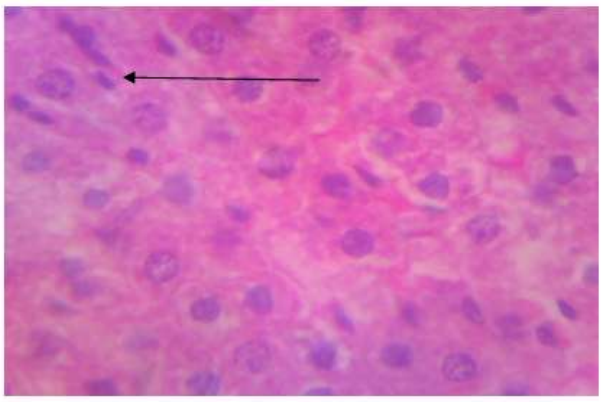


Section shows intra hepatic heamorrhage(IHH)X 600 CML

Plate 3: Liver Hpko, Photomicograph of Cll, Cml And Chl of Liver Section(X150/600) H/E Showing Effects Described Above When Compared With Plate 1 (Conrol).



Section shows poor perfused hepatic tissue X60 DLL



Section shows Mild cytoplamic ground glass appearance (MCGGA) with mild inflammation(MI)X600 DLL

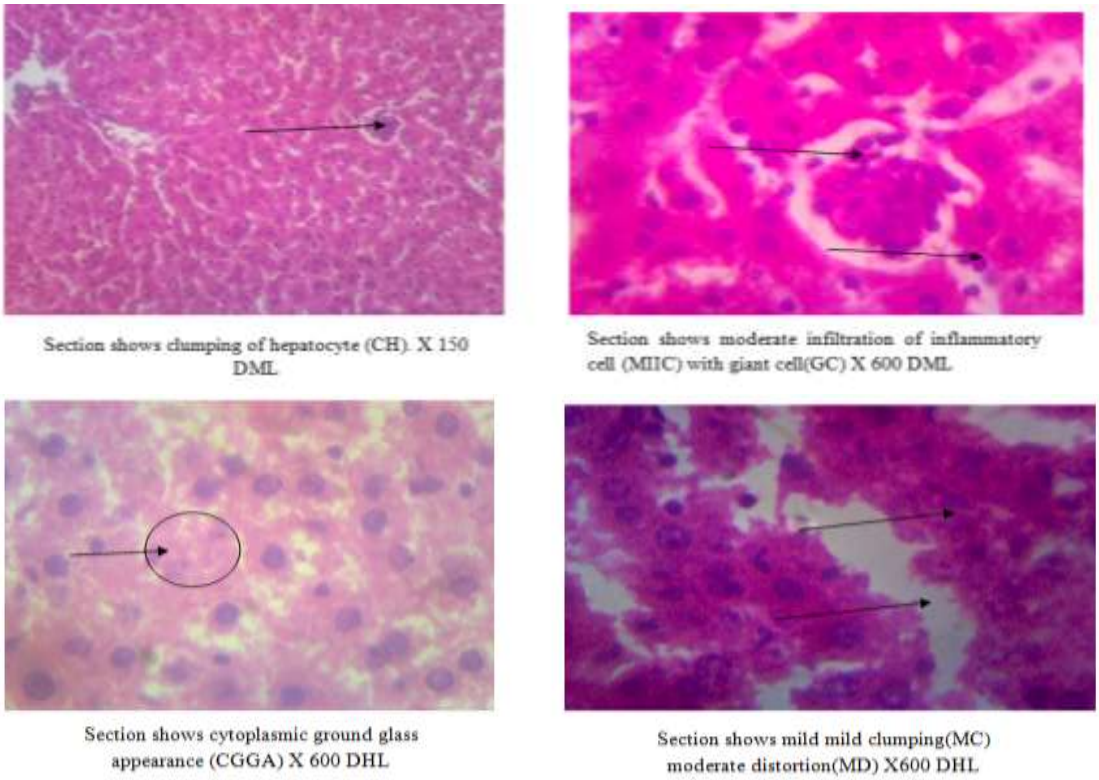


Plate 4: Liver: Uhso. Photomicogaph of Dll, Dml, Dhl of Liver Section (x60/150/600). H/e Showing Effects Described Above As The Were Compared with Plate 1 (Control).

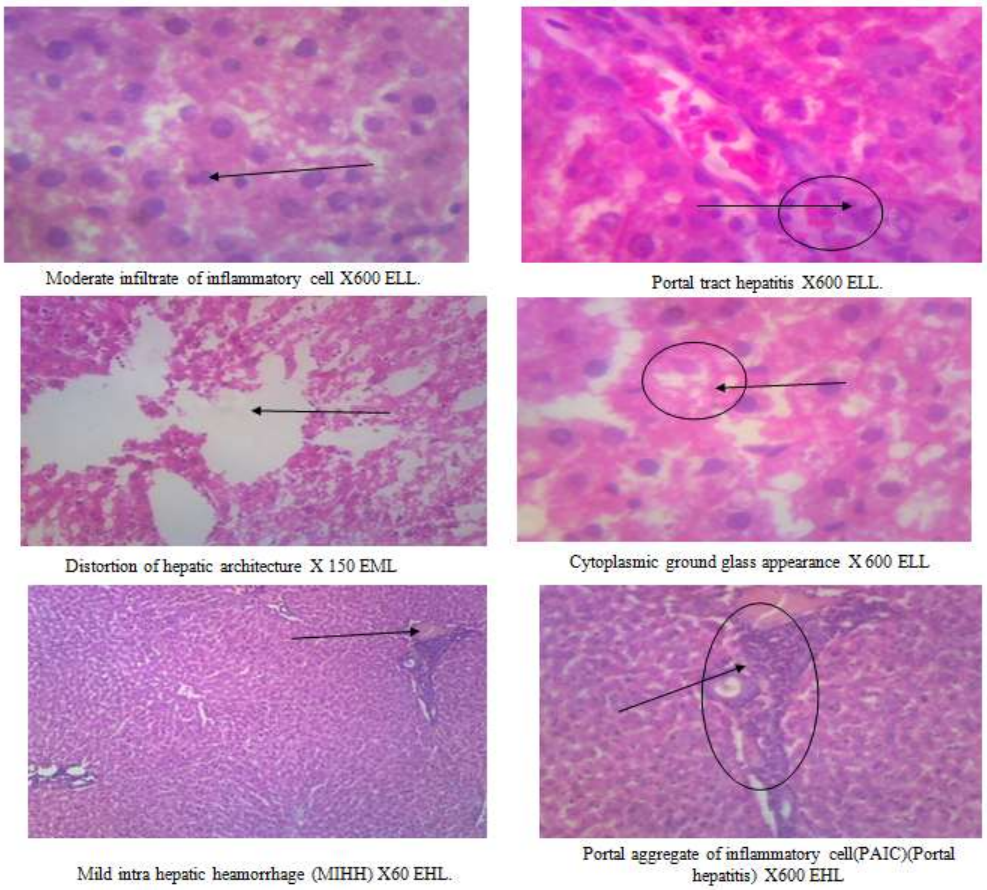
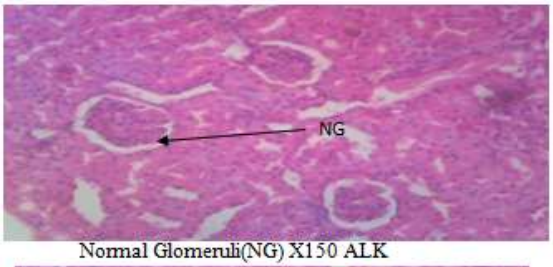
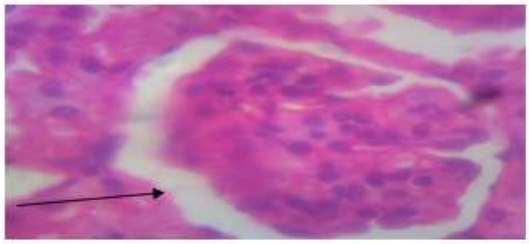


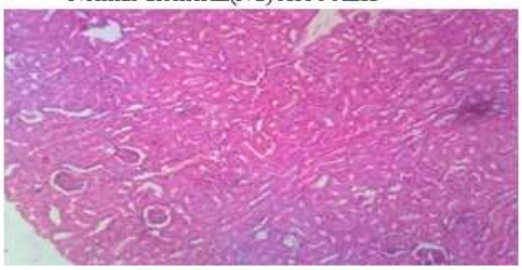
Plate 5: Liver: HSO. Photomicograph Ell, Eml, Ehl of Liver Secton(x60/150/600) h/e section Showing the Effect Described Above When Compared with Plate 1 Control.



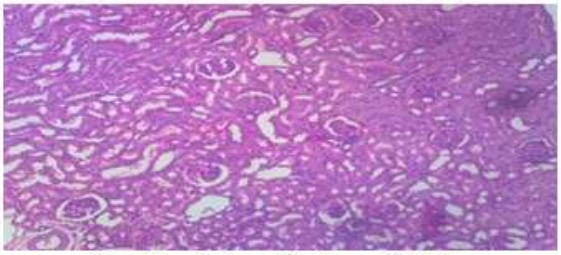
Normal Glomeruli(NG) X150 ALK



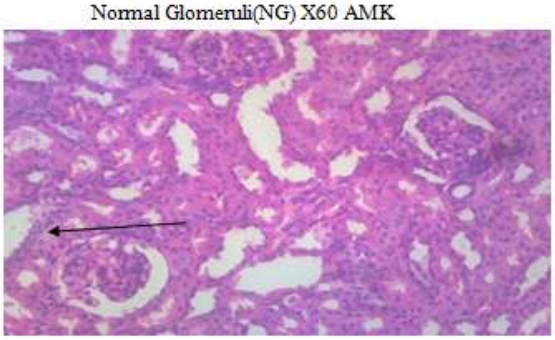
Bowman Space (BS) X600 ALK



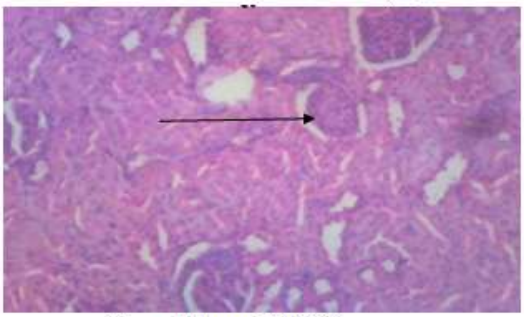
Normal Glomeruli(NG) X60 AMK



Normal renal tissue with Glomerulic(NG)

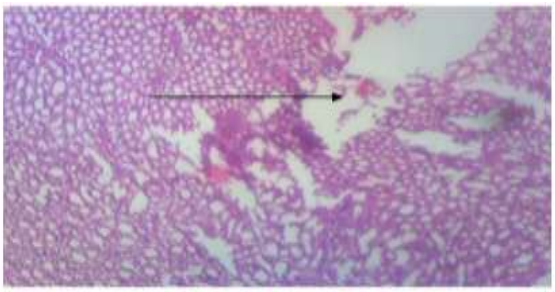


Tubular Cells (TC) X60 AHK

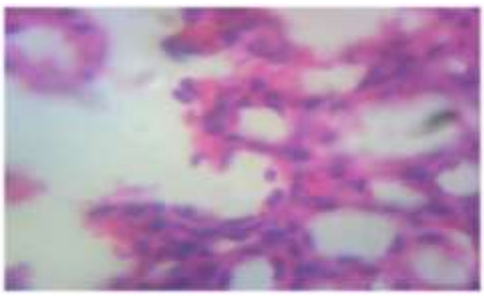


Normal Glomeruli(NG) X

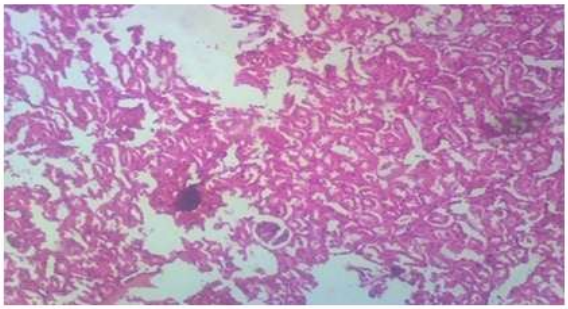
Plate 6: Kidney: Control, Photomicograph Showing Essentialy Normal Kidney Section. H/e At Different Magnification.
KEYS: LK=Least weight kidney, MK=middle weight kidney, HK=Highest weight kidney



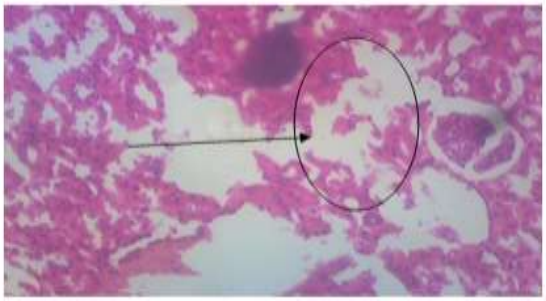
Focal area of tissue loss X 60 BMK



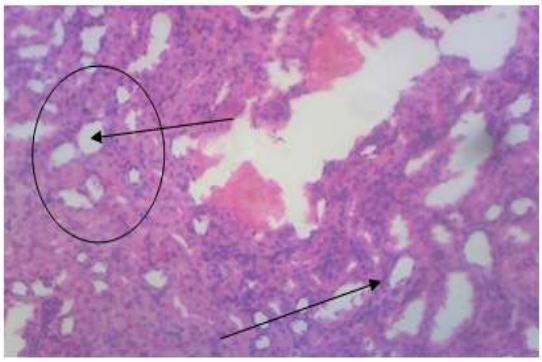
Distortion of Renal Architecture X 150 BMK



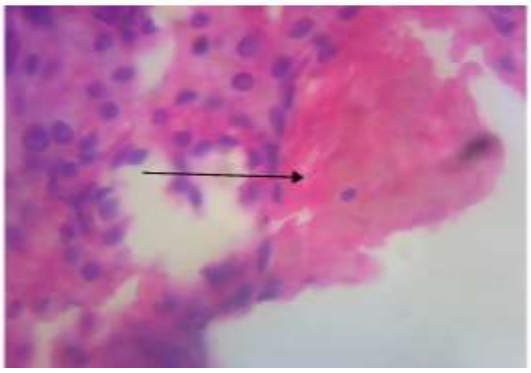
Moderate fatty change X150 BHK



Moderate of intra Renal Hemorrhage X150 BHK



Moderate fatty change (MFC) and mild intra renal heamorrhage (MIRH) X60 CLK



Moderate fatty change X150 CLK

Plate 7: Kidney: Uhpko. Photomicrograph of Blk, Bmk and Bhk of Kidney Section At Differen Magnifications Showing The Effects Described Above When Compared With Plate 6 The Control.

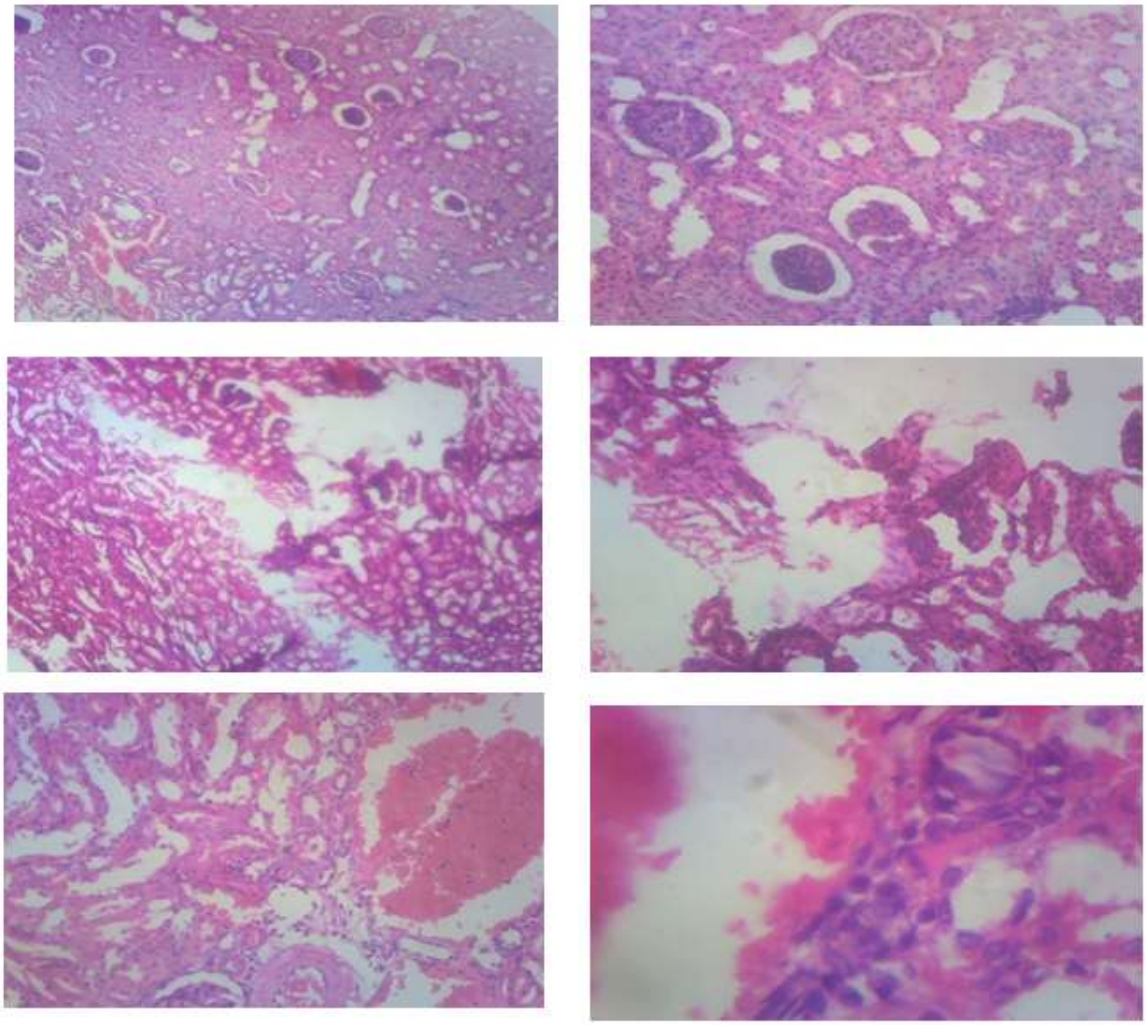
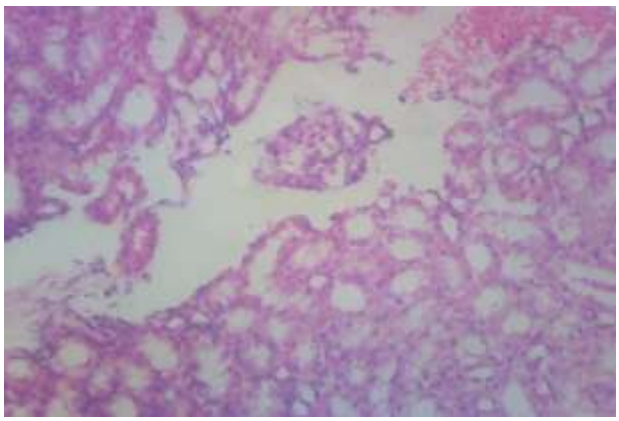
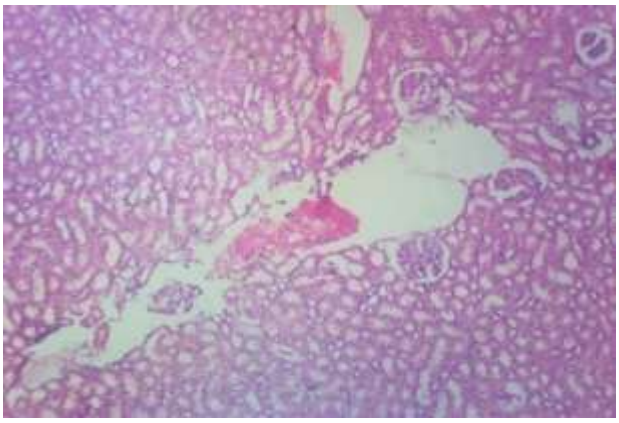


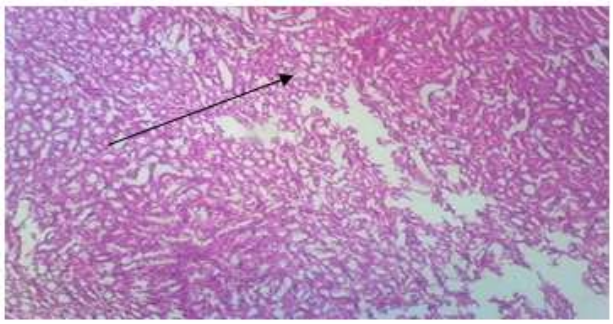
Plate 8: Kidney: Hpko. Photomicograph of Clk, Cmk and Chk of Kidney Section at Different Magnificatios. H/e Showig Effcts Demonstratd Above When Compared with Plate 6 the Control.



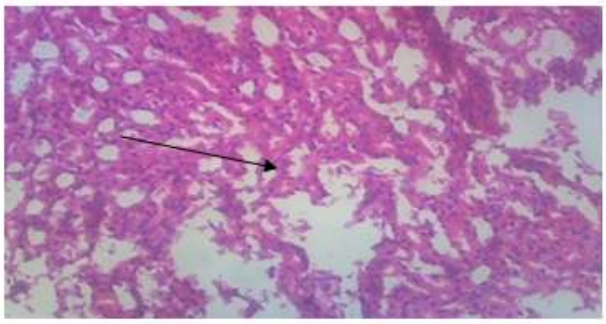
Focal distortion of Renal Architecture X 150 DLK



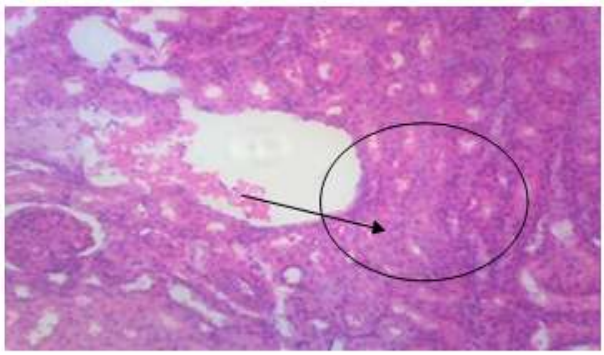
Focal area of intra Renal Heamorrahage X 150 DLK



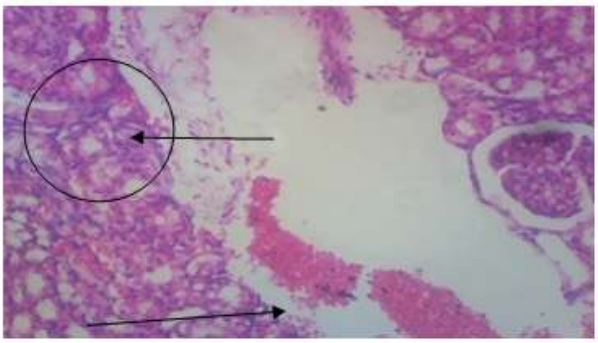
Moderate distortion of Renal Architecture X150 DMK



Moderate fatty change and loss of renal tissue X150M

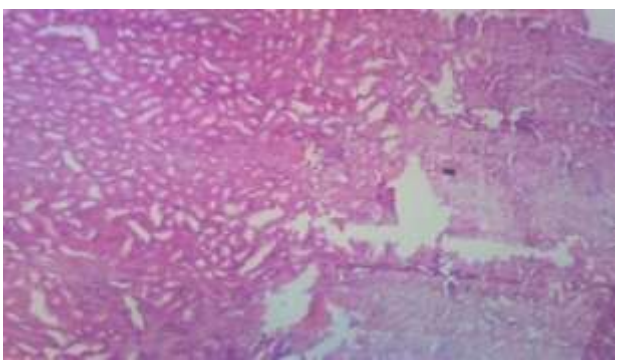


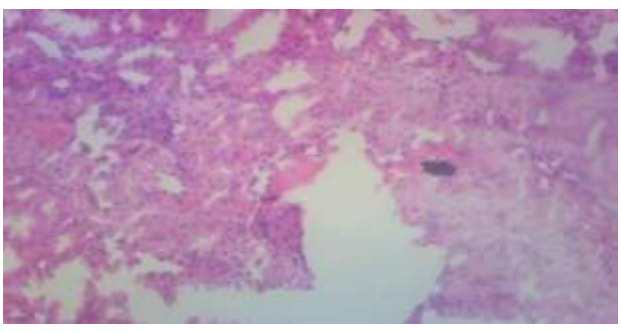
Focal area of renal tissue loss X150 DHK

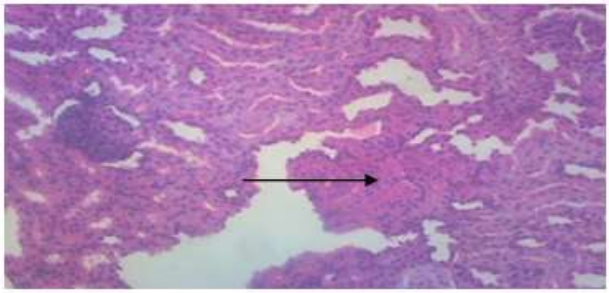


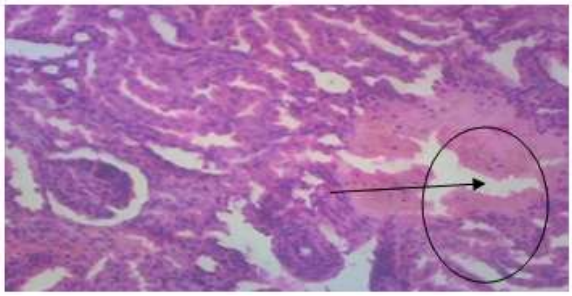
Intrarenal haemorrahge X150 DHK

Plate 9: Kidney: Uhso. Photomicograph of Dlk, Dmk and Dhk of Kidney Secion(X150) H/E Showing Effects Demonstrated Above When Compared With Plate 6 The Control.

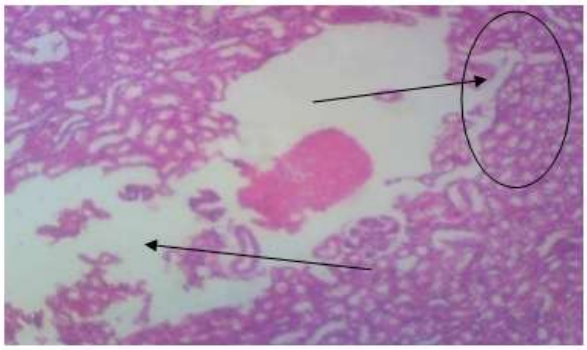




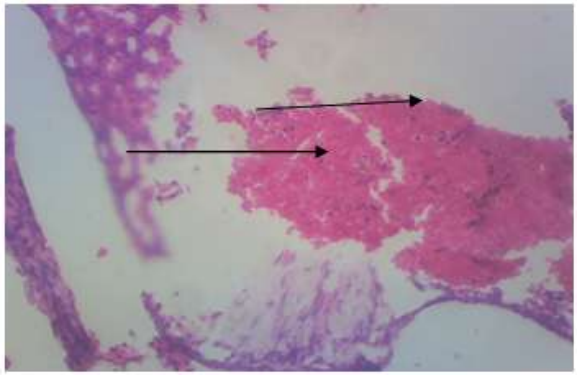




Intra renal hemorrhage

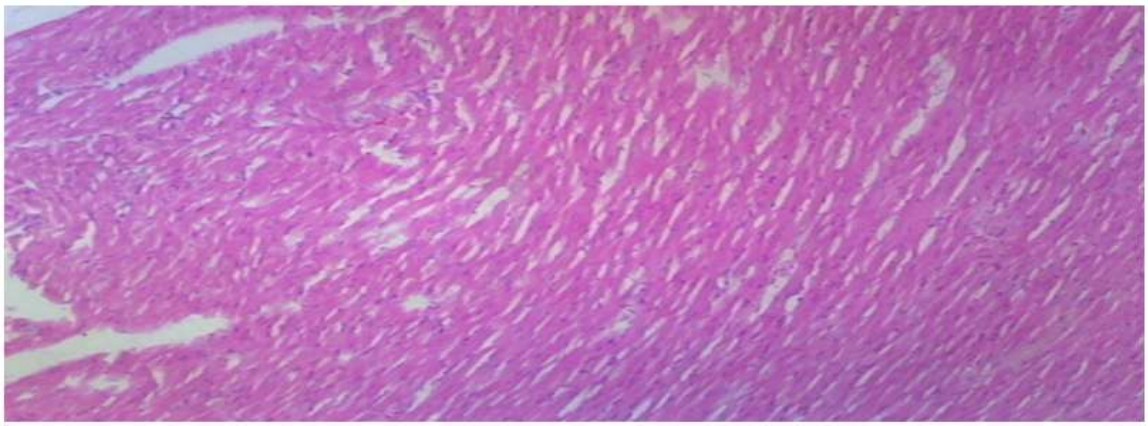


Focal area of renal tissue loss X150 EHK

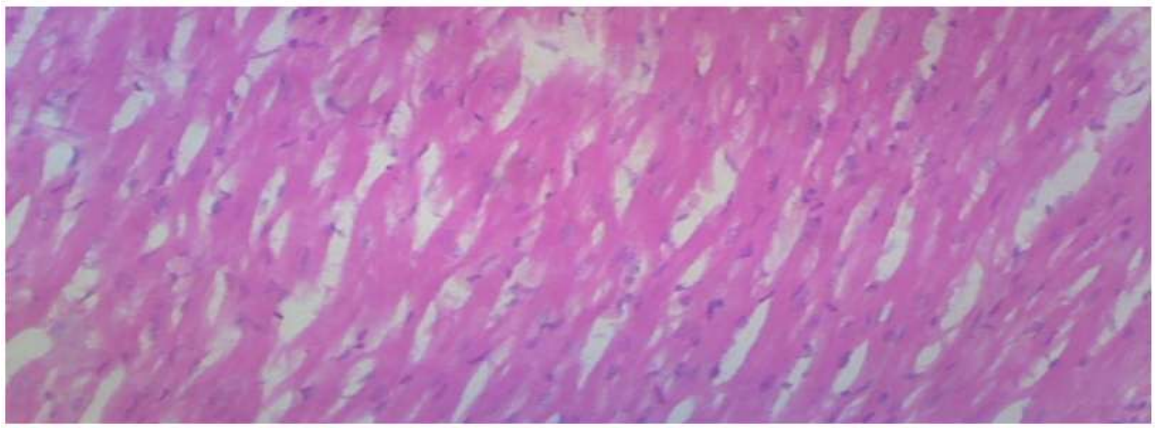


Intra renal hemorrhage X 600 EHK

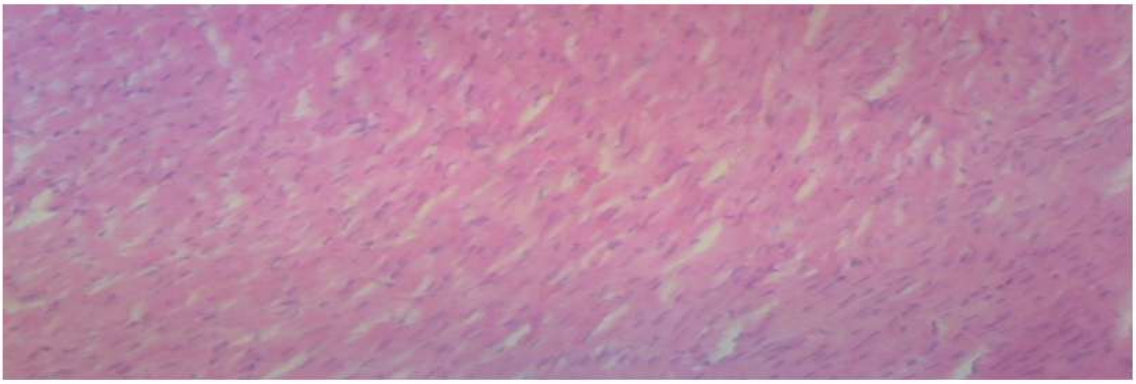
Plat 10: Kidney: Hso. Photomicogaph of Elk, Emk, Ehk of Kidney Section at Different Magnifications H/E Showing Effects Demonstrated Above Compared with Plate 6, The Control.



Section shows normal cardiac muscles that appear in syncytium and well-arranged cardiac fibers X60 ALH.

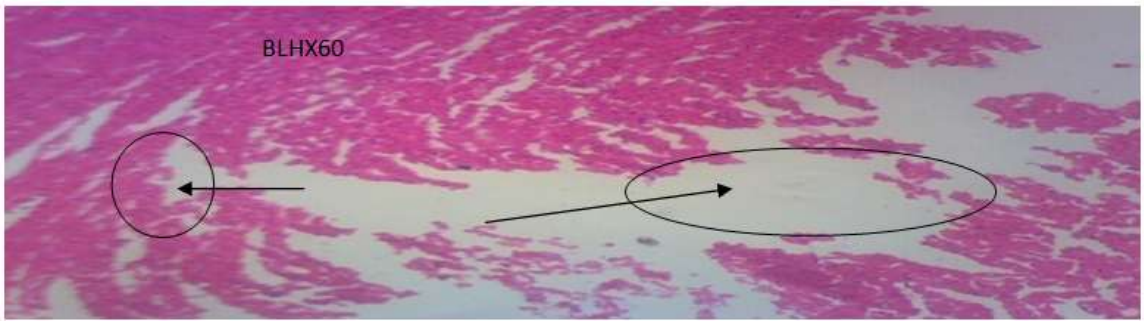


Section shows normal cardiac muscles that appear in syncytium and well arranged cardiac fibers X150 AMH.

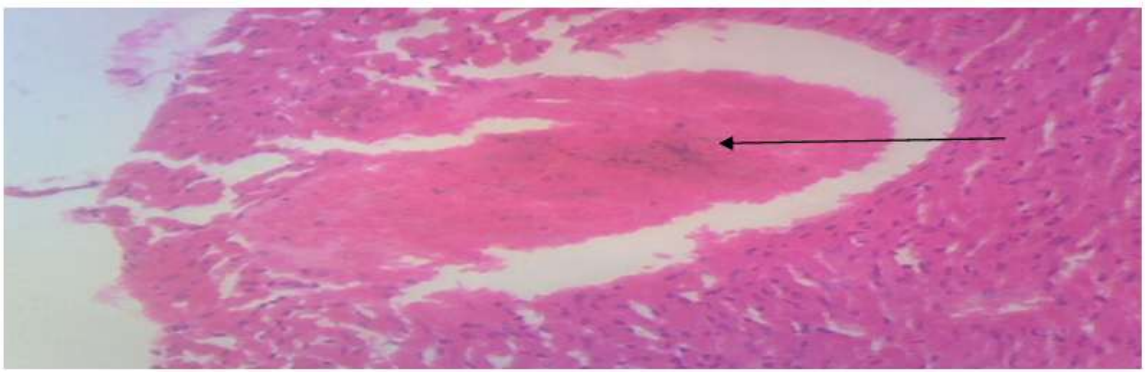


Section shows normal cardiac muscles that appear in syncytium and well-arranged cardiac fibers X150 AHH.

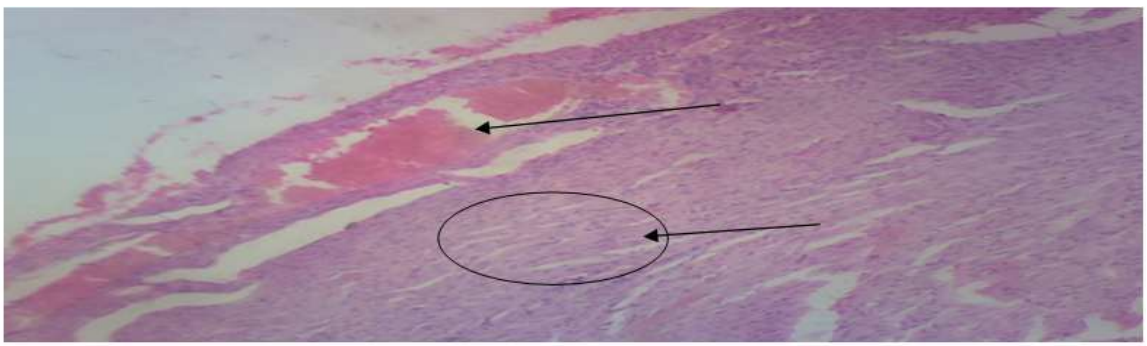
Plate 11: Heart: Control. Photomicograph oF ALH, AMH, AHH Of Heart Section at Different Magnifications H/E Showing Essentially Normal Cardiac Histology. KEYS: LH=Least weight heart, MH=medium weight heart, HH= highest weight heart.



Myocardial loss due to myocardial infarction x60 blh.

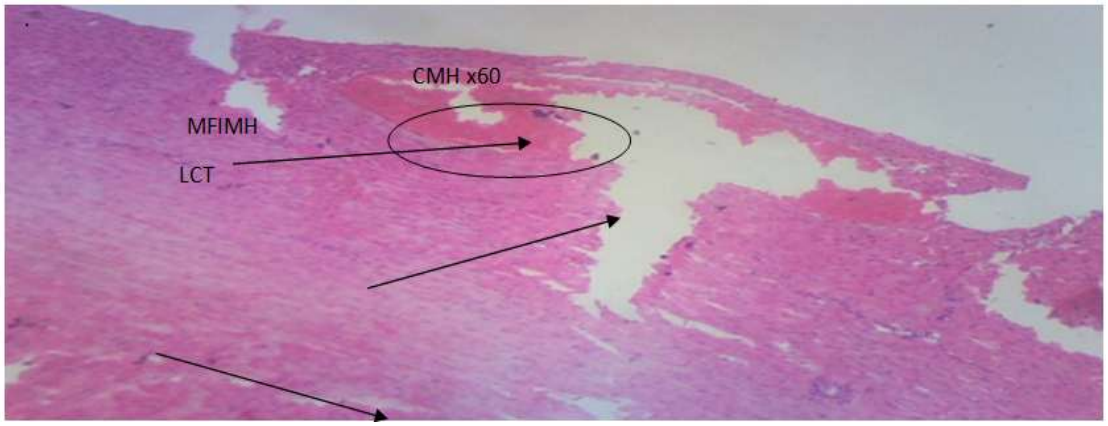


Intra myocardial Bleeding X 60 BMH

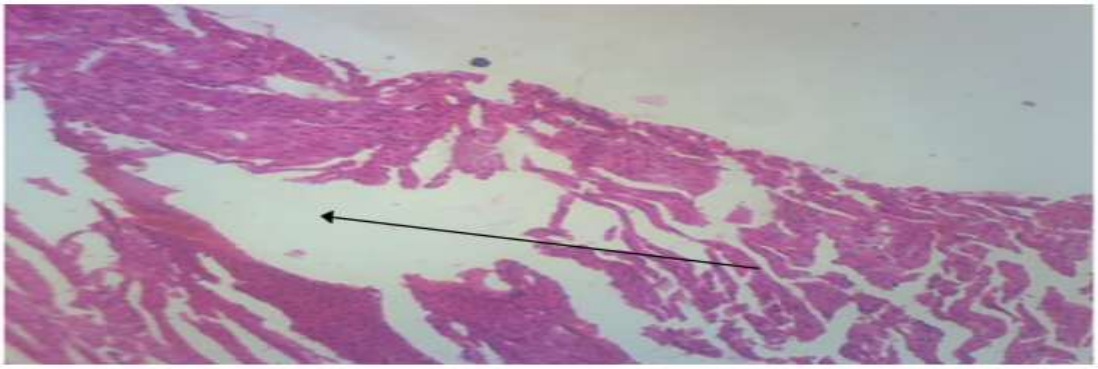


Focal area of intra myocardial bleeding and myocardial infarction X60 BHH

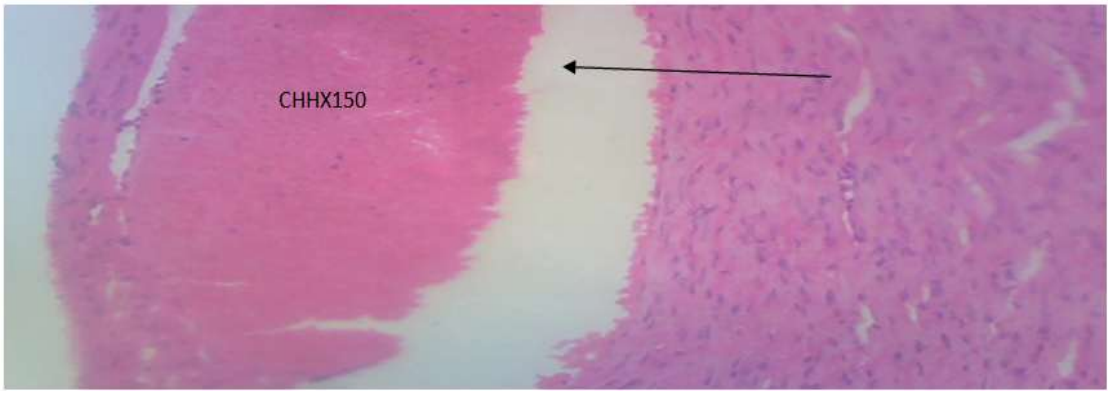
Plate 12: Heart: Uhpko. Photomicograph of Blh. Bmh, and Bhh of Heart Section(x60)h/e Section Showing Effects Demonstrated Above When Compared with Plate 11 the Control.



Focal Myocardial Heamorrhage.X60 CLH.

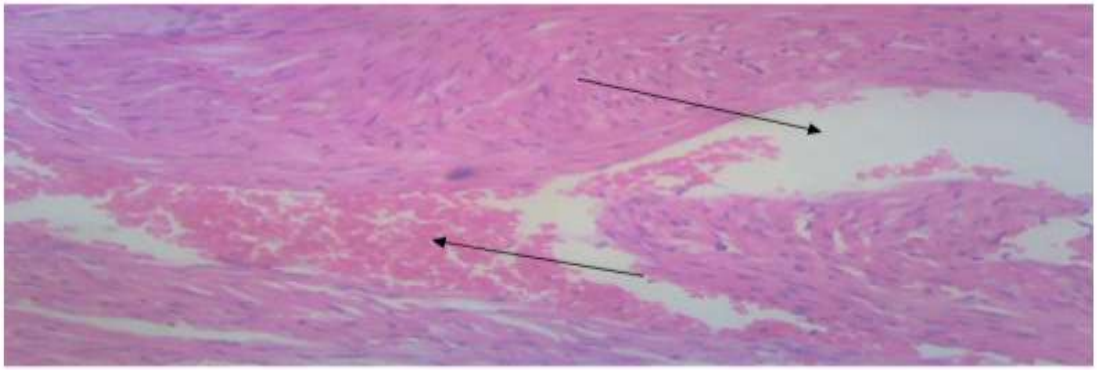


Focal area of tissue loss x150 CMH

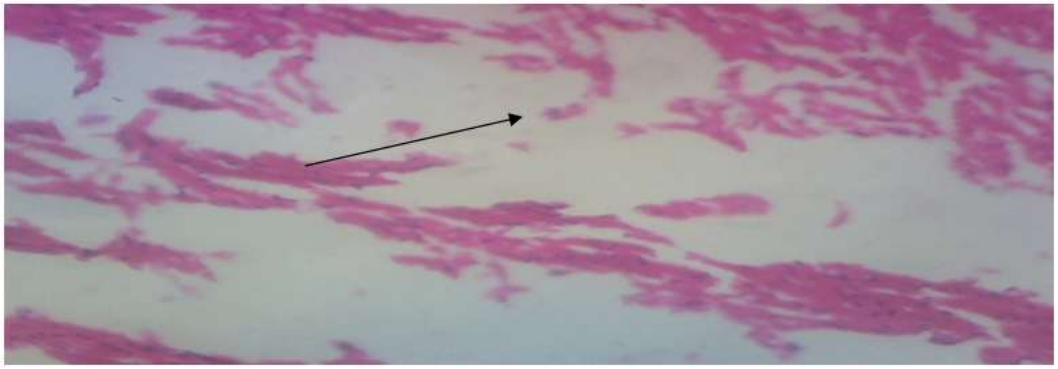


Loss of Cardiac Tissues X CHH

Plate 13: Heart: Hpko. Photomicoraph of Clh, Cmk, and Chh of Kidney Section (x150) H/E Setion S Showing Effects Demonstrated Above When Compared with Plate 11 the Control.



Loss of cardiac tissues and Mild to moderate intra myocardial heamorrhag X60 DLH



Infarction with Loss Myocardial Tissue X60 DMH.

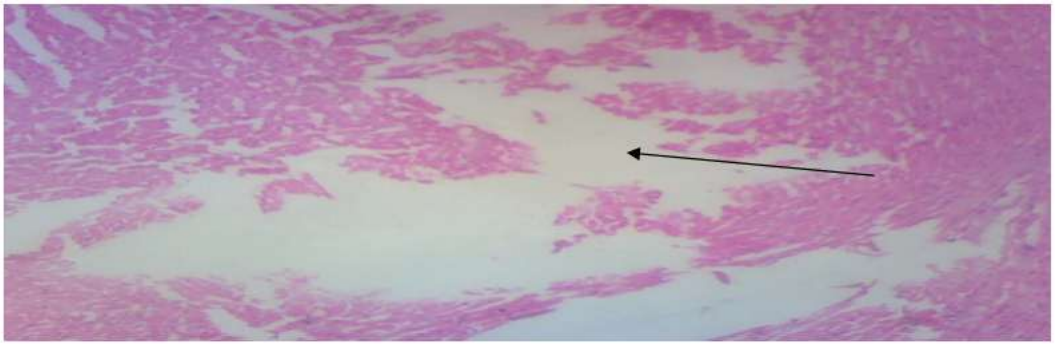
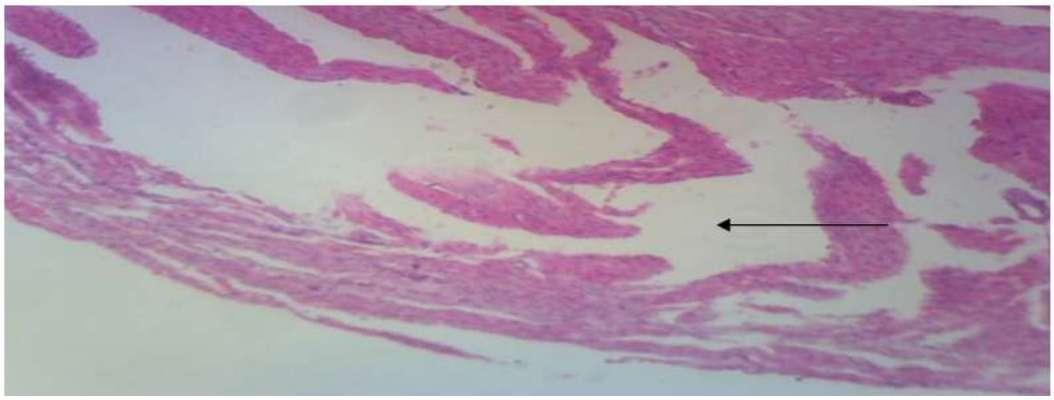
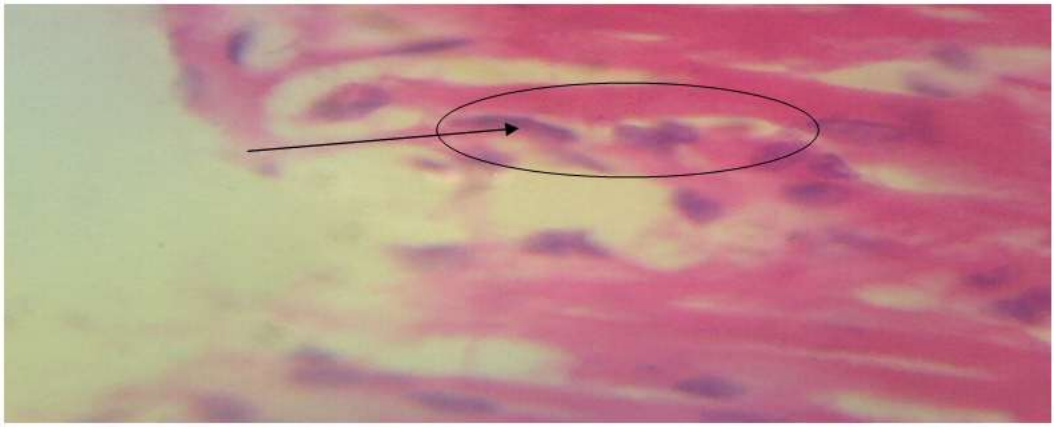


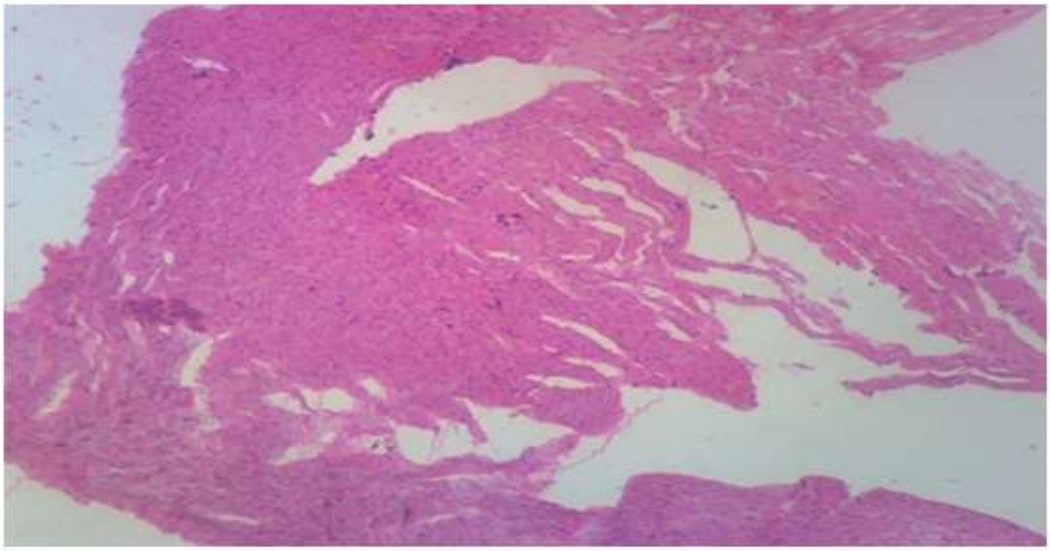
Plate 14: Heart: Uhso. Photomicograph of Elh, Emh, and Ehh of Eart Section(X60) H/E Section Showing Effects Demonstrated Above When Compared With Plate 11 The Control.



Loss of myocardial tissues X60 ELH.



Moderate inflammation (myocarditis) X 600 EMH



Disorganization of cardiac architecture (DCA) with reduction in muscular bulk X60 EHH

Plate 15: Heart: Hso. Photomicograph of Elh, Emh and Ehh of the Heart Section at Different Magnifications. H/E Secton Showing Effects Demonstrated Above When Compared with Plate 11 The Cont Cccontrol Ccccontrol.

DISCUSSION

Recently, to prevent atherosclerosis in human, saturated animal fats were replaced by vegetable oils due to their high content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). However, during deep-heating, cooking oils are repeatedly used at elevated temperatures in the presence of atmospheric oxygen leading to oxidative degradation of these oils. Some of these degradation products can have adverse effects on human health. In this work, organ toxicity study of both unheated and heated palm kernel and Soya oil were evaluated in the albino Wistar rats. Was the histology of the liver, kidney and the heart. Results from the histopathology study of the liver, showed that all the test samples caused hepatic inflammation. This agrees with earlier finding which demonstrated that soya rich diets caused hepatic inflammation. UHPKO, HPKO, and HSO caused distortion in hepatic architecture and UHPKO, UHSO, and HSO caused cytoplamic ground glass appearance. However, the HPKO caused intrahepatic heamorrhage while UHSO caused formation of giant cells. All the test samples caused renal tissue loss, and intrarenal haemorrhage. UHPKO, HPKO and UHSO caused renal fatty changes and distortion in renal architectures. HSO caused renal tissue necrosis and tubular clumping. On the heart, all the test samples caused loss of myocardial tissues. The UHPKO and HPKO caused intramyocardial hemorrhage, and the HSO caused inflammation (myocarditis), disorganization of cardiac architecture and reduction in muscle bulk.

CONCLUSION

The histological evidence of inflammation by all the test samples on all the organs studied is an indication that these products may be toxic to the body organs. Thus consumption of these products especially when heated, may not be completely safe.

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Disclosure of conflict of interest

The authors declare that they have no conflict of interests**.**

Ethical approval

The study received the approval of the ethics committee of the University of Nigeria

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