International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Histological and Ultrastructural Studies on the Kidney of Male Albino Mice after Treatment with Single Dose of LD_{50} Naja Naja Snake Venom

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Abstract: ***The common sign of snake envenomation is Nephrotixicity or renal injury that is dependent on quality and quantity of venom. To clarify the effect of intraperitoneal (i.p.) injection of LD50 dose of Naja naja snake venom on the renal tissues of albino mice after 3 and 24 hr from envenoming respectively. The mice were divided into 3 groups, the first group served as a control group, while the other groups II and III were treated with the snake venom (0.055 µg/g body weight i.p) and sacrificed by decapitation after3 and 24 hours of the snake venom injection respectively. The kidney was isolated and histological and ultrastructural sections were prepared. Intraperitoneal LD50 for Naja naja snake cobra was determined in rats to be equal to 0.055 µg/g body weight. Histopathologicalchanges in kidney tissues after 3 hr from injection were congested of some glomeruli, some glomeruli atrophied and vacuoles in renal tubular. Histopathological changes in kidney tissues after 24 hr from injection were degenerative changes on the renal cortex, hyalinizati of some renal tubule. Besides that observation of the inflammatory cell infilterations. Ultrastructural changes in kidney tissues after 3 hr from injection were condensation of nuclear chromatin endoplasmic reticulum were fragmented in some position, swollen and variable sizes mitochondria. Ultrastructural changes in Kidney tissues after 3 hr from injection were necrotic of some endothelial cells and necrotic of some podocyt. The injection of LD50 dose of Naja naja snake venomin rats caninduce kidney damage and nephrotixicity or renal injury in albino mice.***

Keywords: Naja naja, snake venom, mice, kidney, Histopathological and ultrastructural changes

1. Introduction

Snake envenomation is widely spread in many regions of the world. Snake envenomation involves a series of events that depends on the combined effect of these venom components (1) Envenomation of snake venom causes two main symptoms, one local and the other systemic in humans. The local symptoms are severe pain, swelling, erythema, ecchymosis, bullae, hemorrhage and necrosis and cellulitis (2), while the most serious systemic change are neurotoxic, coagulation, cytotoxic, haemolysis, haemorrhagic activity, hypotension, and necrosis (3,4).The most serious systemic effect and the most common complication in lethal cases is acute renal failure (ARF) secondary to acute tubular necrosis and occasionally glomerulonephritis(5-7). The Naja one of the most genus dreaded snakes of world. Naja naja venom has a lethal potency much higher than venoms of most of other naja species(8).). Naja naja venom contains a mixture of many different proteins, including a variety of enzymes (proteases and phospholipases), non-enzymatic polypeptide toxins (neurotoxins and cardiotoxins), and other substances (9,10). Cobra envenoming is known to induce multiple-organ failure, leading to death in case of severe envenoming (11). There are several reports suggesting nephropathy induced by cobra factor was mentioned by (12- 15), while Abdel Ghani et al (16) added that the cobra venoms caused the histopathological alterations on the renal tissues and different areas of the nephrons including complete necrosis in some glomeruli and in the proximal and distal convoluted tubular cells with the preservation of the basement membranes in envenomed mice. The objective of this study is to determine the ultrastructrual and histological alterations in the kidney of mice following Naja naja envenomation in an attempt to improve our understanding of snake envenomation in mice.

2. Materials and Methods

Preparation of the Naja naja venom
Lyomphilized Naja naja venom was obtained from India (Sigma loeate Ltd).Lyophilized venom was dissolved in phosphate buffered saline(PBS), pH 7.2. The approximate i.p. LD_{50} for Naja naja snake cobra venom was used in mice to be equal to 0.055 µg/g body weight. Morad (17)

Animals and Experimental design
Adult healthy male albino mice weighing 25±2g were taken and used at the animal facility of the High Institute of Infertility Diagnosis and Assisted Reprodetive Technologies, AL-Nahrain University throughout this study. All animals were given free access to standard laboratory chow and tap water. The animals(18mice) were divided randomly into three groups of 6 animals each. Six mice each injected single intraperitoneal(i.p) injection of 0.1 ml phosphate buffered saline and considered as a control group. Groups two and three were injected i.p. 0.055 µg/kg body weight of snake venom in 0.1 ml phosphate buffered saline and was sacrificed by decapitation after 3 and 24 hours of the injection. At the end of the experimental period, the animals from the experimental groups together with the control group were decapitated, and sacrificed and the kidney of the control and treated mice were dissected out for histological and ultrastructural examinations.

For light microscope studies : Small pieces of kidney tissues from each experimental mice were transferred immediately to fixative (Bouin' s solution),paraffin sections (4-5 µm thick) were prepared and then stained with hematoxylin and eosin (H&E) and mathylene blue stain for histological study.

For electron microscopy studies : Small pieces ($1mm^3$) of kidney from each experimental mice were transferred immediately to fresh 2.5% glutraldehyde fixative for 2h and kidney specimens were post-fixed in 1% osmium tetroxide, dehydrated in ascending grades of ethanol and embedded in epoxy resin.

Ultrathin sections were stained with uranyl acetate and lead acetate and to be examined with transmission electron microscope.

3. Results

Light Microscopy

The light microscopy examination of the kidney of the control mice had normal glomerulus, proximal and distal convoluted tubules. (Figure 1-3). Several histopathological changes were observed in the kidneys of the mice treated with the snake venom as compared with those of the control mice. The renal tissues of mice after 3hrs of treated with LD_{50} of Naja naja snake cobra venom(group II) showed congested of some glomeruli, some glomeruli atrophied, vacuoles in renal tubular, moderate inflammatory cellular and some of the renal tubules of the medulla showed nuclear pyknosis(Figure 4-8). The renal tissues of mice after 24hrs of treated with LD_{50} of Naja naja snake cobra venom(group III) showed degenerative changes on the renal cortex. Some glomeruli appeared atrophied and another moderately congested with more expanded Bowman's capsule and patches of hemorrhage, necrosis and hyalinization of some renal tubule(Figure 9-12). Besides that observation of the inflammatory cell infilterations (Figure 13-14).

Electronic Microscopy

There are many degenerative changes in kidney of mice after 3hr from envenoming with 0.055 µg/kg body weight dose of snake venom (group II) were observed, condensation of nuclear chromatin,the endoplasmic reticulum were fragmented and disappearance in some positions, swollen and variable sizes mitochondria, some intercellular vacuoles of different sizes (Figure 15-16). necrotic of some endothelial cells, necrotic of some podocytes, swollen variable sizes of mitochondria(Figure 17). The nucleus irregular shape and abnormal distribution of chromatin (Figure 18) that observed in kidney of mice after 24hr from envenoming with 0.055 µg/kg body weight dose of snake venom (group III)

4. Discussions

In this study, the results show the single dose of LD50 dose of Naja naja snake venom causes histological changes in the glomeruli, renal tubular, nuclear pyknotic and necrotic of some cells. These results were in agreement with the researchers said that snake venom causes nephrotixicity or renal injury, like (15, 18 and19) on different snakes. Some signs of devastation observed in kidney after 3hrs of envenomation included congested of some glomeruli, atrophied of some glomeruli, formation of hyaline and granular tubular casts and vacuoles in renal tubular were also seen In this study. These findings compatible with those described by(16) who had demonstrated that in kidneys of Adult male albino mice treated with ½ LD50 of Naja nigricollis snake venom showed glomerulolysis, tubular necrosis and damage, and formation of hyaline and granular tubular casts as well as signs of intertubular medullary hemorrhage at early stages of envenoming. The degree of damage increased after 24hrs of envenomation included the most of the renal tissues were deteriorated by the venom such as some kidney tubules were vacuolated with glomeruli atrophied and cellular swelling. Cytoplasmic vacuolation and cellular swelling appeared in renal tubules is a clinical sign of irregular lipid inclusions and fat metabolism happening might be due to the action of venom phospholipase (PLA2) because phospholipase responsible for tissue injury by disturbing cell membrane permeability through disorganizing of lipid bilayer on the plasma membrane. All these changes were signals of extensive cellular necrosis. These finding have been detected in renal tissues by (20)who reported that injection of sub-lethal dose of Malayan cobra (Naja sputatrix) venom induced alterations in kidney. In addition, observeation of the cellular swelling might be due to the action of Naja naja venom phospholipase, which hydrolyze phospholipids in the cell membrane and causes to decrease in Na+/K+ ATPase activities and led to greater influx of sodium ions and water molecules into the cell that induces changes to cellular membranes, especially those related to fatty acid changes in the major membrane phospholipids and eventually lead to cell death (21). The main histopathological alterations after 24hrs of envenomation are infiltration of inflammatory cells, glomerulolysis and necrotic of cells probably due to leakage of membrane caused by the action of this phospholipases. in a similar way previously reported by (22) which indicated inflammatory infiltration, glomerular congestion, hemorrhages and focal necrosis. In the present study after 3hrs of envenomation, morphological alterations observed by electron microscopy that are condensation of nuclear chromatin, Increment of the intracytoplasmic vacuoles and swollen and variable sizes mitochondria. The changes obtained in the present study run parallel with the report documented by (23) where the investigator had demonstrated with sub-lethal dose of the Naja haje venom induced after 3hrs of envenomation of rabbit a slight changes were seen in visceral cells and glomerular endothelia. The cortical tubular epithelia revealed an increase of lysosomal structures, cytoplasmic vacuolization, and nuclear irregularity. After 24 hrs of injection of Naja naja venom of this study causes ultrastructural changes in the kidney tissues such as necrotic of some endothelial cells, necrotic of some podocytes, swollen variable sizes of mitochondria and Increment of the intracytoplasmic vacuoles. Similar observation were recorded by(23-24) who mentioned that the proteolytic activity of the venomon in renal glomeruli such as rupture of the capillary endothelia in the glomeruli, necrotic of some podocytes,swollen mitochondria and vacuoles in the endothelial cells. Venom stimulates oxidative stress to induce apoptosis in renal tissue. All that could then contribute to the onset of acute renal failure.

In conclusion, the present study indicate that a direct nephrotoxic action of Naja naja venom on kidney tissues included glomerular structures and tubule cells are the most important physiopathologic factors in Naja naja venom-induced nephrotixicity or renal injury.

5. Acknowledgments

The author thanks Prof. Khattab,Y.I. from the department of histopathology, college of Medicine, AL- Nahrain University for his kind help in reading and confirming the histological alterations.

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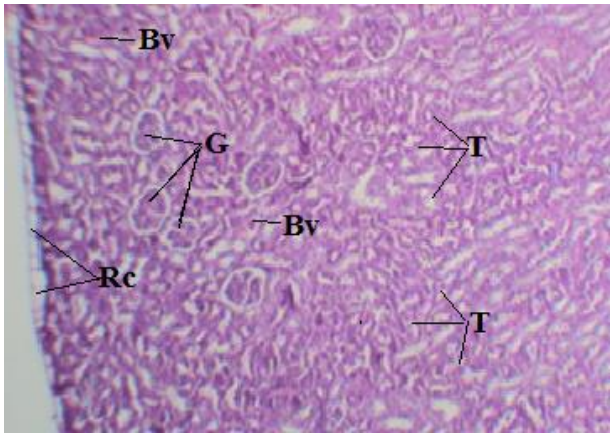

Figure 1: Section of kidney tissues (from control) showing normal histology of the kidney. Renal capsule(Rc) Glomeruli(G) convoluted tubules(T)and Blood vessel (Bv) (H&E 100X).

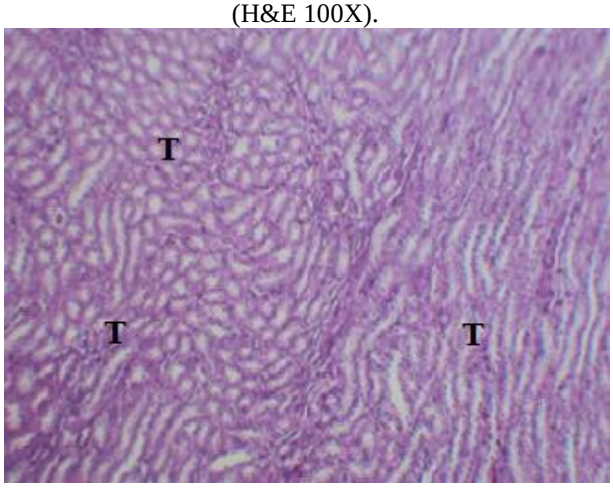

Figure 2: Section of kidney tissues (from control) showing the normal histological structure of the tubules in the medullary portion(T) (H & E x 100).

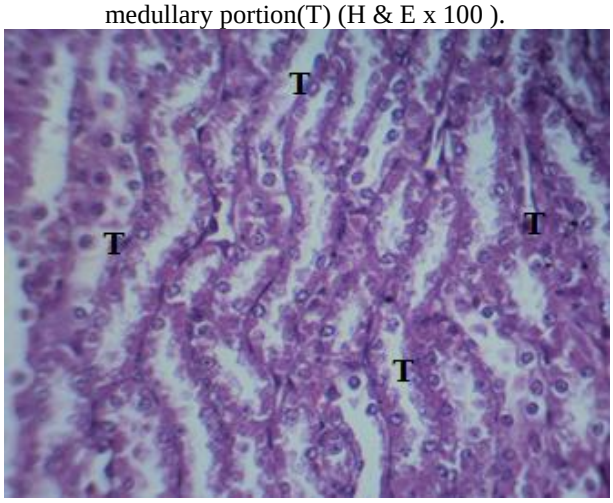

Figure 3: Section of kidney tissues (from control) showing the normal histological structure of the tubules in the medullary portion(T) (H & E x 200).

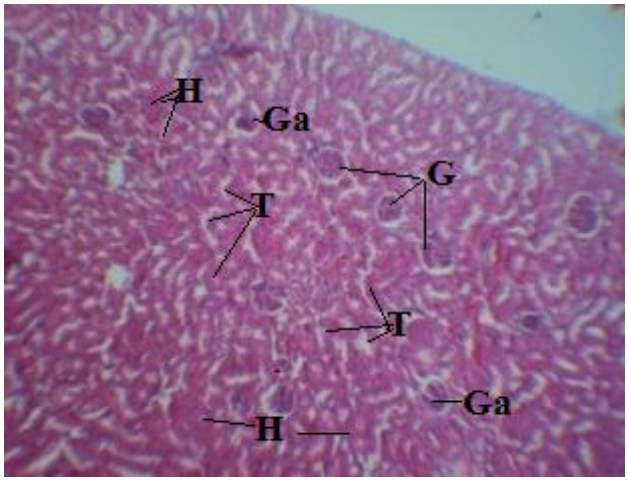

Figure 4: Section of kidney tissues of rat after 3h from envenoming with LD50 snake venom showing atrophied glomeruli (Ga), hemorrhagic (H) and renal tubular (T) (H & E x 100).

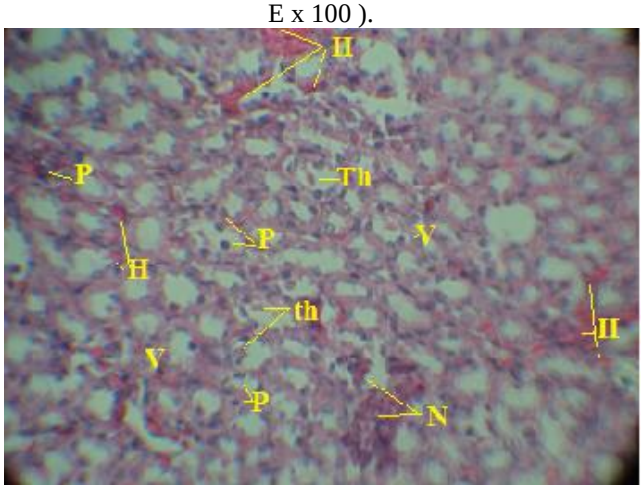

Figure 5: Section of kidney tissues of rat after 3h from envenoming with LD50 snake venom (group II) showing nuclear pyknosis (P), tubular hyaline casts (Th), hemorrhagic (H), vacuoles(V) and necrotic of some cells (N) (H & E; X200).

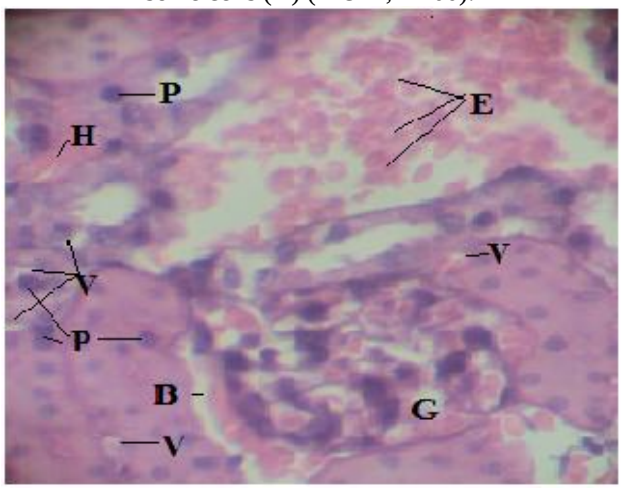

Figure 6: Section of kidney tissues of rat after 3h from envenoming with LD50 snake venom (group II) showing atrophied and segmented glomerulus (G)) erythrocytes(E) nuclear pyknosis (P), vacuoles(V), hemorrhagic (H) and irregular of Bowman's spaces (B) (H & E; X400).

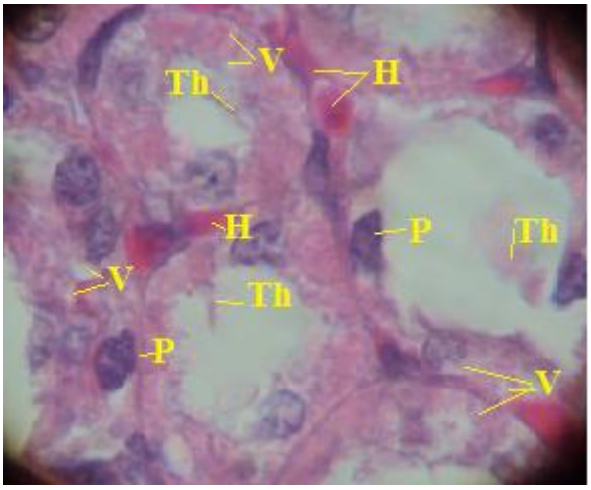

Figure 7: Section of kidney tissues of rat after 3h from envenoming with LD50 snake venom (group II) showing tubular hyaline casts (Th) vacuoles(V) hemorrhagic (H) and nuclear pyknotic (P) (H & E; X400).

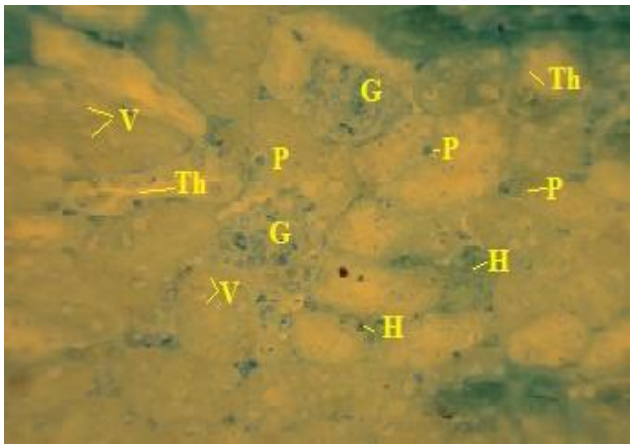

Figure 8: Section of kidney tissues of rat after 3h from envenoming with LD50 snake venom(group II) showing atrophied and segmented glomerulus (G)), nuclear pyknotic (P), vacuoles(V), tubular hyaline casts (Th) and hemorrhagic (H) (Methylene blue ; X200).

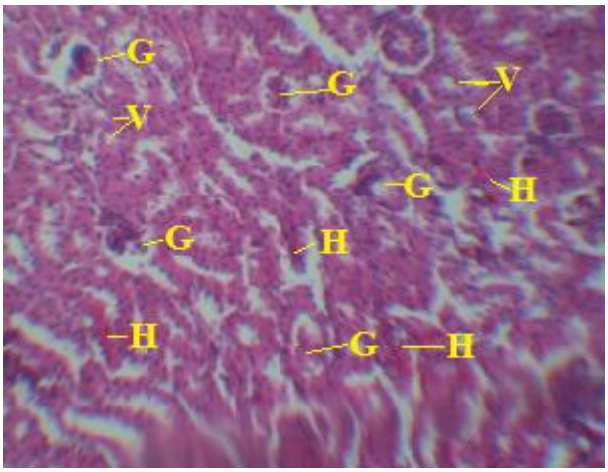

Figure 9: Section of kidney tissues of rat after 24h from envenoming with LD50 snake venom(group III) showing atrophied glomeruli (G) vacuoles(V) hemorrhagic (H) (H&E 100X).

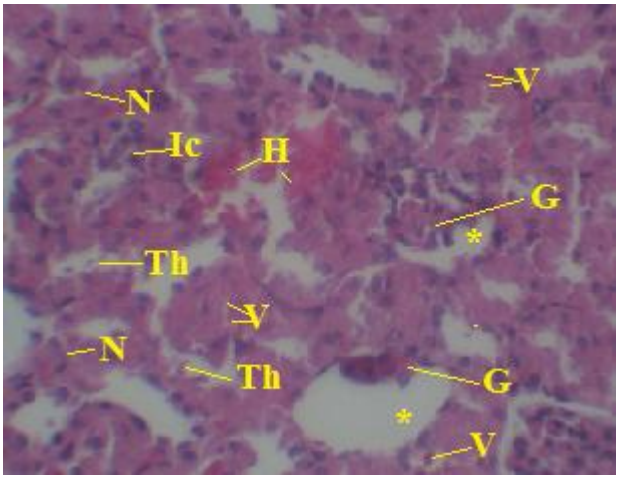

Figure 10: Section of kidney tissues of rat after 24h from envenoming with LD50 snake venom(group III) showing atrophied glomeruli (G) the widened capsular space (asterisk *), tubular hyaline casts (Th) vacuoles(V) and hemorrhagic (H) (H&E 200X).

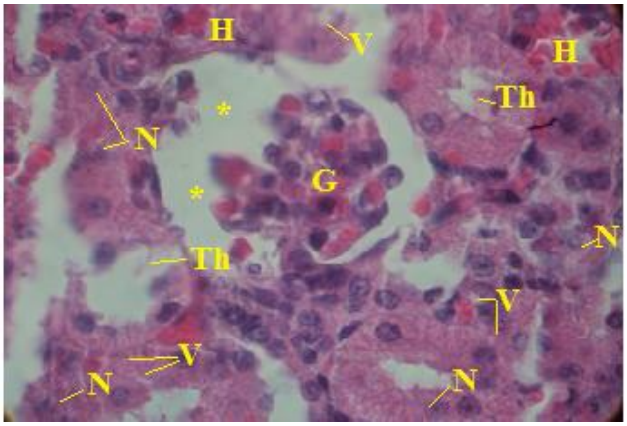

Figure 11: Section of kidney tissues of rat after 24h from envenoming with LD50 snake venom (group III) showing atrophied glomerular (G) the widened capsular space (asterisk *), tubular hyaline casts (Th) vacuoles(V) necrotic of some cells (N)and hemorrhagic (H) (H&E 400X).

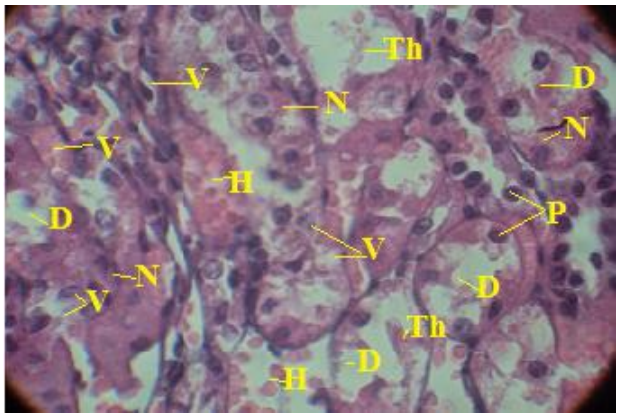

Figure 12: Section of kidney tissues of rat after 24h from envenoming with LD50 snake venom(group III) showing damage of some tubules in the medullary portion(D)) nuclear pyknosis (P) tubular hyaline casts (Th) vacuoles(V) necrotic of some cells (N)and hemorrhagic (H) (H&E 400X).

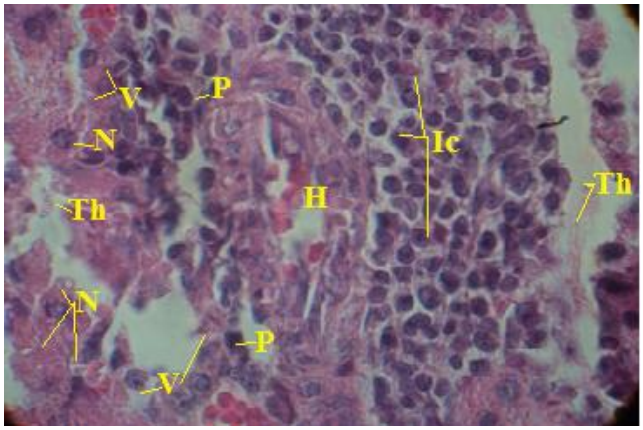

Figure 13: Section of kidney tissues of rat after 24h from envenoming with LD50 snake venom(group III) showing tubular hyaline casts (Th) vacuoles(V) hemorrhagic (H) nuclear pyknotic (P) inflammatory cell infilterations (Ic) necrotic of some cells (N) (H&E 200X).

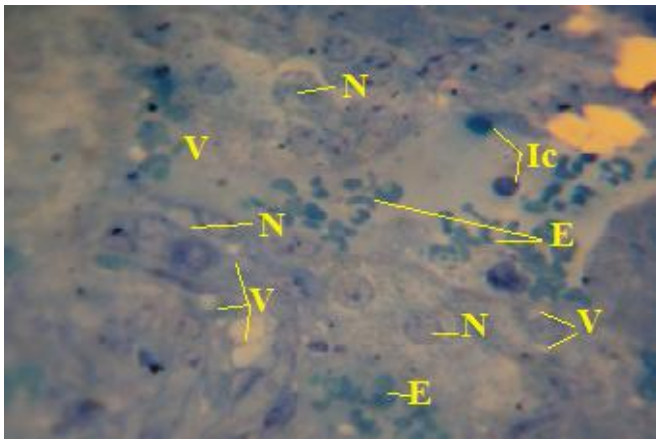

Figure 14: Section of kidney tissues of rat after 24h from envenoming with LD50 snake venom(group III) showing) necrotic of some cells (N), nuclear pyknotic (P), vacuoles(V), erythrocytes(R)and inflammatory cell infilterations (Ic) (Methylene blue ; X200).

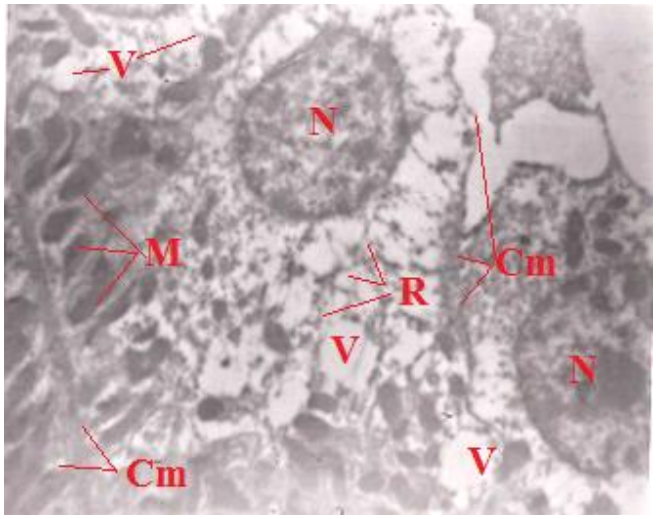

Figure 15: Electron micrograph of kidney tissues of rat after 3h from envenoming with LD50 snake venom(group II) showing variable sizes of nuclei (N) with condensation of nuclear chromatin, swollen variable sizes of mitochondria (M), vacuolation in the renal cytoplasm(V), fragmented of endoplasmic reticulum (R) and disrupted cell membrane(Cm). (X3500).

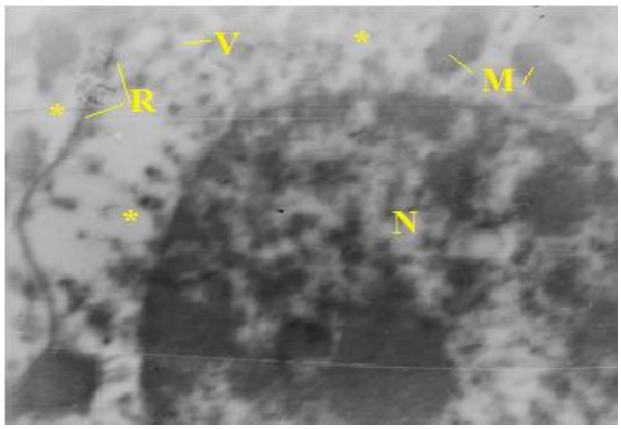

Figure 16: Electron micrograph of kidney tissues of rat after 3h from envenoming with LD50 snake venom(group II) showing nucleus (N) with condensation of nuclear chromatin, irregular nuclear membranes, swollen variable sizes of mitochondria (M), vacuolation in the renal cytoplasm(V) fragmented of endoplasmic reticulum (R) and cytoplasmic lysis (asterisk *)(X4500).

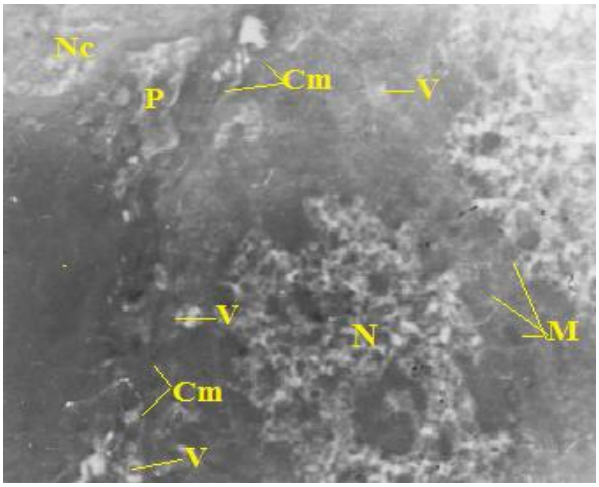

Figure 17: Electron micrograph of kidney tissues of rat after 24h from envenoming with LD50 snake venom(group III) showing nucleus (N) with condensation of nuclear chromatin, irregular nuclear membranes, swollen variable sizes of mitochondria (M), vacuolation in the renal cytoplasm, necrotic podocytes (P) and necrotic endothelial cell (Nc). (X3000).

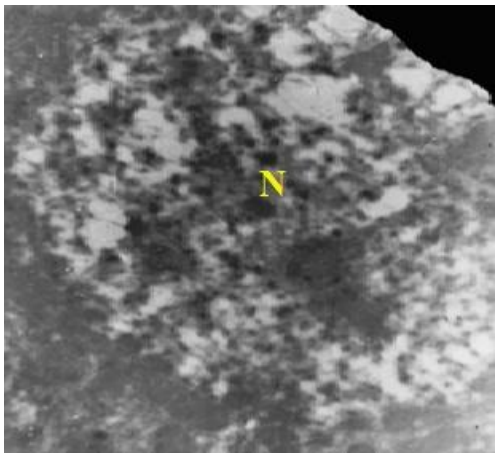

Figure 18: Electron micrograph of kidney tissues of rat after 24h from envenoming with LD50 snake venom (group III) showing necrotic cells with pyknotic nucleus(N). (X5000).