Journal of Agriculture and Environmental Sciences
September 2014, Vol. 3, No. 3, pp. 91-104
ISSN: 2334-2404 (Print), 2334-2412 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jaes.v3n3a8
URL: http://dx.doi.org/10.15640.v3n3a8

Effect of Feeding Dumpsite Forage Calapo (Calopogonium mucunoides) on The Histology of the Kidney and Liver of Rabbits (Oryctolagus Cuniculus)

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Abstract

Effect of feeding dumpsite forage (Calapo -*Calopogonium mucunoides*) on the histology of the kidney and liver of rabbits (*Oryctolagus cuniculus*) was investigated. 24 rabbits; 20 females and 4 males were obtained and distributed randomly into two experimental groups of 10 females and 5 males with each of the groups being fed dumpsite forage and non-dumpsite forage respectively. The forage, specifically Calapo (*Calopogonium mucunoides)* was fed to the two groups' *ad-libitum* with the non-dumpsite fed group serving as the control. After a period of 20 weeks, the rabbits were sacrificed using chloroform soaked with cotton wool placed in the desiccators where the rabbit were place under anaesthesia. Kidney and Liver were harvested and fixed immediately in neutral buffered formalin, they were transported to the laboratory for histopathological analysis. The results revealed moderate areas of inflammation and hyperplasia in the liver of the dumpsite treated animals as compared to the non-dumpsite group which revealed normal cellular pattern with prominent hepatocytes, portal triad and sinusoidal lining with no abnormality being observed. For the kidney results, cellular abnormality with glomerular inflammation, tubular degeneration, degenerated epithelial lining and areas of inflammation were observed in the dumpsite treated groups as compared to the non-dumpsite which revealed normal cellular pattern for both the kidney and liver tissues. In conclusion, feeding of dumpsite forages to rabbits could pose hematoxic and cyto-architectural de-arrangement and pathological alterations with traces of cellular abnormality to the liver and kidney of rabbits, thus posing health risk in animal and human populations exposed to chemical substances from waste dumpsites.

Keywords: Heavy metals, Calapo (*Calopogonium mucunoides),* Rabbits (*Oryctolagus cuniculus*), Histopathology, Kidney, Liver

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1.0 Introduction

In recent times, there has been considerable interest in the level of heavy metallic elements in foods because of their deleterious effects on human health. Apart from those communities exposed to high levels of pollution by industrial effluent or emissions rich in heavy metals, it is evident that, for most individuals, food and diet are the most common sources of these potentially toxic elements. These elements in food or drinking water amount to approximately 80% for cadmium, 40% for lead and 8% for mercury (Bennet, 1984). Metal contamination in foods, especially in meat have been broadly investigated (Sharif *et al.,* 2005). Heavy metal contamination can be transferred to animals through direct exposure, polluted water, crops grown on irrigated sewage, industrial effluents, vehicle emission and dirty slaughter houses (Joseph and Srivastha, 1993; Jukna *et al*., 2006).

The proliferation of open and unsafe dumpsites containing multiple disposals of domestic, municipal, industrial and medical wastes is common practice in most cities in Nigeria. Dumpsite is an old traditional method of waste disposal similar to landfill method of waste management. Dumpsites are often established in disused quarries, mining or excavated pits away from residential areas. However, studies have shown that all forms of waste disposal have negative consequences on the environment, public health, and local economies (Abdus-Salam and Adekola, 2009). These dumpsites contain build up of heavy metals in soils from anthropogenic sources build-up which have been reported to be harmful to crops, animals and human health, their concentrations and transformations to heavy metals in solid municipal waste leads to accumulation in the food web. These dumpsites serve as feeding grounds for disease breeding animals especially rats, birds and stray animals, thereby contributing greatly to their nourishment and growth (Adewuyi and Opasina, 2010). Leachate from dumpsites are of particular interest as they contain potentially toxic heavy metals. These metals are known to bioaccumulate in soil and have long persistence time through plants or animals (Miranda *et al.*, 2005). The risk associated with the exposure to heavy metals present in food product had aroused widespread concern in human health.

Since contamination with heavy metals is a serious threat because of their toxicity, bioaccumulation and biomagnifications in the food chain (Demirezen and Uruc, 2006), it becomes necessary to study the concentrations of toxic heavy metals in rabbits fed from dumpsites in order to assess the levels of exposure of the consumers to toxic metals and henceforth, maintain an ongoing knowledge on the levels of these metals both in the environment and in meat.

Meat is a very rich and convenient source of nutrients including microelements. The reported cases of heavy metal contamination in meat and other animal products is of great concern for both food safety and human health because of the toxic nature of these metals at relatively minute concentrations. Hence, given the prevalence of these pollutants in the environment, there is a clear need for the sources and effects of their contamination to be known, with the aim of reducing both direct effects on animal health and indirect effects on human health (SCAN, 2003) **T**he needed improvement in animal production and performance may be achieved by investigating the effects of some of these heavy metals on the physiological responses in rabbits.

Ingestion of these contaminants by animals causes deposition of residues in meat especially for animals managed extensively or for most nomadic cattle rearers who make use of these dumpsites as feeding sites. As such higher levels of metals have been found in beef and mutton due to the grazing of cattle on contaminated soils (Baykov *et al*., 1996). Umesiobi *et al*. (2000) reported that parameters for assessing the meat quality and physiological performance of these animal based on their feed intake are the histopathological parameters.

The domestic rabbits are primarily herbivorous and consume most types of grains, forages and hay. Diets, whether home grown or commercially prepared consist of ingredients from plant sources. Since rabbits can utilize a certain amount of forage, they have a place in food production by making use of some non competitive feeds (Herbert *et al*., 2005). Forage can contribute up to 50% of rabbit diets (Sanni *et al*., 2005), although there is improvement in the performance of rabbit fed concentrate and forage compared to feeding forage or pellets alone (Taiwo *et al*., 2004). For maximum performance, combination of *Centrosema pubescens* and concentrates in 50:50 ratios is the most efficient and should be used by rabbit farmers to increase production at a reduced cost (Nworgu *et al*., 1998).

Calopo (*Calopogonium mucunoides* Desv.) is a vigorous, hairy annual or short–lived perennial trailing legume. It can reach several meters in length and form a dense, tangled mass of foliage, 30-50 cm deep. The root system is dense and shallow, at most 50 cm deep. The stems are succulent, covered with long, brown hairs. They are creeping in the lower parts, sometimes rooting at the nodes that come in contact with the soil. The upper part of the stem is twining.

The leaves are up to 16 cm long and trifoliate. The hairy leaflets are 4-10 cm long x 2-5 cm broad, ovate to elliptical. The inflorescence is a slender hairy raceme that may be up to 20 cm long and bears 2 to 12 blue or purple small flowers. The fruits are 3-8 seeded hairy pods, 2-4 cm long (Chin Chen Peng *et al.,* 1997).

Calopo is mainly used as cover crop, alone or in mixture with other legumes, especially in rubber, oil palm or in young forest plantations. It serves as grazing legume, green manure, pioneer legume and cover crop. Calopo is used for green manure although its value for this use still needs confirmation. Calopo is a pioneer species: it provides soil protection against erosion, reduces soil temperature, improves soil fertility and controls weeds (Cook *et al.*, 2005). Although not widely used, Calopo is the most popular legume amongst Brazilian farmers and is the legume seed produced in greatest volume in Brazil (Pizarro, 2001).

The liver is a roughly triangular organ that extends across the entire abdominal cavity just inferior to the diaphragm. It is a reddish brown organ with lobes of unequal sizes and shapes. The liver is made of very soft, pinkish-brown tissues encapsulated by a connective tissue capsule. This capsule is further covered and reinforced by the peritoneum of the abdominal cavity, which protects the liver and holds it in place within the abdomen.

Two major types of cells populate the liver lobules, parenchyma and non-parenchyma cells.80% of the liver volume is occupied by the parenchyma commonly referred to as hepatocyte. Non-parenchyma cells constitute 40% of the total numbers of liver cells but only 6.5% of its volume. Sinusoidal endothelial cells (kupffer cell) and hepatic stellate cells are some of the non-parenchyma cells that line the hepatic sinusoid (Kenneth, 1998).

The blood supply of the liver is unique among all organs of the body due to the hepatic portal vein system. The hepatic portal vein then delivers this blood to the tissues of the liver where the contents of the blood are divided up into smaller vessels and processed before being passed on to the rest of the body. Blood leaving the tissues of the liver collects into the hepatic veins that lead to the vena cava and return to the heart. The liver also has its own system of arteries and arterioles that provide oxygenated blood to its tissues just like any other organ.

Histologically, hepatocytes are the epithelial cells grouped in interconnected plates which are arranged in polyhedral hepatic lobules which are the classic structural and functional units of the liver. Each lobule has three to six portal areas at its periphery and a venule called a central vein. The portal zones at the corner of the lobules consist of connective tissue embedded in venule, arteriole and a duct of cuboidal epithelium.

Hepatocytes make up each of the interconnected plates like the bricks of a wall and the plate are arranged radially around the central vein from the periphery of the lobule to its center; the plates of hepatocyte branch and anastomoses freely forming a rather spongy-like structure. The space between these plates contains important microvascular components, the liver sinusoids. The endothelial cells are separated from the underlying hepatocyte by a thin, discontinous basal lamina and a very narrow perisinusoidal space into which project microvilli of the pre-hepatocyte for exchange between these cells and the plasma. This is the key to liver function not only because of the large number of macromolecules secreted into the blood by hepatocytes but also because the liver takes up and catabolizes many of these large molecules. The sinusoids are supported by reticular fibre cells and in the sinusoids are kupffer (Stellate macrophages) cells and fat storing cells (Ito cells) (Mescher, 2010).

The cytoplasm of liver cells contains numerous mitochondria, abundant rough and smooth endoplasmic reticulum, as well as developed ecologic complex, lysosomes, numbering free ribosome and vacuoles containing various enzymes (Singh, 2009). Other functions of the liver include digestion, metabolism, detoxification, storage, production, and immunity.

The kidney has a bean-shaped structure. Each kidney has a convex and concave surface. The concave surface, the renal hilum, is the point at which the renal artery enters the organ, and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perinephric fat, renal fascia and paranephric fat. The anterior (front) border of these tissues is the peritoneum, while the posterior (rear) border is the transversalis fascia.

The nephron is the functional unit of the kidney and each consists of one renal corpuscle and its associated tubule.

The essential tissue composition of kidney is that of a gland with highly modified secretory units and highly specialized ducts. The secretory units of the kidney, called renal corpuscles, comprises of a relatively small portion of the kidney. The bulk of the kidney consists of highly specialized tubules, which correspond to a typical gland. Together, the renal corpuscle and its associated tubule is called a nephron. In the kidney, each corpuscle is a highly modified secretory acinus as it secretes a filtrate of blood plasma while each tubule functions as exaggerated striated ducts. Renal tubules have wiggly portions called convulated tubules, straighted segments called loop of henle, and collecting ducts. A cross section of the kidney reveals the cortex (which consists of convulated tubules), the renal corpuscles, the medulla, (consisting of the loops of henle) and collecting ducts.

The kidney accomplishes various homeostatic functions both independently and in concert with other organs such as excretion of wastes, e-absorption of vital nutrients, acid-base homeostasis, osmolality regulation, blood pressure regulation and hormonal secretion.

This study was carried out to investigate the effect of these heavy metals on liver and kidney histology of rabbits fed forages from dumpsites with the view to elucidate the risk of contamination to the environment, the living organisms and the secondary consumers being humans, especially of animals that obtain their feeds from these sites.

2.0 Materials and Methods

2.1 Drugs and Chemicals

Sodium chloride, formaldehyde, sodium trioxocarbonate V, sodium bicarbornate, xylene, paraffin wax, 70% alcohol, 90% alcohol, absolute alcohol, distilled water, hutches, concentrate feed, latex hand glove, weighing scale, graduated vials, measuring tape. All chemicals were procured from BDH Chemicals, England and were of analytical grade.

2.2 Experimental Animals and Management

The animals were sourced from the University of Uyo Teaching and Research Farm, Use-Offot, Akwa Ibom, Nigeria.

A 2- week experimental period was used to get the animals (rabbits) acclimatized with the experimental procedures. The experiment lasted 20 weeks (June, 2013 to November, 2013). The animals used in this study were 24 crossbred rabbits (4 bucks and 20 does) aged 6-7 months. The males weighed between 1350 g and 1650 g, while the females weighed between 1400 g and 1800 g.

Four bucks and twenty does respectively were divided into two groups of 12 animals each. When placing the animals into groups care was taken in order to balance the groups such that there were no significant differences between them on the basis of age and weight and the animals were identified individually with the aid of a permanent marker on their ears**.** The groups were randomly assigned to the two (2) treatment diets: dumpsite feed and non-dumpsite feeds.

The experimental animals were housed in a wooden hutch with a wire mesh floor and in-built waste trays. The management techniques employed for all the experimental animals included regular cleaning of the hutch, feeding and watering of the experimental animals on a daily basis. The experimental animals were managed well.

Drinkers and feeders were made of plastics and concrete with narrow but blunt opening to discourage fed wastage and injuries. Forage (experimental diets) and clean water were also supplied *ad libitum.* Permission and approval for animal studies were obtained from Animal Ethnics Committee, College of Health Sciences, University of Uyo.

2.3 Experimental Animal Health

The rabbits acquired were treated against internal and external parasites by subcutaneous injection of ivomec (0.2 ml per rabbit) and a broad spectrum antibiotic (Oxytetracyclin L. A.) was also administered at the rate of 0.2 ml per rabbit. Sulphur powder was given for the occurrences of mange and neomycin was given for diarrhea at the rate of 10 g per four (4) liters of drinking water.

2.4 Experimental Designing and Feeding of Experimental Diets

Two treatments being the waste dumpsite fed and the non-dumpsite fed. Forages were obtained from two sites, one being the waste dumpsite within Uyo metropolis and the other being a land, which is the non- waste dumpsite. Forage chosen was *Calapogonium mucunoides* due to its palatability to the rabbits. The forages were supplied daily to the animals and fed *ad-libitum*. Alongside with the forage, the concentrate of pelleted poultry grower's mash meal (20% CP and 2700Kcal/kg) were fed routinely to facilitate the growth of the animals.

Table 1: Showing the Experimental Designing of the Study

Group	Female	Male	Treatment (site)	Duration
1(NDS)	10	2	Non-dumpsite forage	20 weeks
2(DS)	10	2	Dumpsite forage	20 weeks

2.5 Sample Collection for Histopathological Analysis

At the end of the stipulated 20 weeks feeds were withdrawn, the rats were subjected to a 12 hours fast but had access to water, sacrificed using chloroform vapour.

Liver and Kidney were harvested from all rabbits respectively, harvested organs were carefully dissected, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were immediately fix in Neutral Buffered Saline and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the kidney, liver and pancreas. The sections were designated "vertical sections". Serial sections of 5 µm in thickness were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides and later stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed Histopathologically under digital light microscope.

2.6 Photomicrography

Records of the histological and histochemical results were obtained by photomicrography using digital photomicrographic microscope at the Gross Anatomy Research Laboratory, Department of Human Anatomy, College of Health Sciences, University of Uyo, Uyo, Akwa- Ibom, Nigeria as illustrated in Plate.1 to 4.

4.0 Results

4.1 Liver Histopathology of Rabbits Fed Dumpsite and Non – Dumpsite Forage

Non - Dumpsite – (NDS) - Plate A(X100) and B(X400) of Liver tissue from the NDS revealed normal cellular pattern with prominent hepatocytes, portal triad and sinusoidal lining, no abnormality seen.

Dumpsite (DS) - Plate C(X100) and D(X400) of Liver tissue from the DS revealed moderate area of inflammation hyperplasia

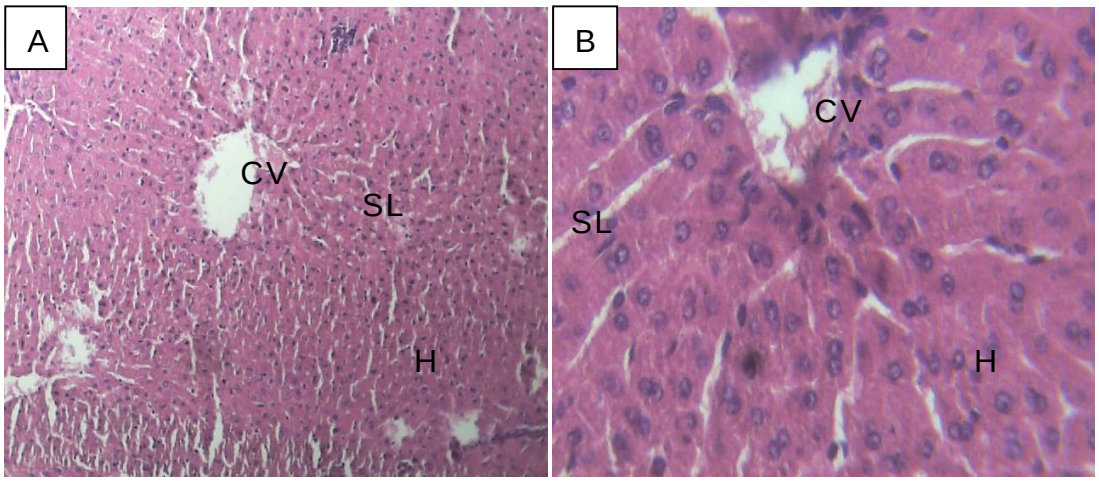

Plate 1 : *Photomicrographs of the Liver tissue from Non- dumped sites at Magnification A (**X100**) and B (**X400**) stained with H and E technique*

Keys: H *–Hepatocytes,* ***VC*** *–Vascular congestion,* ***RBC*** *– Red Blood cells, VC-Vascular congestion.*

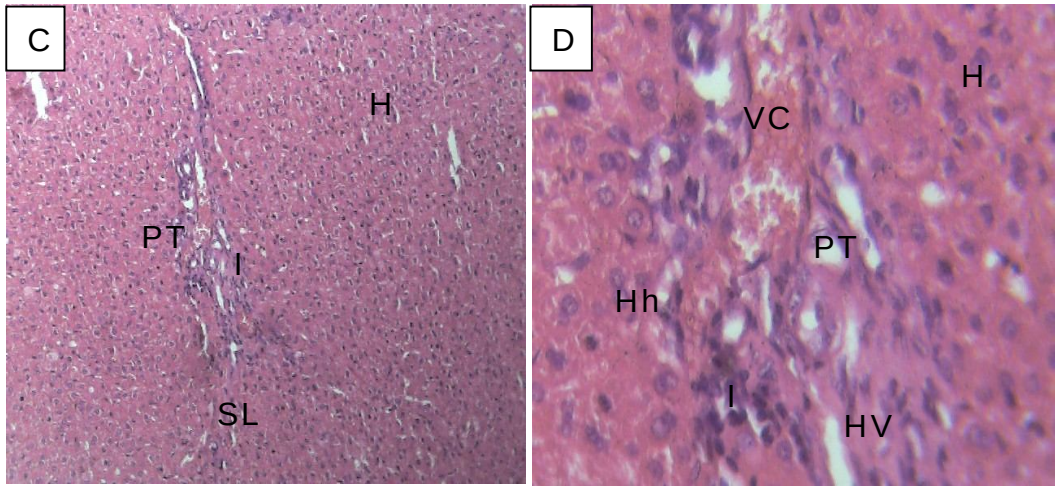

Plate 2: *Photomicrographs of the Liver tissue from Dumped sites at Magnification C (**X100**) and D (**X400**) stained with H and E technique*

Keys: H *–Hepatocytes,* ***VC*** *–Vascular congestion,* ***RBC*** *– Red Blood cells, VC-Vascular congestion,* ***SL*** *– Sinusoidal lining,* ***HA*** *–Hepatic artery,* ***CV*** *– Central vein.****PT****- Portal triad,* ***HV****-Hepatic vein,* ***Hh****- hepatic hyperplasia,* ***I****- Inflamation,****VC****- vascular congestion.*

4.2 Kidney Histopathology of Rabbits Fed Dumpsite and Non – Dumpsite Forage

Non - Dumpsite – (NDS) - Plate E(X100) and F(X400) of Kidney tissue from the NDS revealed normal cellular pattern with area of distal and proximal convoluted tubules, renal corpuscle.

Dumpsite (DS) - Plate G(X100) and H(X400) of Kidney tissue from the DS revealed cellular abnormality with glomerular inflammation, tubular degeneration, degenerated epithelial lining and area of inflammation.

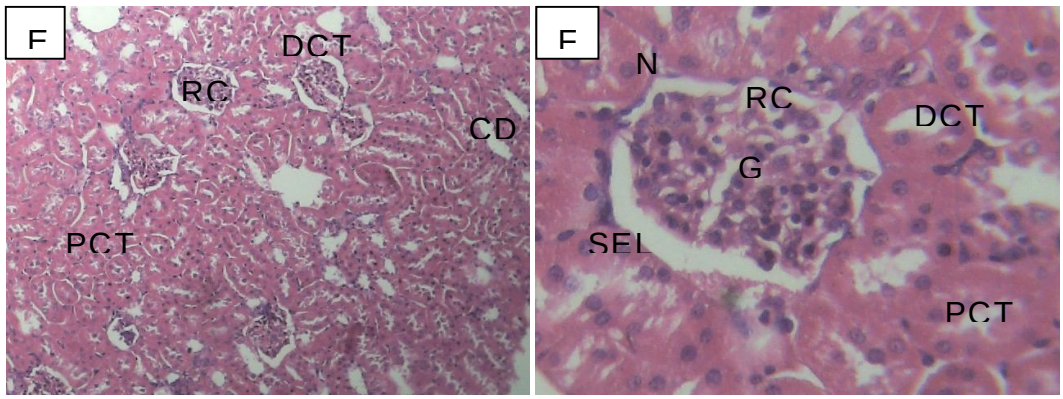

Plate 3: *Photomicrographs of the Kidney tissue from Non - Dumped sites at Magnification E(**X100**) and F (**X400**) stained with H and E technique*

Keys: RC *–Renal corpuscle,* ***GI-*** *Glomerular inflammation* ***PCT****- proximal convoluted tubules,* ***DCT****- Distal convulated tubulesmg,* ***G****- glomerulus,* ***SEL*** *–Squamous epithelial lining.*

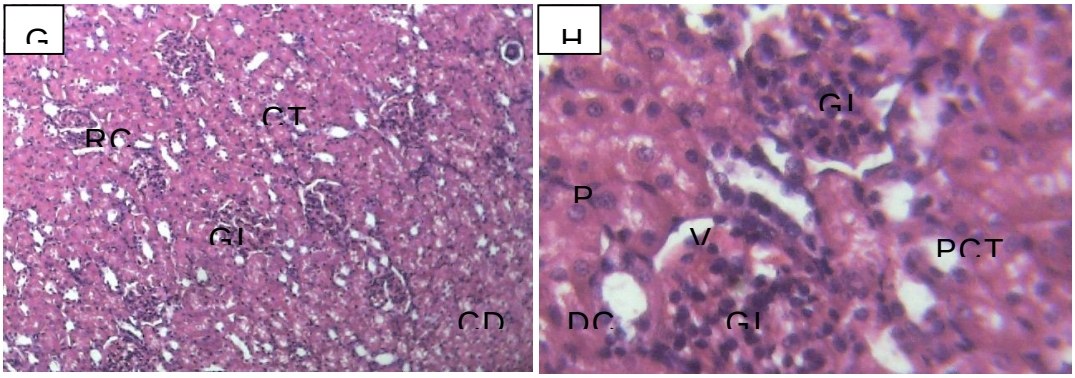

Plate4: *Photomicrographs of the Kidney tissue from Dumpsites at Magnification G (**X100**) and H(**X400**) stained with H and E technique*
Keys: Pn *–pyknotic nucleus,* ***RC*** *–Renal corpsule,* ***GI-*** *Glomerular inflammation* ***VD****-Vascular degeneration,* ***SL*** *– Sinusoidal lining,* ***CD*** *–collecting duct ,* ***TD*** *– Tubular degeneration.*

5.0 Discussion

Histopathological examination of tissues is useful in identifying the type of lesions caused by xenobiotics and is acknowledged as the most sensitive end point for detecting organ toxicity (Rio, 2001). From this analysis, Liver histology from the dumpsite group revealed areas of inflammation, cellular abnormalities such as degeneration, vacuolation and hyperplasia at magnifications of X100 and X400 in Plates C and D while the Non- Dumpsite group at X100 and X400 of Plates A and B revealed normal cellular pattern with prominent hepatocytes, portal triad and sinusoidal lining, no abnormality was observed.

In the analysis of the kidney tissues, Plate G(X100) and H(X400) from the Dumpsite group both revealed cellular abnormality with glomerular inflammation, tubular degeneration, degenerated epithelial lining and area of inflammation. For the Non - Dumped site - Plate E(X100) and F(X400) revealed normal cellular pattern with area of distal and proximal convoluted tubules, renal corpuscle.

Hepatic necrosis and cellular infiltrations with inflammatory cells observed in leachate treated rats had been previously reported in workers exposed to chemicals from industrial wastes due to hepatotoxic substances in the generated wastes (Swarup *et al.,* 2005). Proximal tubular cells are the most susceptible to toxic xenobiotic since they are the first renal epithelial cells to be exposed to filtered toxic compounds Hence, degeneration of epithelia of the renal tubules and renal necrotic changes observed in dumpsite treated rabbits indicates damage to the kidney.

This result agrees with the findings of Kjellstrom *et al*.,1984 whereby cadmium exposure lead to renal tubular dysfunction. This is primarily a re-absorption defect in the proximal tubules and the critical effect of cadmium and hepatic dysfunction (Massányi and Uhrín, 1996). It may be deduced from these findings that forages from dumpsite induced hematotoxicity, hepatotoxicity and nephrotoxicity in the rabbit. This is supported by reports on the activities of heavy metals analysed herein (Cd, Pb, Hg and As) that they exhibit their damaging effects in the liver and kidney.

5.1. Conclusion

From the results of this study, it is hereby suggested that heavy metals is an environmental stressor which causes depression of total white cell and red cell count, thus, having serious consequences on hematological parameters and also causes cyto-architectural abnormality in gross macroscopy and microscopic histology of the liver and kidney of rabbits. Hepatotoxicity and Nephrotoxicity of liver and kidney respectively are the likely implications in a situation where there are chronic consumptions of these forages due to the presence of heavy metal accumulations from dumpsites as the investigation showed toxicity in liver and kidney. Considering the role of liver in detoxification and kidney as excretory organ, the damages induced by the forage pose a serious deleterious effect on animal as well as human within food chain. Designation and provision of the health programs to limit causal exposure to these toxic elements is highly important for human health and in animal production.

Conflict Interests

The authors declared that they have no competing interests.

Authors' Contributions

All the Authors contributed equally

Acknowledgements

This research was not supported by any organization or funding body, however the authors contributed collectively to carry out the experiment.

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