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HISTOLOGICAL EFFECTS OF RICIN POISON FROM CASTOR OIL SEEDS ON THE KIDNEY OF ALBINO RATS

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ABSTRACT

The Dichloromethane extract derived from castor oil seed (*Ricinus communis L. Euphorbiaceae*) was used in a Laboratory experiment for its toxicity. The study was undertaken to evaluate the rodenticidal potentials of ricin poison on histology of experimental rat's kidney. Thirty experimental rats (Albino) were divided into 3 replicates and each replicate had 5 cages. Each cage had 2 rats of both sexes. The age of the rats used for this experiment was 9 to10 weeks. The rats were fed ad libitum and fresh water was given once daily from a suspended water feeder. 1000g of castor oil seed were crushed into coarse meal using pestle and mortar prior to extraction. The coarse meal of castor seed were then soaked into 2000ml of Dichloromethane for 24 hour before it was

Introduction:

The castor oil plant (*Ricinus communis L.*) is a member of the Spurge family of plants (Euphorbiaceae). This beautiful plant is sometimes used to decorate gardens. It is an oil seed crop cultivated mainly in India, Mozambique, Brazil, and China (FAOSTAT 2014). It is grown commercially for the oil contained in the seed, which is used primarily for industrial purposes and in the manufacture of pharmaceuticals, cosmetics and as a laxative (Cosmetic Ingredient Review Expert Panel, 2007), in the textile and leather industries, and for manufacturing plastics, fibres soaps, printing inks,

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later sieved with muslin chloth, the Dichloromethane extract derived from castor oil seed was air-dry completely, 10ml, 20ml, 30ml and 40ml Dichloromethane extract derived from castor oil seed were weighed, each was mixed thoroughly with 50 g of commercial rat feed, fifth one is Commercial rat feed only which was used as the control. At the time of sacrifice, the kidney were removed immediately after dissected and fixed or preserved in a 10% neutral buffered formalin for preservation before it was later sent to histopathological studies at the Histopathology Laboratory, University of Ilorin Teaching Hospital Ilorin, Kwara state. The result of Photomicrographs of the tissue taken were provided. Data were collected on fresh Kidney Weight organ of treated rat against fresh Kidney weight organ of untreated (control) rat which were analyzed using analysis of variance (ANOVA) and means were separated with Duncan Multiple Range Test (DMRT) at 5% level of probability using Statistical Analysis Software (SAS). The result show no significant difference in the fresh kidney weight of all the rat treated with 10ml, 20ml, 30ml and the control group (Rats fed with the normal feed). There was significant difference in fresh weight of rat kidney fed with the highest concentration of 40ml extract of castor oil seed when compare with the rat treated with 10ml, 20ml, 30ml extract of castor oil seed and the control group. The result of all test kidney showed histological features of lethal tissue damage in all the rat treated with ricin toxin examined. The control group showed normal tissue. The results indicated that highest concentration of 40ml extract of castor oil seed has major effect on fresh kidney weight. Also, histological features of a very low dose of ricin poison can altered cellular change.

Key words: Dichloromethane extract, ricin poison, Castor oil seed, Albino rat, Histology of kidney

Wetting agents, and lubricants (Weiss, 2000). It can also be used as a purgative (Bradberry et al., 2003; Johnson et al., 2005; Musshoff, F and Madea, B. 2009). The seeds are approximately

17 mm long and 8 mm broad. Present-day cultivars have seeds weighing approximately 3 g. The seed mantle accounts for approximately ¼ of the weight. Most of the world's castor oil is produced in India, China and Brazil, but commercial production also occurs on a smaller scale in many other tropical countries. World production of castor oil increased from 0.4 million tonnes in 1970 to 0.8 million tonnes in 2000 (Weiss, 2000). Most of the global oil extraction occurs in the countries in which it is produced. The EU uses 10,000 tonnes of castor oil annually (Anonymous, 2006), of which approximately 8,000 tonnes of castor oil are produced in the EU

The seeds contain the highest concentration of poison which is ricin. Other poisonous components are tricinin and ricinin (Hartley and Lord 2004). Protein Ricin toxin is the most important component. Ricin is a ribosome inactivating protein that is manufactured in the endosperm. Ricin has both an A and a B chain which are approximately 30-kDa in size that are linked together by a disulfide bond (Olsnes and Pihl 1973). RCA120 is composed of two A-chains and two B-chains linked together by disulfide bonds. These causes cell death by attacking ribosomes, resulting in inhibition of protein synthesis (Bradberry et al., 2003).

Even though RCA120 is very similar in both amino acid sequence and structure to ricin, it is much less toxic (Hartley and Lord 2004).

The kidney of the vertebrate is the main organ which balances the body fluid homeostasis (Ojeda, Icardo and Domezain, 2003) or partially responsible for regulation of the chemical composition of blood and tissue fluids. Body fluid homeostasis is a vital process that encompasses the intertwined mechanisms through which the body maintains an appropriate composition and volume of extracellular fluid (Bourque 2008). It has two major functions. 1. Removal of nitrogenous waste products (urea, uric acid) from the blood. 2. Maintenance of normal fluid (water) and salt balance in blood and in the body as a whole. The kidney is the main target of many toxic agents due to its biochemical and physiological functions which make it susceptible to many xenobiotics and chemicals. The kidney is the major site of conversion of chemical and their route of excretion (Armstrong *et al.,* 1984). Chemical exposure induced renal

dysfunctions which includes cortical necrosis, renal failure, cancer, proteinuria and hematuria (Clarkson, 1991).

MATERIALS AND METHODS

The experiment was carried out in the year 2015 at the Toxicology Laboratory in the Department of Crop and Environmental Protection, Faculty of Agricultural Sciences, Ladoke Akintola University of Technology, Ogbomosho.

Animals and experimental design. The albino rats were obtained from the Virology Department, University College Hospital (U.C.H) Ibadan, Oyo State. They were kept in rat cages and fed with commercial rat feed (Growers mash) (Bova Jay Livestock Feeds, Nigeria Ltd). Thirty (30) experimental rats were divided into 3 replicates and each replicate had 5 cages. Each cage had 2 rats of both sexes. The age of the rats used for this experiment was 9 to10 weeks.

Preparation of the solvent crude extracts of the plants. The castor oil seed used for this experiment were obtained from the Botanical Garden Ladoke Akintola University of Technology, Ogbomoso. The seeds were collected when ripe as the capsule, becomes dry, open and discharge the seeds. The 1000g of castor seed were crushed into coarse meal using pestle and mortar prior to extraction. The coarse meal of castor seed were then soak into 2000ml of Dichloromethane for 24 hour before it was later sieved with muslin cloth, the Dichloromethane extract gotten from castor oil seed was air-dry completely, 10ml, 20ml, 30ml and 40ml Dichloromethane extract gotten from castor oil seed, each was mixed thoroughly with 50 g of commercial rat feed, fifth one is Commercial rat feed only which was used as the control.

The treatments were as follows:

Treatment I: 10 ml Dichloromethane extract derived from castor oil seed plus 50g of Commercial rat feed

Treatment II: 20 ml Dichloromethane extract derived from castor oil seed plus 50g of Commercial rat feed

Treatment III: 30 ml Dichloromethane extract derived from castor oil seed plus 50g of Commercial rat feed

Treatment IV: 40 ml Dichloromethane extract derived from castor oil seed plus 50g of Commercial rat feed
Treatment V: Commercial rats feed only served as control

All the treatments were replicated three (3) times. The experiment lasted for 5 days
The rats were fed ad libitum (feeding without restriction) once daily with a baited powdered castor oil seed plus fried fish to treated rat and commercial feed to control rat, They were given fresh water once daily from a suspended water feeder.

Histopathological Studies

At the time of sacrifice, the kidney were removed immediately after dissected and fixed or preserved in a 10% neutral buffered formalin for preservation before it was later sent to histopathological studies at the Histopathology Laboratory, University of Ilorin Teaching Hospital Ilorin, Kwara state. The stained with hematoxylin and eosin (H&E) following standard procedures. The result of Photomicrographs of the tissue taken were provided. This is done just to determine the mode of action of ricin poison used i.e. if it is the treatments applied or not, responsible for the tissue damage. A histopathological examination was performed to monitor the morphological changes or the study of tissues at a microscopic level and its also termed microanatomy. Histopathology, on the other hand is the study of injured or affected tissues. Tissues examined first should be from animal that are clinically healthy. As a final portion of the exercises, examine tissues from infected animal and try to identify changes associated with infection.

Data Collection:

a. Fresh Kidney Weight organ of treated rat.
b. Fresh Kidney weight organ of untreated (control) rat.

Statistical Analysis

Data collected were analyzed using analysis of variance (ANOVA) and means were separated with Duncan Multiple Range Test (DMRT) at 5% level of probability using Statistical Analysis Software (SAS).

RESULT:

No significant difference was observed in the histology of fresh kidney weight for all the rat treated with 10ml, 20ml, 30ml and the control group i.e. Rats fed with the normal feed (Fig 1). There was a significant difference in the histology of fresh kidney weight fed with the highest concentration of 40ml extract of castor oil seed when compare with the rat treated with 10ml, 20ml, 30ml extract of castor oil seed and the control group (Fig 1).

Histology of kidney in plate 1 show glomerulus surrounded by bowman's capsules (BC). Both the cortical and collecting tubules (CT)appear normal. There is normal mesangial cellularity.Features are in keeping with normal renal histology.

This section shows the necrosis of the glomerulus (G), collecting tubules (CT) as well as the Bowman's capsule (BC).This section of the kidney show necrosis of glomerulus (G), the Bowman's capsule (BC).the distorted histological architecture as evidenced by numerous foci of haemorrhage within the interstitium, There is extensive necrosis of the tubular structues. The glomeruli are of poor outline. Feature agrees with ongoing renal damage in plate 2 and it is in line with Olayioye, A., *et al.* (2014)

Section of plate 3 show enlargement of the cell as well as the glomerulus (G), collecting tubules (CT) and there was mild injury both at the Bowman's capsule (BC) and centre of the glomerulus.

Section of plate shows a great lysis of the cell as well as the enlargement of the collecting tubules (CT), glomerulus (G) and Bowman's capsule (BC).Feature are in keeping with acute tubulo-interstitial necrosis of the kidneys in plate 4.

The section in plate 5 show an enlargement of the cell, lysis occur at the collecting tubules (CT), bowman's capsule and also, there is fusion of

glomerulus (G) as well as the emulsification of the cell.Feature are in keeping with severe necrosis of the kidneys.

DISCUSSION

Plant have been reported as the major source of phytochemicals and biologically active natural agents (koksal *et al,* 2009). The result from this study indicate that the effect of extract from castor oil seed on fresh kidney weight led to degeneration of renal tubules as evident as necrosis occur to the glomerulus and Bowman's capsule, The changes observed in this study, especially on the fresh kidney weight level, confirm that orally effects of higher doses of ricin poison from castor oil seeds exert renal injury (Fig 1). This is in line with the global upsurge in the prevalence of many diseases including organ failure and cancer in recent years has been associated with increase in toxic chemical exposures (Briggs, 2003). Evidences from experimental research indicates that exposure of human and animals to xenobiotics and other chemical substances resulted in serious adverse effects including liver and kidney failure as well as cancer (Klassen, 2013). Ricin is quite stable and extremely toxic to the cells of different organs such as the liver, kidney, lung, pancreas, intestine, and thyroid (Sadani, 1997; Franz, 1997; Greenfield, 2002; DaSilva, 2003). Glomerular lesions and lysis of the collecting tubules and glomerular tuft occur. (Plate 4).

Ingestion of ricin results in gastrointestinal hemorrhage, necrosis of the liver, spleen and kidneys; severe localized muscle pain; regional lymph node necrosis, and moderate involvement of visceral organs. Intravenously administered ricin is found in the spleen followed by kidneys, heart, liver, and thymus (Fodstad, 1976, 1979; Ramsden, 1989; Franz, 1997; Stirpe, 2004; Bismuth, 2004). Dichloromethane seed extract of castor oil seed caused toxicity in renal system by showing distorted histological architecture as evidenced by numerous foci of haemorrhage within the interstitium (Plate 2, 3 and 5),the immune system cannot makes a good role for defending against foreign particles.

In summary, the histology of the kidney, showed an extensive necrosis and haemorrhage. Again, ricin poison extracted from castor oil seeds on histology of experimental rat's kidney can be effectively used as rodenticide instead of synthetic rodenticides and it is clearly showed the histological features of lethal tissue damage in all the rat kidney treated with ricin poison extract from castor oil seeds examined. The control group showed normal tissue.

Conclusion

According to these results, it is suggested that extract from castor oil seed is capable of inducing histopathological changes in the kidney of the exposed rats. Based on our histological observations, we therefore conclude that ricin poison exposure was detrimental to the kidney of albino rats and hence it can be used as rodenticides.

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Glyceryl Ricinoleate SE, Ricinoleic Acid, Potassium Ricinoleate, Sodium Ricinoleate, Zink Ricinoleate, Cetyl Ricinoleate, Ethyl Ricinoleate, Glycol Ricinoleate, Isopropyl Ricinoleate, Methyl Ricinoleate, and Octyldodecyl Ricinoleate. International Journal of Toxicology 26(suppl. 3): 31-77.

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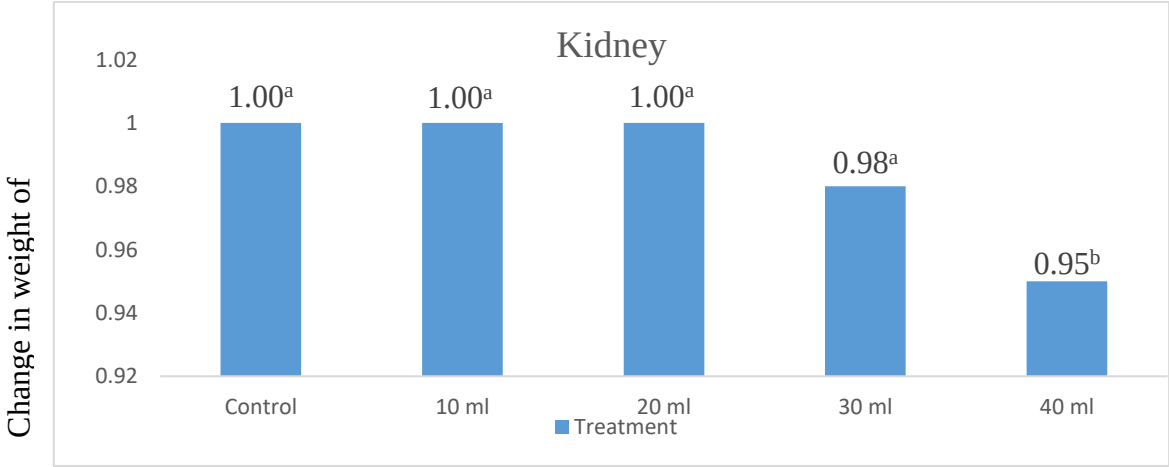

Fig 1: Effect of the ricin poison from castor oil seed on Kidney weights of the experimental rat.

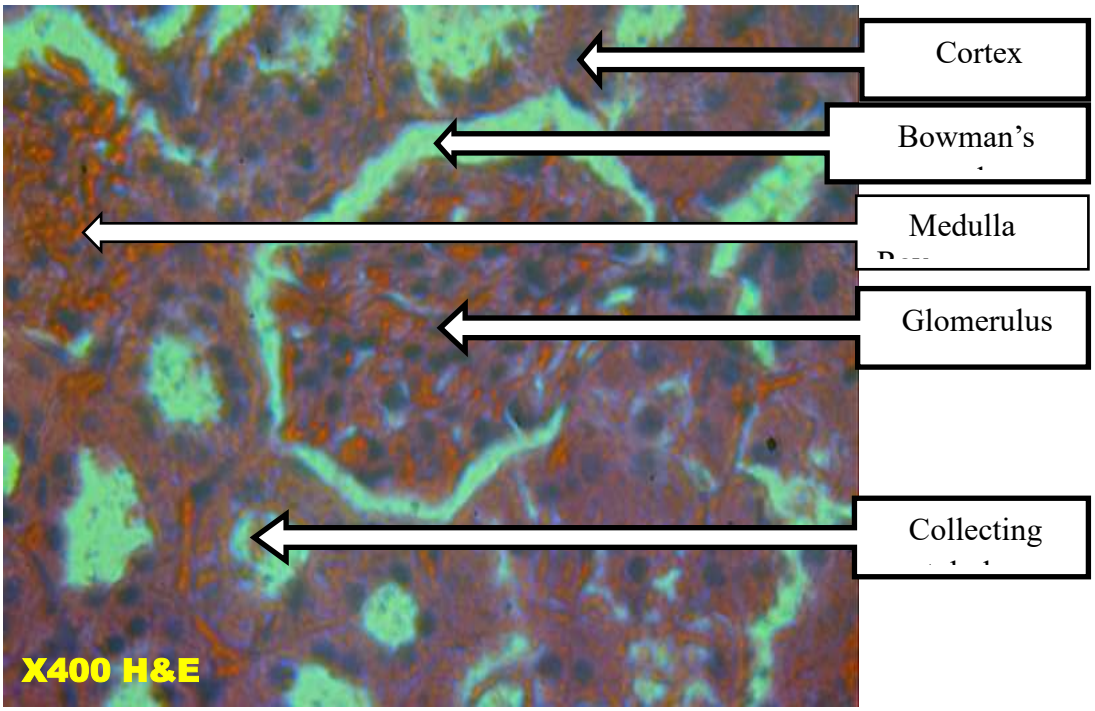

Plate 1: Histology of the normal kidney (control group) of Albino (wistar rats). Mag X400 H & E Stain

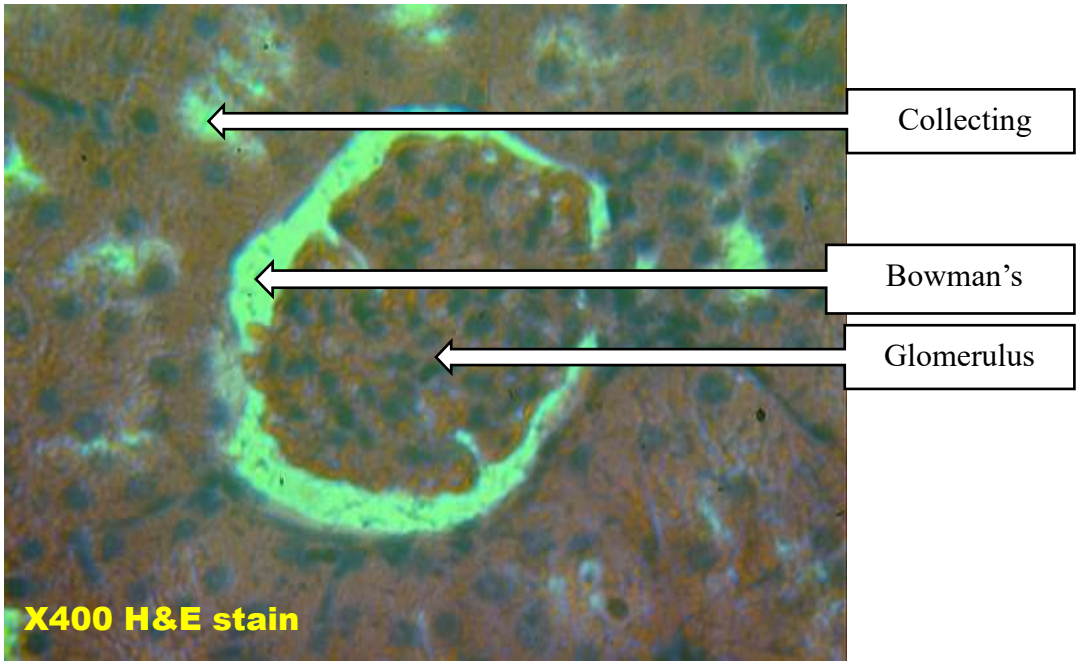

Plate 2: Histology of Albino kidney treated with 10ml extract of castor oil seed. Mag X400 H & E Stain.

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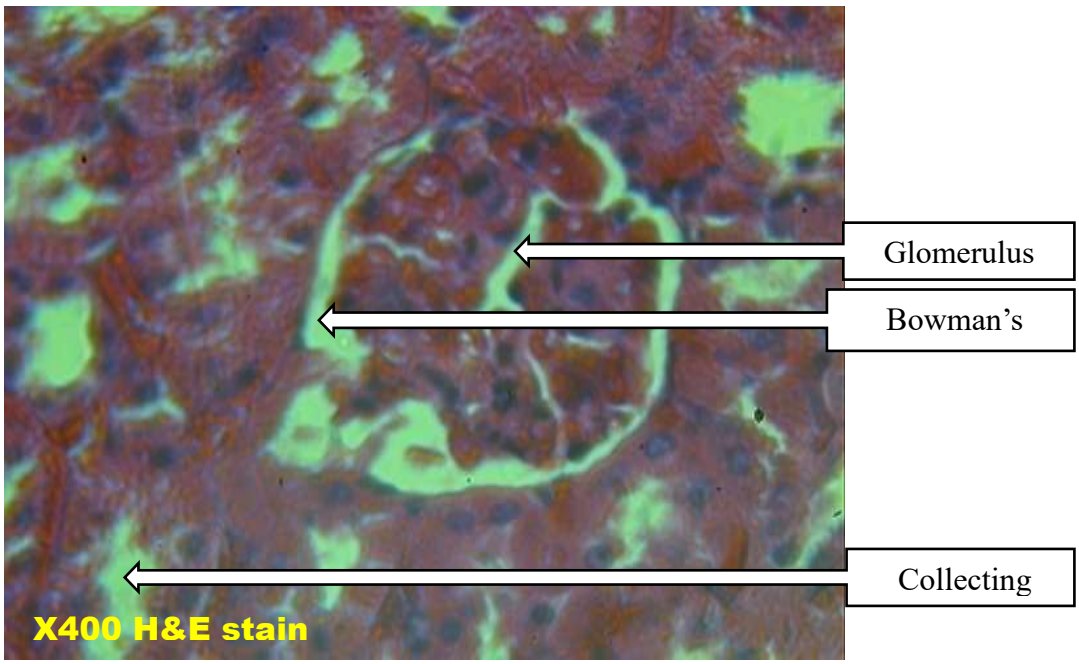

Plate 3: Histology of Albino kidney treated with 20ml extract of castor oil seed Mag X400 H & E Stain.

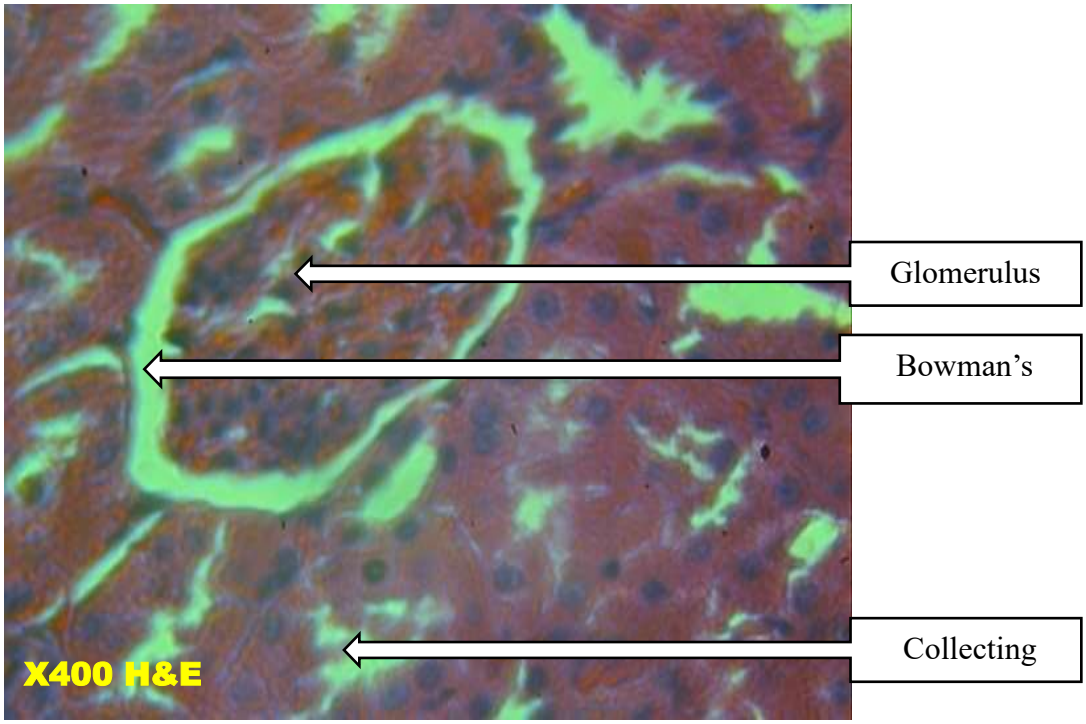

Plate 4: Histology of Albino kidney treated with 30ml extract of castor oil seed Mag X400 H & E Stain.

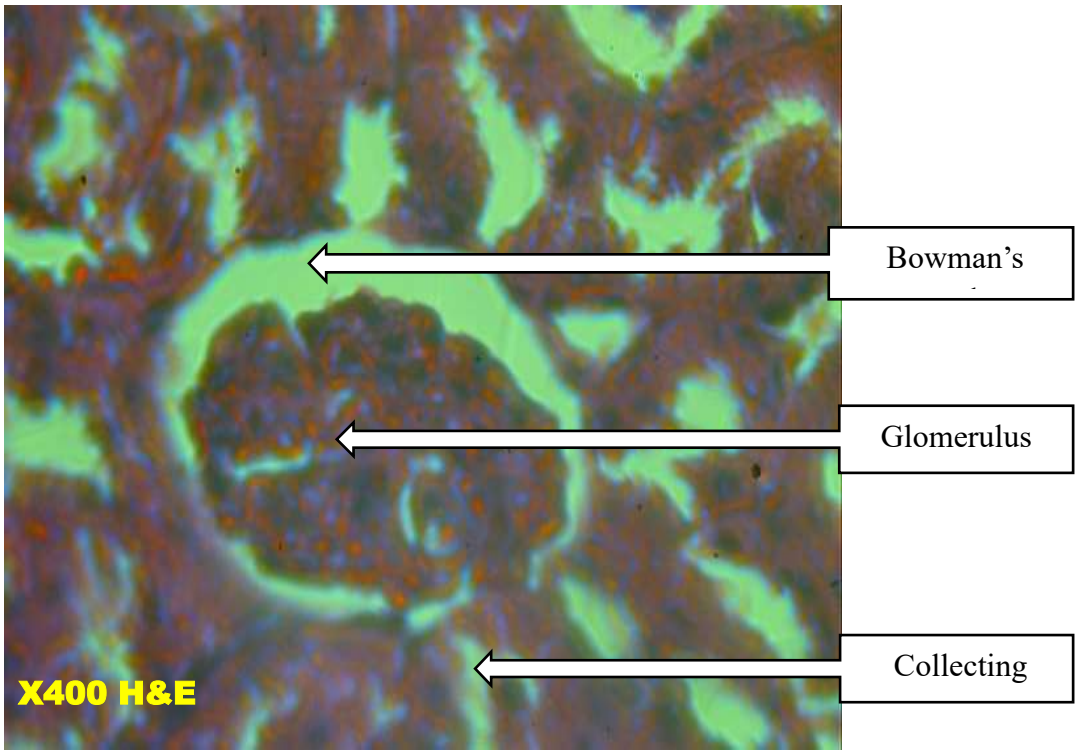

Plate 5: Histology of Albino kidney treated with 40ml extract of castor oil seed Mag X400 H & E Stain.