Introduction

Nephrotoxicity is one of the most common kidney problems and occurs when the body is exposed to a drug or toxin that causes damage to the kidneys **(Chaudhary and Paranjape, 2014).** Nephrotoxicity is recognized a clinical liability of certain classes of drugs; in particular, antibiotics which represent the major class of nephrotoxic drugs, gentamicin is an example of antibiotics that impair glomerular ultrafiltration and decrease glomerular filtration rate (GFR) **(Manahan, 2003).** Nephrotoxicity is a major complication characterized by functional alterations including prevention of protein synthesis, reduced glutathione (GSH) depletion and elevation in the lipid peroxidation production accompanied with mitochondrial damage **(Monika *et al*., 2014).**

Gentamicin is probably the most commonly used and studied of all the aminoglycosides, however, it causes severe renal toxicity**.** Gentamicin inhibits phosphorylation and reduces ATP levels in renal tubular cells. Oxidative stress has also been reported in the tubular toxicity of gentamicin. Thus, gentamicin increases reactive oxygen species (ROS) formation and ROS-induced cell death. They were found to have a role in gentamicin mediated acute renal failure **(Nasri *et al*., 2013)**. Gentamicin causes nephrotoxicity by inhibiting protein synthesis in renal cells. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting in acute tubular necrosis which can lead to acute renal failure **(Sundin *et al.*, 2001)**and decrease urine concentration capacity, tubular proteinuria, mild glucosuriaand ammonium excretion as well as lowering of GFR **(El-Badwi *et al.*, 2012).** In the kidney, proximal tubule cells are mostly affected by ROS and oxidative stress. This can lead to apoptosis and necrosis and ultimately this is the reason of nephrotoxicity **(Monika *et al*., 2014)**. One of the most common manifestations of nephrotoxic damage is acute renal failure **(Bonventre, 2010).**

Oxidative stress has been recognized as an important contributory factor in a number of pathogenic processes including those affecting kidney leading to the possibility of utilizing the antioxidants for the prevention of nephrotoxicity **(Peres and Junior,**

2013). Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules which are generally very reactive **(Prasad *et al.*, 2012).**

Aminoglycoside antibiotics have been shown to elevate the generation of superoxide anion and hydrogen peroxide by renal cortical mitochondria. The interaction between superoxide anion and hydrogen peroxide in the presence of metal catalyst can lead to the generation of hydroxyl radical **(Pal *et al.*, 2013).** The mitochondrial DNA is susceptible to oxidative damage by the increasing levels of ROS and free radicals in the mitochondrial matrix. Spillage of ROS into the cytoplasm can further aggravate damage to the various subcellular structures **(Acharya *et al.*, 2013).** Oxidative damage is thought to be one of the main mechanisms involved in nearly all chronic renal pathologies **(Kalyanaraman, 2013).**

Antioxidants are substances capable to repair or prevent the damage produced by oxidative stress. L-carnitine exerts a substantial antioxidant action **(Ye *et al.*, 2010).** Carnitine is a quaternary ammonium compound **(Steiber *et al.*, 2004).**

L–carnitine is biosynthesized mainly in the liver and kidney from the amino acids lysine and methionine. The kidney plays the major role in carnitine biosynthesis, excretion and acylation. Kidney contains all enzymes needed to form carnitine from trimethyllysine in activities exceeding that of the liver **(Cibulka *et al.*, 2006).** L-carnitine is a compound widely distributed in nature and obtained primarily from the diet. It is involved in intermediary metabolism and is important to mammalian bioenergetic processes. It has been shown that L-carnitine plays an essential role in multiple primary functions **(Sirolli *et al.*, 2012)** and providing a protective effect against lipid peroxidation and oxidative stress **(Ye *et al.*, 2010).**

Carnitine plays a key role in lipid metabolism by facilitating transport of long chain fatty acids in the mitochondria where fatty acid can undergo β-oxidation in the matrix of the mitochondria. It also assists the membrane stability of red blood cells (RBCs) **(Demizieux, 2005).** Apart from its main role in energy metabolism, L-carnitine is an antioxidant that prevents the accumulation of end-products of lipid

peroxidation. It also exhibits powerful protective effects against different kidney injury models including ischemia and reperfusion (IR) injury, myoglobinuric acute kidney injury and chronic renal failure. It has been reported that L-carnitine deficiency destabilizes the erythrocyte membrane and leads to dysfunction in the erythrocyte sodium potassium pump with reducing erythrocyte survival time **(Aydogdu *et al.*, 2013).**

Aim of the work

The current investigation was designed to evaluate the possible therapeutic role of L–carnitine on some hematological and biochemical parameters in gentamicin-induced nephrotoxicity in adult male albino rats dependent on the dose and time of treatment.

Review of literature

The renal system consists of the kidneys, ureters, urinary bladder and urethra. The kidneys constitute the glandular component; the remainder of the renal system forms the excretory passages.The ureters conduct urine from the kidneys to the bladder, where it is stored temporarily. In turn, the bladder is drained by the urethra, through which the urine ultimately is voided from the body **(Martini *et al*., 2012).**

1-Kidney anatomy and physiology:

-Kidney anatomy:

The kidneys are organs that lie in a retroperitoneal position against the posterior abdominal wall, one on either side of the upper lumbar vertebrae. Each kidney contained within a thin but strong connective tissue capsule that contains fat. The renal artery and nerves enter the kidney on the medial border at the hilum, a concavity that also serves as the point of exit for the renal vein, lymphatics, and ureter. The hilum is continuous with the renal sinus, a large central cavity surrounded by the parenchyma of the kidney and filled with loose areolar connective tissue that normally contains much fat. Nerves, lymphatics, and branches of the renal artery and vein run through the sinus. The renal pelvis is a funnel shaped expansion of the ureter where it joins the kidney; it also passes through the sinus, dividing into two or three short tubular structures called the major calyces. These in turn divide into smaller units called minor calyces. Each minor calyx forms a cylindrical attachment around a conical projection of renal tissue called a renal papilla (Figure 1)**(Marieb and Hoehn, 2012)**.

Two layers form the kidney are cortex and medulla, the darker granular appearing outer region is the cortex, which forms a continuous layer beneath the capsule. The inner region, or medulla, is paler smoother in texture and consists of cone-shaped structures called medullary pyramids, which are separated from each other by inward extensions of cortical tissue. The cortex that separates adjacent

medullary pyramids makes up a renal column. The bases of the pyramids are directed toward the overlying cortex, while their apices are oriented toward the renal sinus and form the renal papillae. From the bases of the pyramids, groups of tubules extend into the cortex, giving it a striating appearance. These striations represent a continuation of medullary tissue into the cortex and constitute the medullary rays. The arrangement of cortex and medulla allows subdivision of the kidney into smaller units, the lobes and lobules. A medullary pyramid, together with its closely associated cortical tissue, forms a renal lobe, while a medullary ray, together with its associated cortical tissue and, forms a renal lobule **(Marieb and Hoehn, 2012)** (Figure 1).

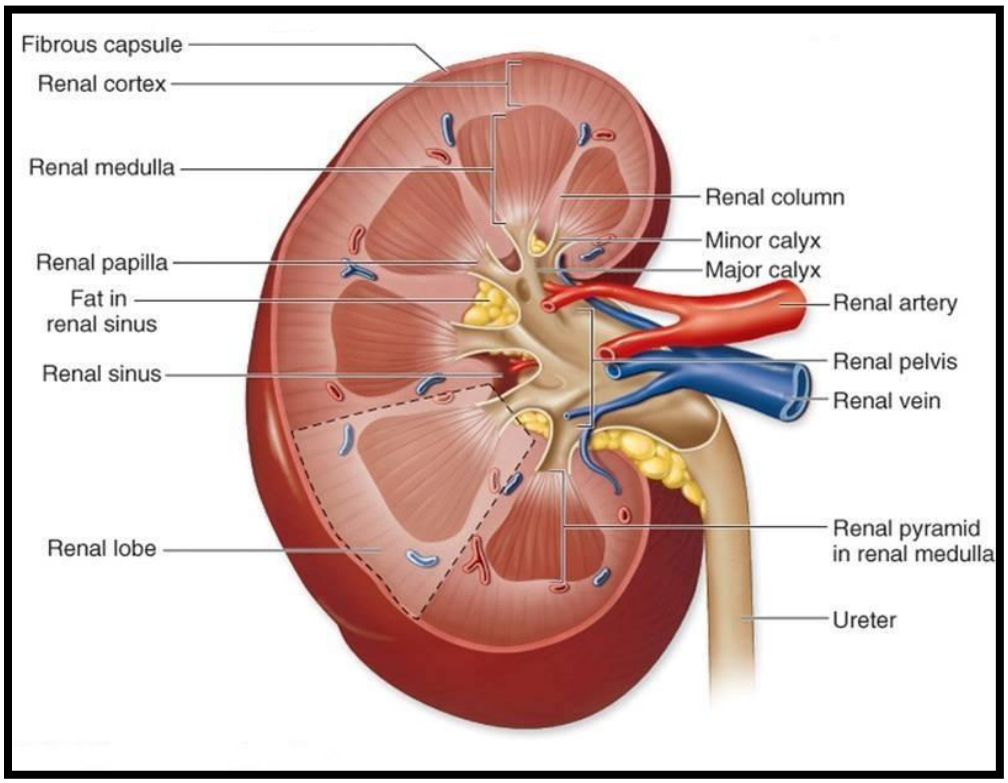

Figure 1: Cross section of the kidney (Quoted from **Mescher, 2010).**

The nephron:

The nephron is the functional unit of the kidney. Approximately one million nephrons comprise each kidney.

The nephron consists of a renal corpuscle and renal tubule:

- The renal corpuscle is composed of Bowman's capsule and the glomerulus.
- The renal tubule consists of :
    - Proximal convoluted tubule.
    - Loop of Henle.
    - Distal convoluted tubule.

Each nephron empties into the collecting system: a series of tubes that carries tubular fluid away from nephron.

- Collecting ducts receive: fluid from many nephrons

Each collecting duct begins in cortex, descends into medulla and carries fluid to papillary duct that drains into a minor calyx.

Types of nephron:

- Cortical nephrons:
    - Approximately 85% of all nephrons
    - Located in the cortex
    - Perform excretory and regulatory functions

- Juxtamedullary nephrons:
    - Approximately 15 %
    - Closer to renal medulla.
    - Loops of Henle extend deep into renal pyramids.
    - Responsible for concentration and dilution of urine

(Figure 2) **(Martini *et al.*, 2012).**

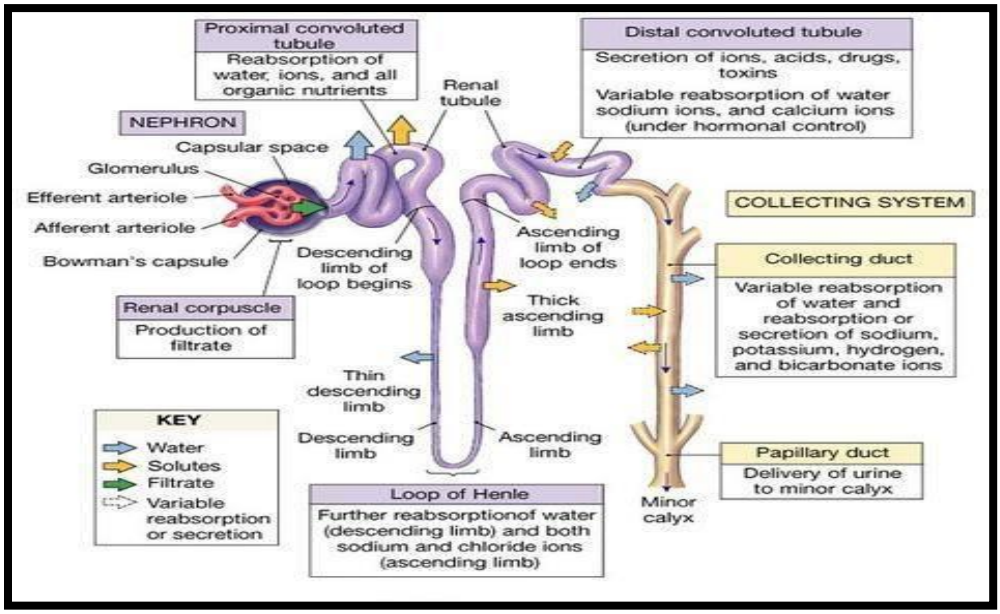

Figure 2: The functional anatomy of nephron and the collecting system (Quoted from **Martini *et al.*, 2012**).

Kidney function:

Kidney is the organ that serves several essential regulatory roles in most animals, it plays an indispensible role in the regulation of the whole organism water balance, electrolytes balance, and acid base balance, as well as in the excretion of metabolic wastes and toxins. Additionally, the kidneys produce or activate hormones that are involved in erythrogenesis, calcium metabolism, and the regulation of blood pressure and blood flow**.**Renal dysfunction can negatively impact all these roles **(Raghavendra *et al*., 2013).**

Many of the kidney's functions are accomplished by relatively simple mechanisms of filtration, reabsorption and secretion which take place in the nephron. Filtration takes place at the renal corpuscle, is the process by which blood pressure forces water and small solutes across membrane into capsular space. Larger solutes, such as plasma proteins are excluded in this process occurs passively**.** Basically, everything smaller than a protein enters capsular space including metabolic wastes, excess ions, glucose, free fatty acids, amino acids and vitamins **(Martini *et al*., 2012).**

The kidney generates 180 liters of filtrate a day, while reabsorbing a large percentage**,** allowing for the generation of only approximately 2 liters of urine. Reabsorption is the transport of useful molecules from this ultrafiltration into the blood occurs in the renal tubule. Secretion is the reverse process occurs in the renal tubule. Secreted waste products that failed to enter renal corpuscle through filtration at glomerulus, are transported in the opposite direction, from the blood into the urine **(Snigdha*et al*., 2013).**

2.Kidney diseases:

Diseases of the kidney are diverse. Common clinical conditions involving the kidney include the nephritic and nephrotic syndromes (renal cysts, acute kidney injury, chronic kidney disease, urinary tract infection, nephrolithiasisand urinary tract obstruction). Various cancers of the kidney exist; the most common adult renal cancer is renal cell carcinoma. Cancers, cysts and some other renal conditions can be managed with removal of the kidney (nephrectomy). When renal function, measured by GFR, is persistently poor, dialysis and kidney transplantation may be treatment options **(Raghavendra *et al*., 2013).**

Glomerulonephritis:

Glomerulonephritis (GN) is an inflammation of the glomerulus, can be caused by immunologic abnormalities, drugs or toxins, vascular disorders and systemic disease. GN can be acute, chronic or progressive. Two major changes in the urine are distinctive of GN: hematuria and proteinuria with albumin as the major protein. There is also a decrease in urine as there is a decrease in GFR. In some cases, GN may progress to renal failure. Renal failure is associated with oliguria (less than 400 ml of urine output per day) **(Marieb and Hoehn, 2012).**

Acute renal failure:

Acute renal failure is a clinical syndrome, characterized by an abrupt decline in GFR. There is a subsequent retention of metabolic waste products and an inability to maintain electrolyte and acid-base homeostasis. Regulation of fluid volume is also affected**.** Acute renal failure occurs rapidly resulting in fifty percent or more nephrons to

lose functionand as this occurs quickly thebody is unable to compensate. There are three classifications of acute renal failure based on the location of the cause, they are prerenal, intrarenal and postrenal **(Motsoaledi, 2012).**

Prerenal in which renal dysfunction is largely related to systemic factors that limit blood flow and reduce GFR such as:

- Hypotension.
- Hypovolaemic shock- dehydration, blood loss.
- Cardiogenic shock – post myocardial infarction.
- Septic shock.
- Bilateral renal vascular obstruction- thrombosis **(Attwood and Cullis, 2012).**

Intrarenal in which renal impairment occurs secondary to damage that is sustained at the site of the nephrons. This may be the result of a number of conditions or nephrotoxins such as:

- Acute tubular necrosis.
- Acute glomerulonephritis.
- Acute pyelonephritis.
- Acute cortical necrosis.
- Malignant hypertension.
- Acute vasculitis.
- Rhabdomyolysis - drugs, trauma.
- Nephrotoxins - intravenous contrast, aminoglycosides **(Attwood and Cullis, 2012).**

Postrenal in which renal failure occurs secondary to obstruction that prevents excretion of urine. This may be the result of a number of conditions such as:

- Prostatic hypertrophy.
- Renal calculi.
- Tumour.
- Blocked urinary catheter **(Attwood and Cullis, 2012).**

Chronic renal failure:

Chronic renal failure is usually the end result of conditions such as diabetes mellitus, hypertension, primary glomerulonephritis,

autoimmune disease, obstructive uropathy, polycystic disease, renal artery stenosis, infections, tubular dysfunction and the use of nephrotoxic drugs. In most cases of acute oliguric renal disease there is diffuse damage involving the majority of nephrons **(Crook, 2012).**

A patient who survives long enough to develop chronic renal disease must have some functioning nephrons. Histological examination shows that not all nephrons are equally affected, some may be completely destroyed and others almost normal. Also, some segments of the nephrons may be more affected than others. The effects of chronic renal disease can be explained by this patchy distribution of damage, acute renal disease may sometimes show the same picture. Inchronic renal failurethe functional adaptive effects can be divided into three main categories: diminished renal reserve, renal insufficiency, and end–stage uremia. This helps to explain why the loss of 75 percent of renal tissue produces a fall of GFR by 50 percent **(Crook, 2012).**

3.Animal models of acute renal failure:

Acute renal failure is a common complication of critical illness, which is associated with high mortality and has a separate independent effect on the risk of death **(Abdel-Zaher *et al*., 2008 and Adeneye *et al*., 2008).**

The animal models of acute renal failure are pivotal for understanding the characteristics of acute renal failure, the pathophysiology of acute renal failure in these different conditions and to explore the drug therapeutics and development of effective therapy for its optimal management. Since; the etiology for induction of renal failure is multifold, therefore, a large number of animal models have been developed to mimic the clinical conditions of renal failure **(Singh *et al*., 2012).**

3.1 Chemotherapeutic and immunosuppressive agents:

Chemotherapeutic and immunosuppressive agents such as cisplatin, cyclosporine and ifosfamide.

- Cisplatin, an anticancer drug, is broadly used for the therapy of cancers. Administration of 5 ml/kg cisplatin (0.1% of saline solution) in the abdominal cavity is associated with development of acute renal failure in rats within 72 hrs of administration **(Izuwa *et al*., 2009).**
- Cyclosporine, formerly called cyclosporine A (CsA), can be considered the prototype of immunosuppressant that has revolutionized the management of allotransplantation **(Tirkey *et al*., 2005). Heibashy *et al*. (2009)** reported that development of acute renal failure in rats by subcutaneous injectionwith CsA at a dose of 20 mg/kg/day dissolved in olive oil for 21 days.
- Ifosfamide (IFO), a synthetic analog of cyclophosphamide, is an alkylating oxazaphosphorine and is widely used as first-line combination therapy for a variety of malignancies including metastatic germ-cell testicular cancer and some sarcomas. It has been demonstrated that, intraperitoneal administration of IFO at a dose of 50 mg/kg for 5 consecutive days induces the renal damage in rat **(Singh *et al*., 2012).**

3.2 Angiotensin converting enzyme inhibitors:

Angiotensin converting enzyme inhibitors (ACEI), these classes of drugs were found to be successful for managing cardiovascular disease, hypertension and chronic nephropathy. Initiation of ACEI in patients with advanced renal failure can precipitate uremia, hyperkalemia and dialysis dependence. Previously stable patients taking chronic ACEI therapy can become dehydrated and develop profound renal failure that requires temporary dialysis **(Aronow *et al*., 2011). Cao *et al*. (2000)** reported that effects of treatment with ACEI on renal function and glomerular injury in subtotal nephrectomized rats. The nephrectomized rats were treated with ACEI (perindopril at a dose of 8 mg/L in drinking water for 12 weeks).

3.3 Heavy metals:

Heavy metals such as lithium, uranium, mercuric chloride and potassium dichromate.

- Lithium administration is the most popular therapeutic approach to treat bipolar disorders **(Chiu and Chuang, 2010**). **Efrati *et al.*** (**2005)** reported that development of renal failure in rats by receiving Lithium carbonate 20 mg/kg/day with feed for four weeks.

- Uranium exposure causes the development of renal failure. **Priyamvada *et al.***(**2010)** reported that development of renal failure in rats within 5 days by intraperitoneal administration of single dose of uranyl nitrate (5 and 10 mg/kg) dissolved in 0.9% of saline.

- Mercuric chloride ($HgCl_2$) is a well-known renal toxicant that causes acute renal failure. The development of acute renal failure in rats performed within 24 hrs by administration of a single subcutaneous dose of $HgCl_2$ at 4.0 mg/kg and 5.0 mg/kg **(Augusti *et al.*, 2008)**.

- Potassium dichromate is another animal model of acute renal failure. Chromium is a naturally occurring element found in volcanic dust, rocks, soil, plants and animals. Cr^{6+} and Cr^{3+} are widely used in industrial and chemical processes. In some regions, waste disposal of chromium compounds to the environment contributes to increase its presence and potential toxicity. **Khan *et al.*** (**2010)** demonstrated that a single injection of potassium dichromate (15mg/kg, subcutaneous) causes development of acute renal failure within 48 hrs.

3.4 Non-steroidal anti-inflammatory drugs:

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most common prescription medicines.

- Acetaminophen is most widely used in the world as an analgesic and antipyretic drug that is safe at therapeutic dosages. However, it is also known to cause hepatic necrosis and renal failure. **Adeneye *et al.*** (**2008)** reported that development of acute renal failure within 24 hrs in rats by administration of a

single dose of acetaminophen 800 mg/kg, intraperitoneal, which was dissolved in normal saline.

- Diclofenac sodium, diclofenac is widely used NSAID for the management of pain and inflammation associated with arthritis**. Efrati *et al*.** (**2007)** noted that intraperitoneal administration of diclofenac (15 mg/kg) for 3 day has been reported to induce renal failure in rats.

3.5 Glycerol:

Glycerol-induced acute renal failure is characterized by myoglobinuria, tubular necrosis and enhanced renal vasoconstriction ***(*Yousefipour *et al.*, 2007).Izuwa *et al*.**(**2009)** postulated in their comparative study that, using single dose 8–10ml/kg, intramuscularis used for induction of acute renal failure in rats.

3.6 Radiocontrast media:

Radiocontrast media are very commonly used in radiology particularly for cardiac catheterization. **Yen *et al*.(2007)** noted that development of acute renal failure in rats within 1hrs by administration of 10ml/kg of ditriazoate with an iodine load of 3700mg/kg *via* the tail vein.

3.7 Osmotic nephrosis:

Osmotic nephrosis is due to intravenous infusion of hypertonic sucrose, hydroxyethyl starch, dextrans, and contrast media to reduce intra-cranial pressure. **Zhang *et al*.** (**1999)** showed that, a single dose administration of mannitol (4%, 9%, 19% and 27%) with dose of 5ml/kg leads to induction of renal apoptosis and acute renal damage in spontaneous hypertensive rats.

3.8 Folic acid:

Folic acid (FA) induced acute renal failure is a conventional animal model of human acute renal failure. FA-induced renal injury is associated with the rapid appearance of FA crystals within renal tubules and subsequent acute tubular necrosis, followed by epithelial regeneration and renal cortical scarring. An intravenous injection of

folic acid (250mg/kg) is reported to induceacute renal failure after 48hrs in mice **(Wan *et al.*, 2006).**

3.9 Ferric-nitrilotriacetate:

Ferric-nitrilotriacetate (Fe-NTA) complex causes acute nephrotoxicity in animals. Nitrilotriacetic acid (NTA), a synthetic chelating agent, is used as a household and hospital detergent in various countries. NTA is a low-toxic agent; however, (Fe-NTA) complex cause acute nephrotoxicity in animals as well as in humans, administration of the single dose of Fe-NTA (8mg iron/kg, intraperitoneal) has also been reported to produce renal failure in rats **(Gupta *et al*., 2008).**

3.10 Sepsis:

Sepsis is also employed to induce acute renal failure in rats. Cecal ligation and puncture (CLP) induced polymicrobial sepsis is also employed to induce acute renal failure in rats.**Jesmin *et al*.(2009)**noted that a single intraperitoneal injection of lipopolysaccharide (LPS) derived from *Escherichia coli* (*E. coli* 055: B5) (15mg/kg) induces the potential acute renal failure in rats.

3.11 Antifungal agents:

Antifungal agents like amphotericin B (AmB) considered the drug of choice for the treatment of systemic fungal infections. Nephrotoxicity is a major complication associated with its use and appears to be related to higher cumulative doses **(Laniado-Laborn and Cabrales-Vargas, 2009).** Animals treated intraperitoneal with AmB deoxycholate at a dose of 10mg/kg of body weight (b.wt.) for 5 days ped to a pronouncing of acute renal failure in rats **(Odabasi *et al.*, 2009).**

3.12 Renal Ischemic/ reperfusion injury:

Renal ischemic/ reperfusion injury is the major cause of renal injury in ischemic acute renal injury. Rats were anesthetized with isoflurane inhalation. A midline laparotomy incision was performed to expose the abdomen. Intestines were covered with warm, moist gauze