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Histopathological study effect of morphine on liver and kidney in rats

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Abstract

Morphine is one of the most commonly used opioid analgesics however its chronic use during pregnancy may cause histological and functional damage to maternal organs and their fetuses. The aim of study the effect of morphine sulphate on rats on different organs especially brain adult and puppies. The number of animals 50 were taken female rat 40 and male 10 and divided into four groups.G1: 10 female rat treated by morphine sulphate dosage 15 mg/kg.Bw orally 14 day before mating till the 5th of pregnancy.G2: 10 female treated by morphine sulphate dosage 15 mg/kg.Bw orally (6-16 day). G3: 10 female will be treated by morphine sulphate dosage 15 mg/kg.Bw orally(17-21 day) of pregnancy and G4 control group the female rats of group one will be mated by male rats which be received orally morphine 15 mg/kg.Bw for 2 months before mating. Histotopathological examinations that revealed marked in liver that appear congestion ,activation of kuffer cells, and inflammatory infiltration and dilation of central vein while the kidney that appear fragmentation of glomerular tuft ,degenerative change ,protonatious material, inflammatory cells and dilation of renal tubules. Morphine shows clear effects on both the liver and kidneys. Chronic use or high doses may lead to functional disturbances due to the metabolic and excretory processes occurring in these organs. The liver, being the primary site of morphine metabolism, is particularly vulnerable to oxidative stress and hepatocellular damage with excessive exposure. The kidneys can also be affected by the accumulation of metabolic byproducts and reduced excretion efficiency, which may result in impaired renal function. Therefore, morphine should be used with caution, and liver and kidney functions should be regularly monitored, especially in patients with pre-existing chronic conditions in these organs.

Keywords: *Morphine ,rats, liver ,kidney*.

Introduction

Morphine is one of the natural opioid compounds extracted from the poppy plant (*Papaver somniferum*) and it is widely used in medicine as a potent analgesic to relieve moderate to severe pain [1]. Despite its therapeutic effectiveness chronic or uncontrolled use is associated with a range of serious side effects, especially on the central nervous system [2].Morphine a potent opioid analgesic is widely employed in clinical settings for the management of moderate to severe pain [3].Its mechanism of action involves binding primarily to μ-opioid receptors within the central nervous system leading to significant modulation of nociceptive pathways[4].

Despite its high effectiveness as a painkiller repeated or excessive use of morphine is associated with numerous toxic side effects that may affect vital organs of the body particularly the liver and kidneys, due to their crucial roles in drug metabolism and excretion [5]. Morphine is primarily metabolized in the liver, where it is converted into metabolic derivatives by specific enzymes,and these metabolites are then excreted through the kidneys in the urine However, chronic exposure to morphine or administration of high doses leads to oxidative stress as a result of the formation of reactive oxygen species (ROS), which attack cellular membranes and cause both structural and functional damage in the liver and kidneys [6]. Experimental studies on animals, especially rats, have shown that morphine induces histological and biochemical alterations in the liver such as vascular congestion, fatty degeneration, and elevated liver enzymes (ALT and AST) indicating hepatocellular damage [7].It also causes renal dysfunction, manifested by increased levels of urea and creatinine and tubular degeneration in the kidneys due to the accumulation of metabolic products and oxidative stress [8].Therefore investigating the effects of morphine on the liver and kidneys is essential to understanding the toxic mechanisms resulting from its use, as well as to developing possible preventive and therapeutic approaches—particularly in cases that require long-term morphine treatment [9].

Materials and Methods

Chemicals

Ec Germany (17,81925) provided the morphine solution by patient suffering from cancer and after died which was taken orally by gavage. It is given for 120 days at a dose of 15 mg/kg.

Ethics

Procedures in this experiment were approved by the local Committee of Use and Care of Animals at the University of Baghdad, College of Veterinary Medicine (Approval Number 1173 on February 2, 2024.

Animals housing

The present study was applied to (40) Laboratory animals (Dawliya male and female rats) were involved in this study the weights were ranged between (250-300) grams and age about 2-3 months rats were placed in a plastic cages these plastic cages containing hard-wood chip as bedding and the bedding was changed continuously to ensure a clean environment and rat were kept in the animal house of the College of Veterinary Medicine at University of Baghdad for 7 days all animals were kept under similar management conditions included room temperature 22 ℃ and light cycle 12/12 hrs. The rats were fed on standard palate and tap water ad libitum the rats were left for 1 week for adaptation before the beginning of the experiment. Rats were divided as following:

Experimental protocol:

1.G1: 10 female rat treated by morphine sulphate dosage rate 15 mg/kg.Bw orally 14 day before mating till the 5th of pregnancy [10].

2.G2: 10 female will be treated by morphine sulphate dosage rate 15 mg/kg.Bw orally (6-16 day) of pregnancy.

3.G3: 10 female will be treated by morphine sulphate dosage rate 15 mg/kg.Bw orally(17-21 day) of pregnancy.
4.G4: Control rat treated with normal saline.
-The female rats of group one which mated by male rats were received orally morphine 15 mg/kg.Bw for 2 months before mating
-The female rats of G2 ,G3 and G4 will be mated by control male rat would be received vehicle only .

Sample collection:

Samples were collected from experimental animals of female rat and tissue pieces were taken from the liver kidney and brain the samples was preserved in buffer formalin solution 10% for the purpose of conducting histopathological change of liver and kidney lesion and the other part of the brain samples will be ground .The purposes of grinding brain tissue is for molecular study.

Molecular study:

For detection of Apoptosis and Necrosis in in tissue homogenates brain in rats Using Annexin V-FITC/PI After Morphine Treatment [11].

Histopathological study:

On days 5, 6-16, and 17-21 female rats from each group were anesthetized and euthanized by Diethyl Ether (ether solvent) C4H100. performed dissections on a groups of rats the internal organs were extracted including the liver, kidney and brain these organs were then taken tissue for examined microscopic histopathological change(H&E) and macroscopic changes for observation any abnormal alterations in these organs such as variations in the organs' size, color, or shape These organs were sliced into 2 cm-thick to facilitate detection of pathological change, While the brain tissues sample is collected for the purpose of grinding the brain tissue using a homogenizer[12].

Results and Discussion

Histopathological study of liver in rat:

The liver of G1 at 5 day showing slight degenerative changes of hepatocyte with focal aggrcation of inflammatory cell adjacent central vein (Figure1-1). showing dilation of central vein and aggregation of mononuclear inflammatory cells adjacent to central vein with degenerative changes of hepatocyte (Figure1-2). These findings are consistent with those of [13], who reported marked central venous congestion and significant fatty degeneration in liver tissue after morphine administration.

These pathological features were also accompanied by inflammatory cell infiltration because of oxidative stress caused by the liver's metabolism of morphine, which generates reactive oxygen species (ROS) due to morphine degradation, which damages hepatocyte membranes [14].In contrast, While the liver of G2 at 6-16 showing showing infiltration of inflammatory cells with activation of kuffer cells , vascular congest and dilated of central vein (Figure1-3).

In addition showing sever aggregation adjacent pericentral of inflammatory cells mononuclear and activation of kuffer cell (Figure1-4). These findings are consistent with those of [15], who examined the histopathological alterations of liver tissues after morphine was administered for 6–16 days. They found significant pathological alterations, such as central vein congestion, inflammatory cell infiltration, and kupffer cell proliferation.

The reason for these alterations is believed to be oxidative stress brought on by the liver's metabolism of morphine, which produces reactive oxygen species (ROS) that harm hepatocyte membranes and trigger the activation of kupffer cells,which are the liver's resident macrophages [16].Mononuclear cell infiltration and pericentral inflammation are caused by proinflammatory cytokines released by activated kupffer cells, including tumor necrosis factor-alpha (TNF-α) [17].Furthermore, morphine's effect on hepatic microcirculation is probably what causes endothelium thickening and central vein congestion. Morphine can impede blood flow

and increase vascular permeability, which causes dilated and congested central veins with an accumulation of inflammatory mediators and cells. [18].However,

While the liver of G3 at 17-21 day showing sever infiltration of inflammatory cells in portal area with congested of central vein (Figure 4-15). The presence of congested in central vein (Figure1-5). In addition infiltration of inflammatory cells in central vein and with activation of kuffer cells and congestion of blood vessel (Figure1-6). And presence dilation of portal area and infiltration of inflammatory in portal area and activation of kuffer cells (Figure1-7).

The observed vascular congestion and multifocal inflammatory cell aggregation may be a secondary response to both oxidative injury and immune activation. These findings are consistent with[19],who reported hepatic vascular congestion and intense leukocytic infiltration.

Morphine is metabolized in the liver into compounds like morphine-3-glucuronide, which can have hepatotoxic effects when accumulated, especially with chronic exposure. The accumulation of these metabolites stresses hepatocytes and triggers the activation of local immune cells, which leads to inflammation and liver tissue damage [20]. The kidney of G1 at 5 day showing slight alteration with fragmentation of glomerular tuft (Figure 1-8). In addition the presence slight RBCs in urinary tubules with vaculation of glomerular tuft and degenerative changes of epithelial lining in urinary tubules and widening of Bowman's space (Figure 1-9). These findings support [21], who found that tubular dilatation and vacuolar degeneration in tubular epithelial cells closely match experimental models of cystic tubulopathy, a pathological condition characterized by tubular lumen epithelial degeneration. They also support [22], who suggested that oxidative stress may be a key factor in morphine-induced nephrotoxicity. The idea that morphine damages renal function not only by directly harming cells but also by encouraging inflammation and oxidative damage is supported by the observation of hydropic and vacuolar degeneration, bleeding, and vascular congestion.

While The kidney of G2 at 6-16 day showing infiltration of inflammatory cells with cystic dilatation and present of protonatious material of urinary tubules (Figure1-10) .In addition showing interstitial infiltration of inflammatory cells with focal aggregation in necrotic parenchyma (Figure 1-11). In addition infiltration of inflammatory cells with congestion of blood vessel with cystic dilation neutrophil and mononuclear cell (Figure 1-12) .

These findings are consistent with those of [23],who found that administering morphine caused cystic tubulopathy, renal tubule vacuolation, proteinaceous cast accumulation, and inflammatory infiltration. These alterations imply that morphine not only compromises the structural integrity of renal tubules but also triggers a potent inflammatory response that exacerbates renal damage over time.

Additionally, the infiltration of inflammatory cells and focal necrosis seen in both studies point to an ongoing immune-mediated injury that is probably made worse by oxidative stress and the immunomodulatory effects of morphine. The presence of proteinaceous material within the tubules as seen in both the current study may indicate impaired tubular reabsorption and filtration barrier dysfunction[24].According to the histological alterations seen, morphine has direct nephrotoxic effects, particularly after extended exposure, and these effects are mediated by a confluence of vascular impairment, inflammation, and tubular injury [25].

The kidney of G3 at 17-21 day showing congestion and infiltration of inflammatory mononuclear cells with widening of intertubular spaces (Figure 1-13). In addition the presence vaculation, congestion, protonatious material with infiltration of inflammatory cells and cystic dilatation (Figure 1-14). These findings are consistent with those of [21], who documented renal tubule dilatation, peritubular congestion, proteinaceous casts in the tubular lumen, infiltration of mononuclear inflammatory cells,

and bleeding. These alterations point to morphine's long-term nephrotoxic effects, which may cause functional abnormalities in the nephron units. Tubular damage, interstitial inflammation, and the eventual development of fibrosis and renal failure may be facilitated by elevated glomerular permeability and protein leakage into the tubules [26].

These findings concur with those of [27],who noted significant tubular dilatation in addition to vacuolar and hydropic degeneration in the epithelial cells lining the renal tubules. These changes are thought to be linked to oxidative stress and mitochondrial dysfunction, which results in ionic and fluid imbalance within the cells and the formation of intracellular vacuoles due to water accumulation.

These structural changes are thought to be obvious signs of acute tubular injury, most likely brought on by the direct toxic effects of morphine or its metabolic byproducts [28].In people with compromised antioxidant defenses or pre-existing renal conditions, where compensatory mechanisms are insufficient to counteract the injury, morphine, particularly when administered over a medium to long duration, may contribute to direct tubular toxicity, which is an early phase of drug-induced nephropathy [29].

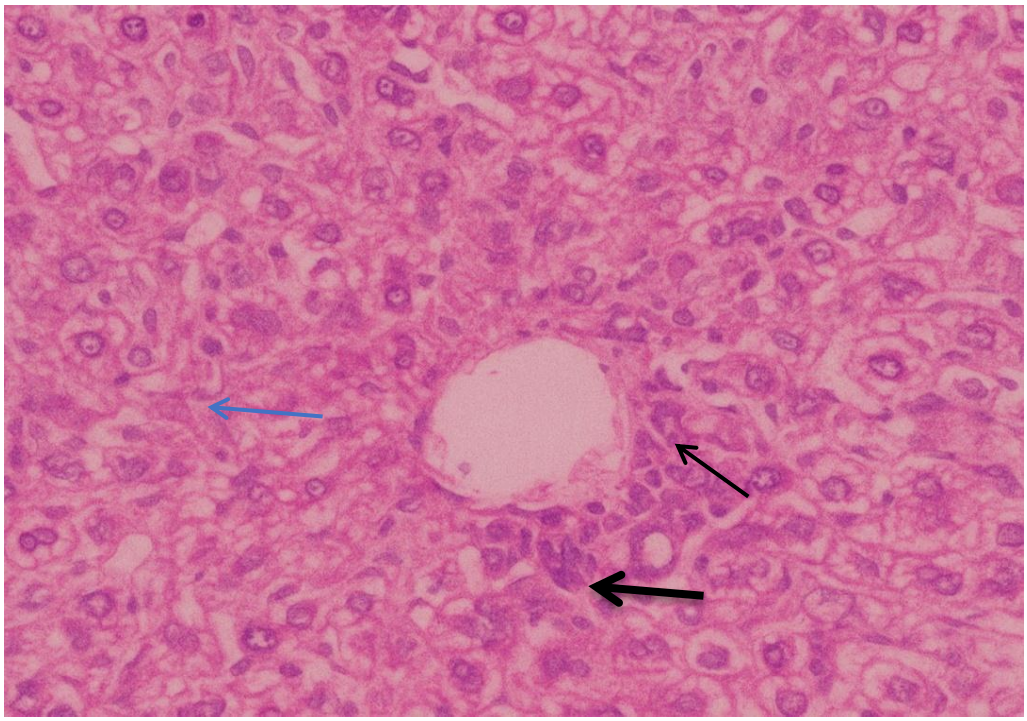

Figure 1-1:Histopathological section of Rat liver tissue from (G1) following 5 days showing slight degenerative changes of hepatocyte(blue arrow) with focal aggrcation of inflammatory cell adjacent central vein(black arrow) (H&E 10X).

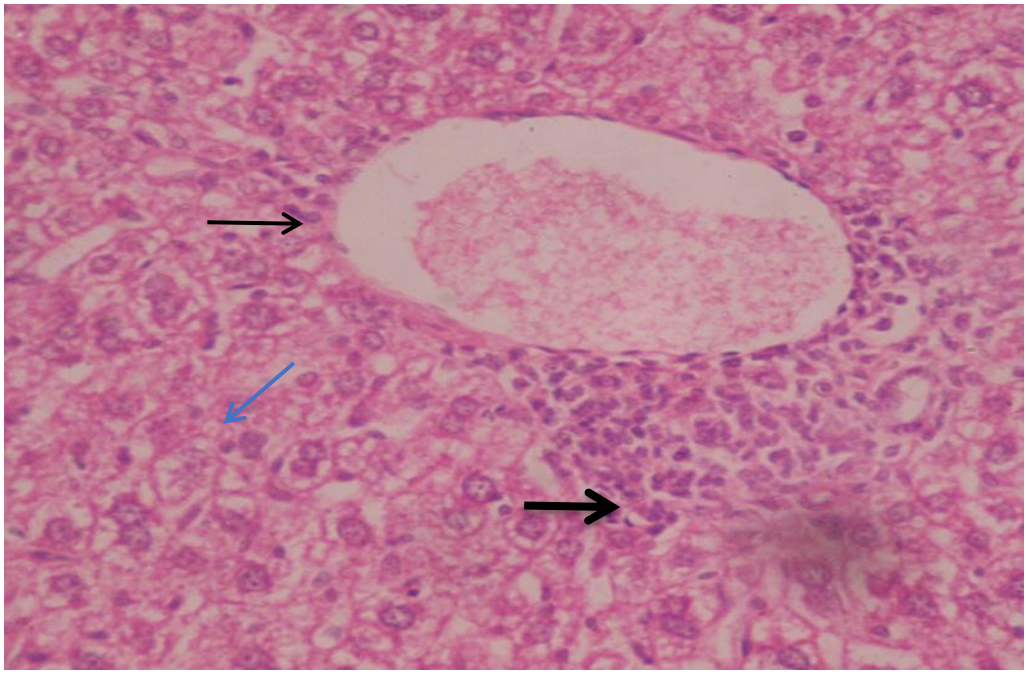

Figure 1-2: Histopathological section Rat liver tissue from (G1) following 1-5 days, showing dilation of central vein and aggregation of mononuclear inflammatory cells adjacent to central vein (black arrow)with degenerative changes of hepatocyte (blue arrow) H&E **40X).**

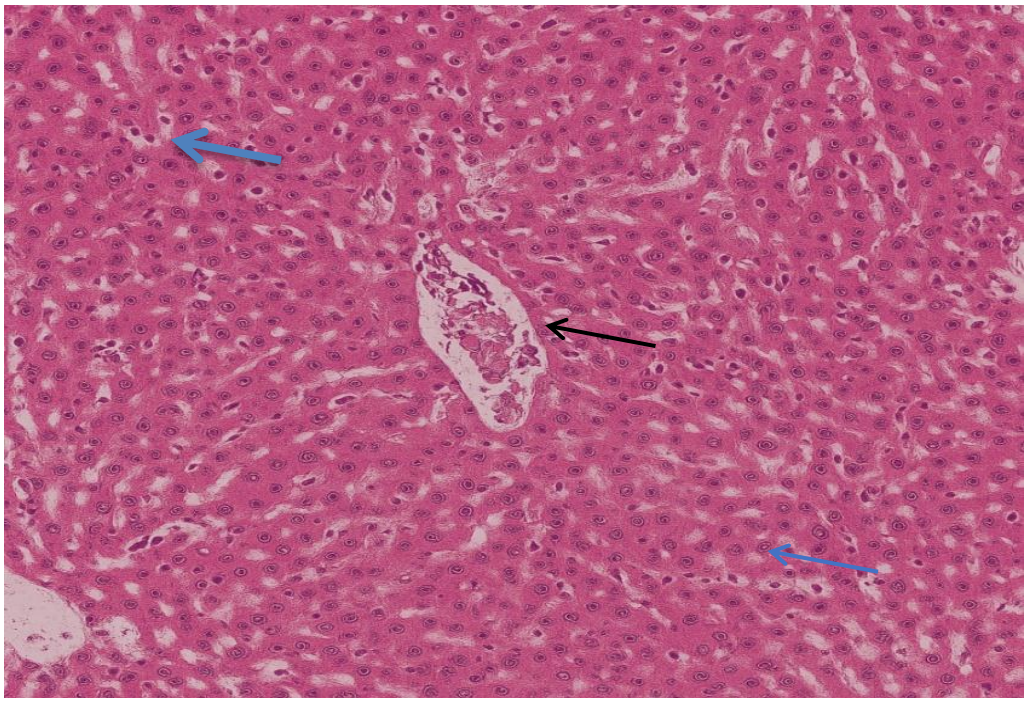

Figure 1-3: Histopathological section of Rat liver tissue from (G2) following 6-16 days, showing infiltration of inflammatory cells with activation of kuffer cells vascular congest (black arrow) and dilated of central vein (blue arrow) (H&E 40X).

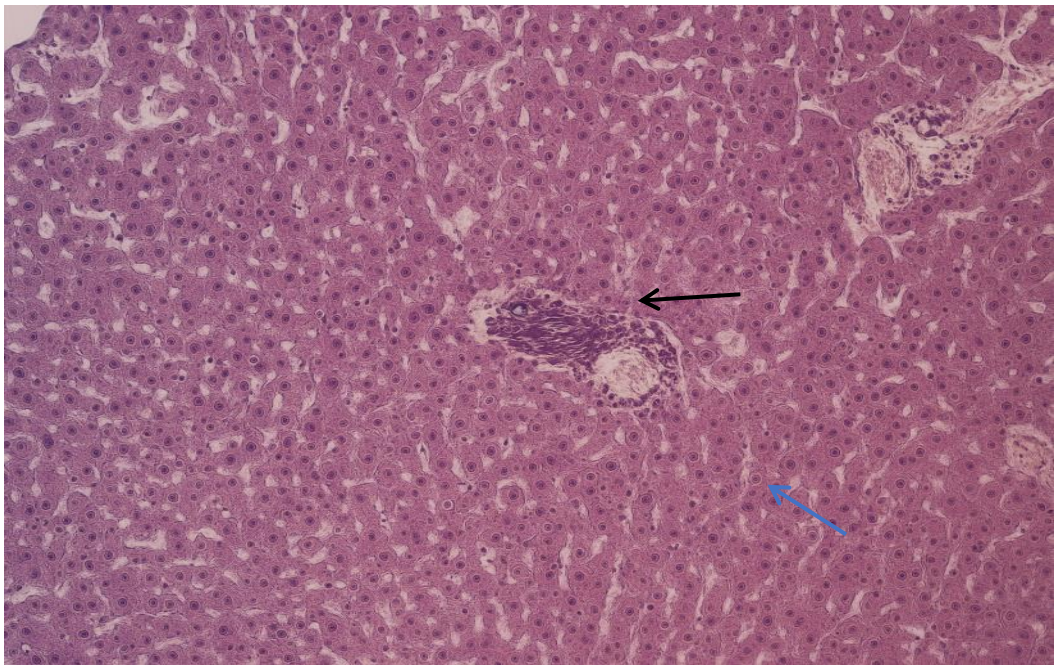

Figure 1-4: Histopathological section of Rat liver tissue from (G2) following 6-16 days, showing sever aggregation adjacent pericentral of inflammatory cells mononuclear (black arrow) and activation of kuffer cell (blue arrow) (H&E 40X).

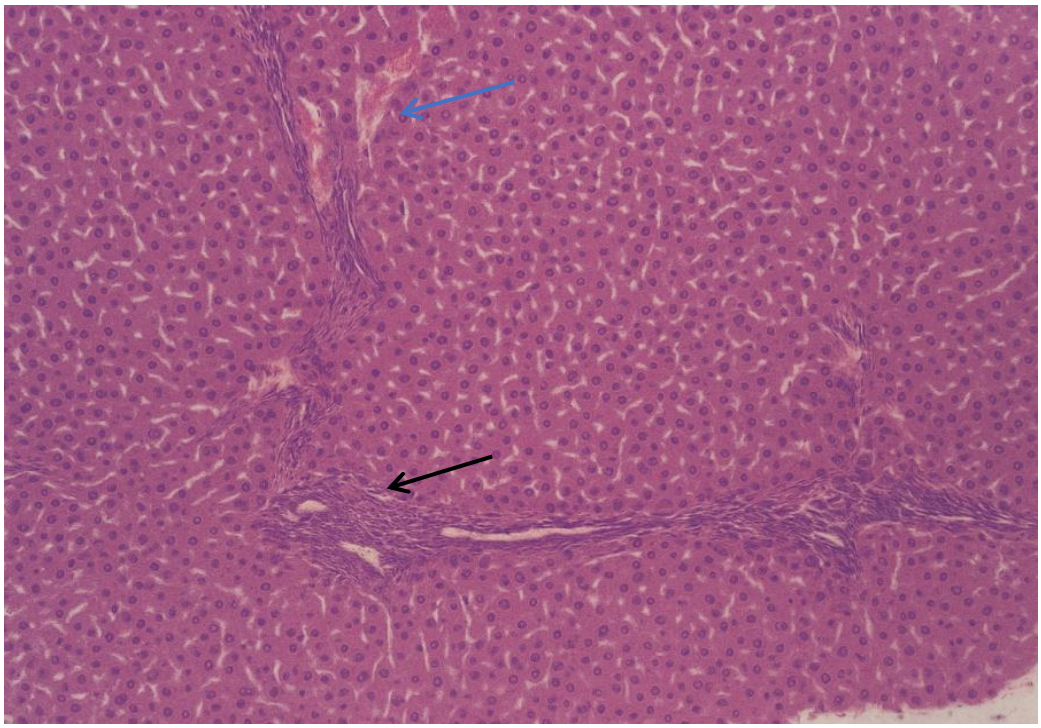

Figure 1-5 :Histopathological section of Rat liver tissue from (G3) following 17-21days, showing sever infiltration of inflammatory cells in portal area(black arrow) with congested of central vein (blue arrow) (H&E 40X).

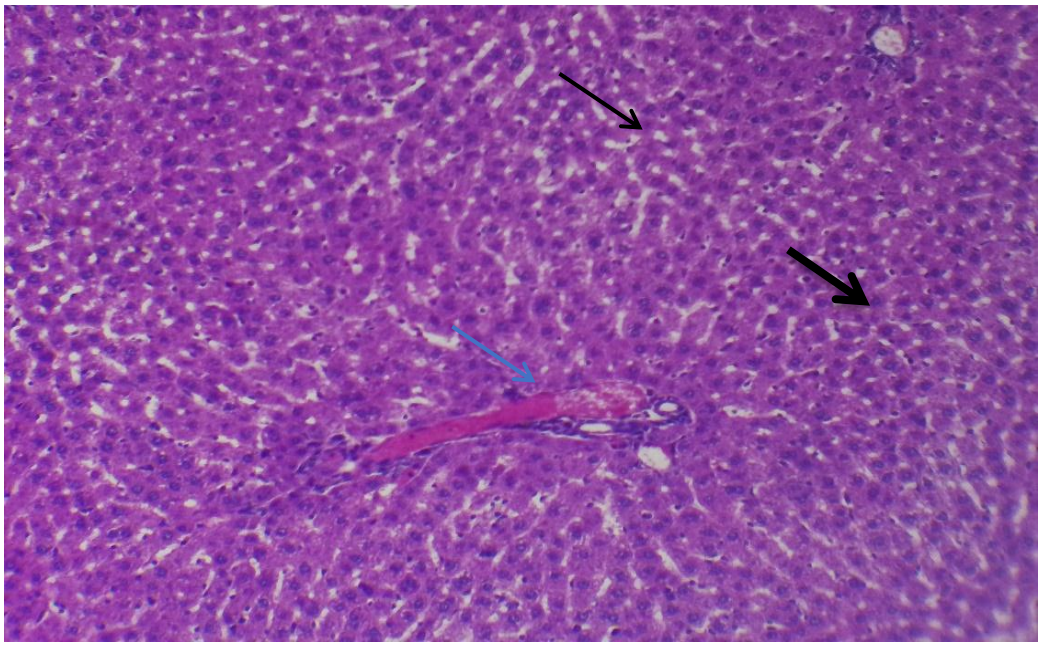

Figure 1-6:Histopathological section of Rat liver tissue from (G3) following 17-21 days, showing infiltration of inflammatory cells in central vein with activation of kuffer cells (black arrow)and congestion of blood vessel (blue arrow) (H&E 10&40X).

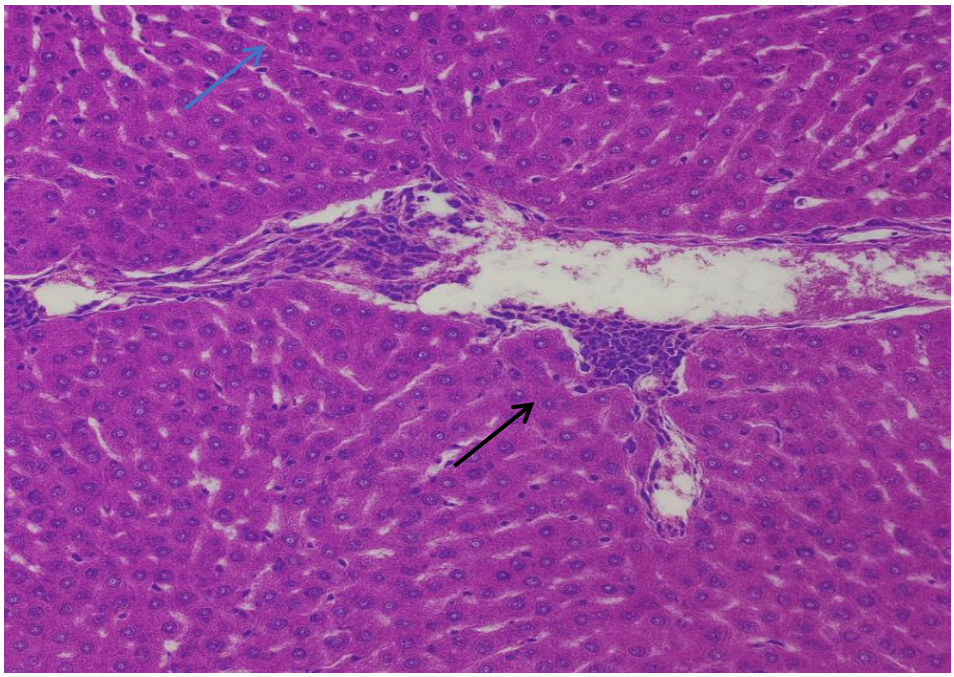

Figure 1-7:Histopathological section of Rat liver tissue from (G3) following 17-21 days, showing dilation of portal area with filtration of inflammatory in portal area (black arrow)and activation of kuffer cells(blue arrow) (H&E 10&40 X).

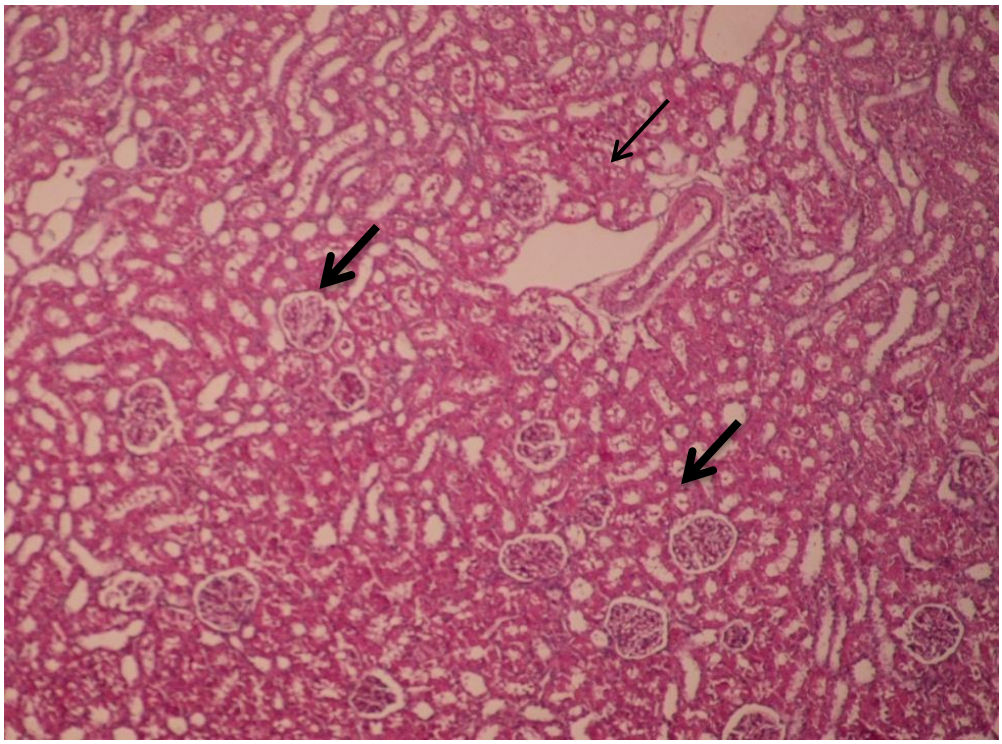

Figure 1-8:Histopathological section of Rat kidney tissue from (G1) following 5 days, showing slight alteration with fragmentation of glomerular tuft (black arrow) (H&E stain 40X).

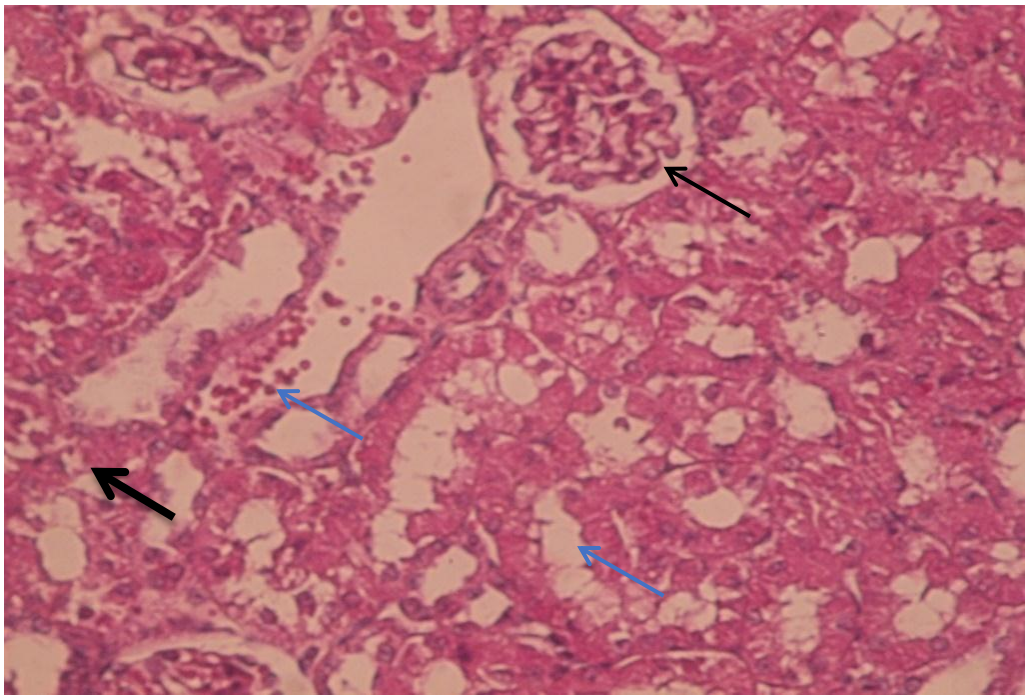

Figure 1-9:Histopathological section of Rat kidney tissue from (G1) following 5 days showing slight RBCs in urinary tubules(red arrow) with vaculation of glomerular tuft with widening of Bowman's space (black arrow) and degenerative changes of epithelial lining in urinary tubules (blue arrow) (H&E stain 40X).

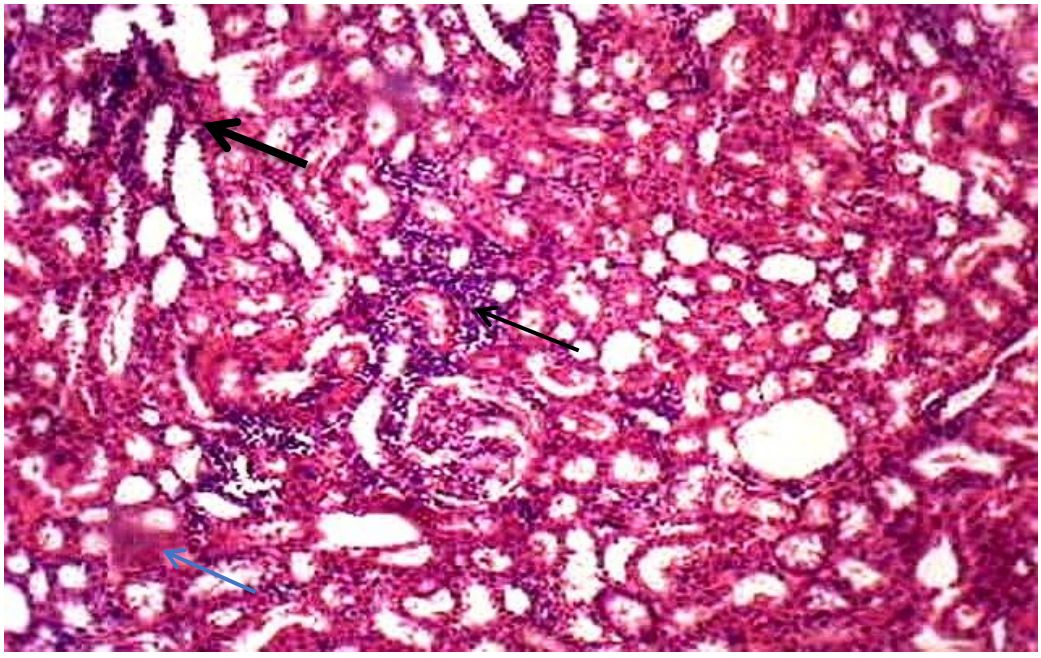

Figure 1-10: Histopathological section of Rat kidney tissue from (G2) following 6-16 days showing infiltration of inflammatory cells with cystic dilatation(black arrow) and present of protonatious material of urinary tubules(blue arrow) (H&E stain 40X)

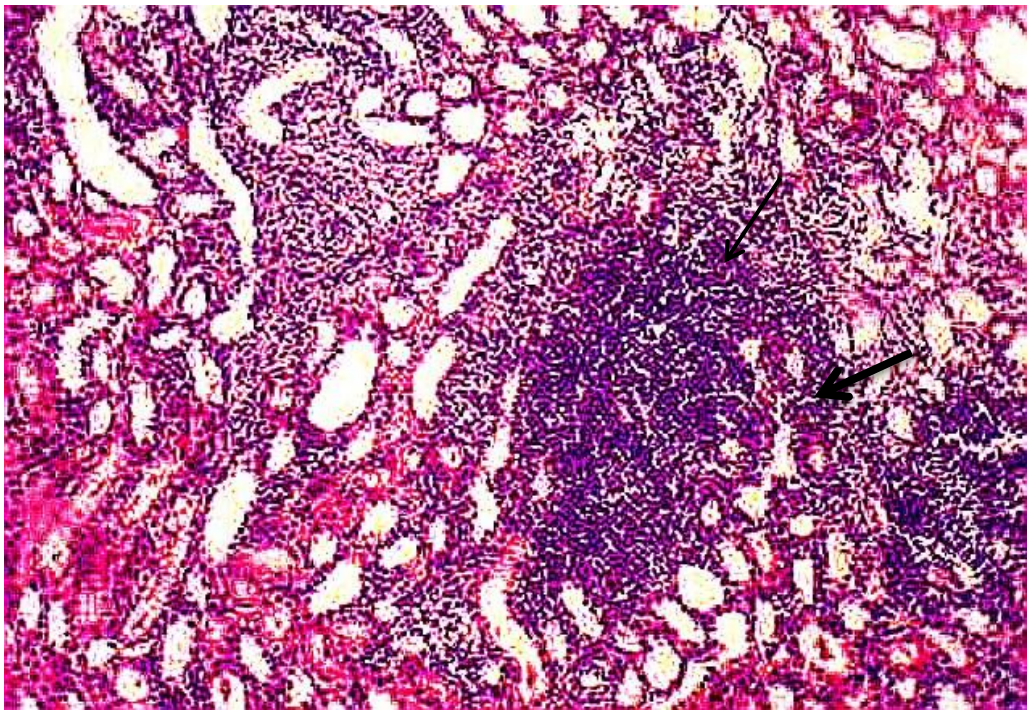

Figure 1-11:Histopathological section of Rat kidney tissue from (G2) following 6-16 days, showing interstitial infiltration of inflammatory cells with focal aggregation in necrotic parenchyma(black arrow) (H&E stain 40X).

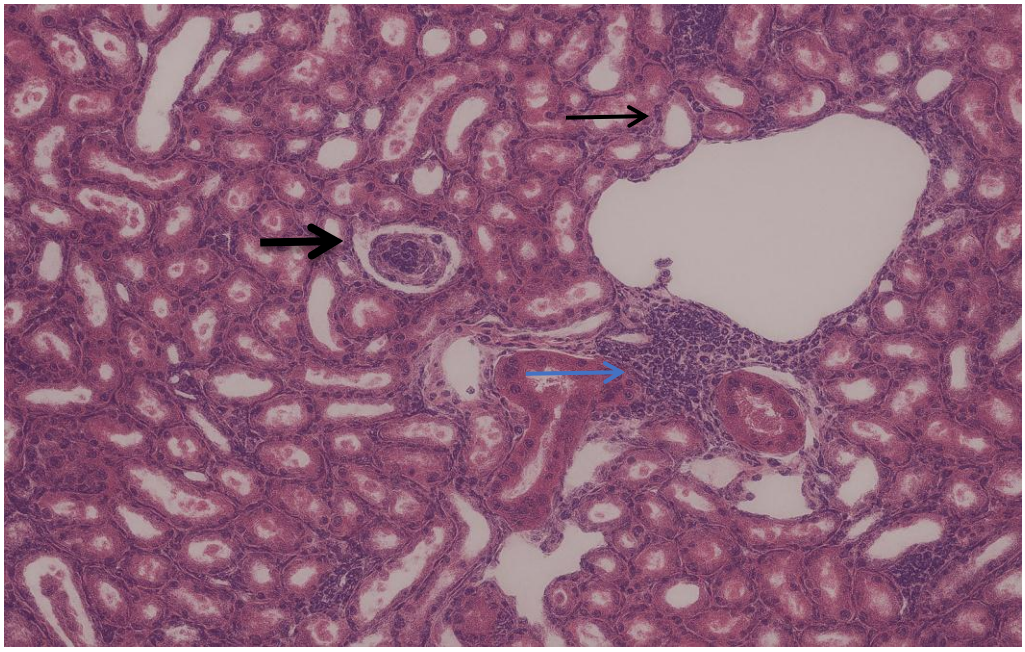

Figure:1-12: Histopathological section of Rat kidney tissue from (G2) following 6-16 days, showing infiltration of inflammatory cells neutrophil and mononuclear cells (blue arrow)with congestion of blood vessel with cystic dilation(black arrow) (H&E 40X).

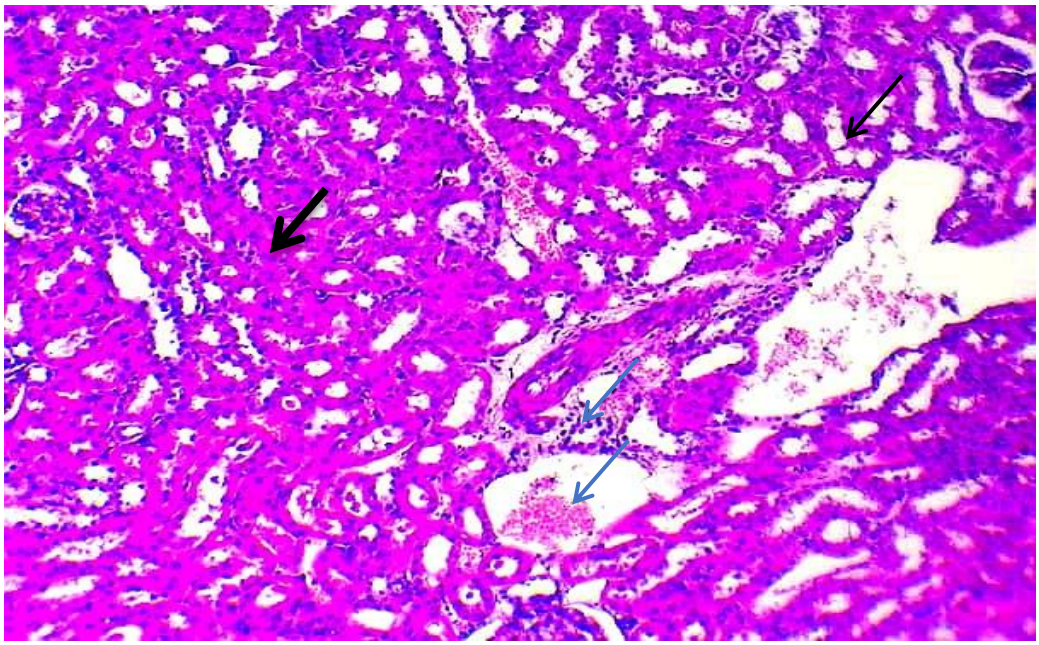

Figure 1-13:Histopathological section of Rat kidney tissue from (G3) following 7-21 days showing congestion and infiltration of inflammatory mononuclear cells (blue arrow)and interstitial edema with widening of intertubular spaces (black arrow) (H&E stain 40X).

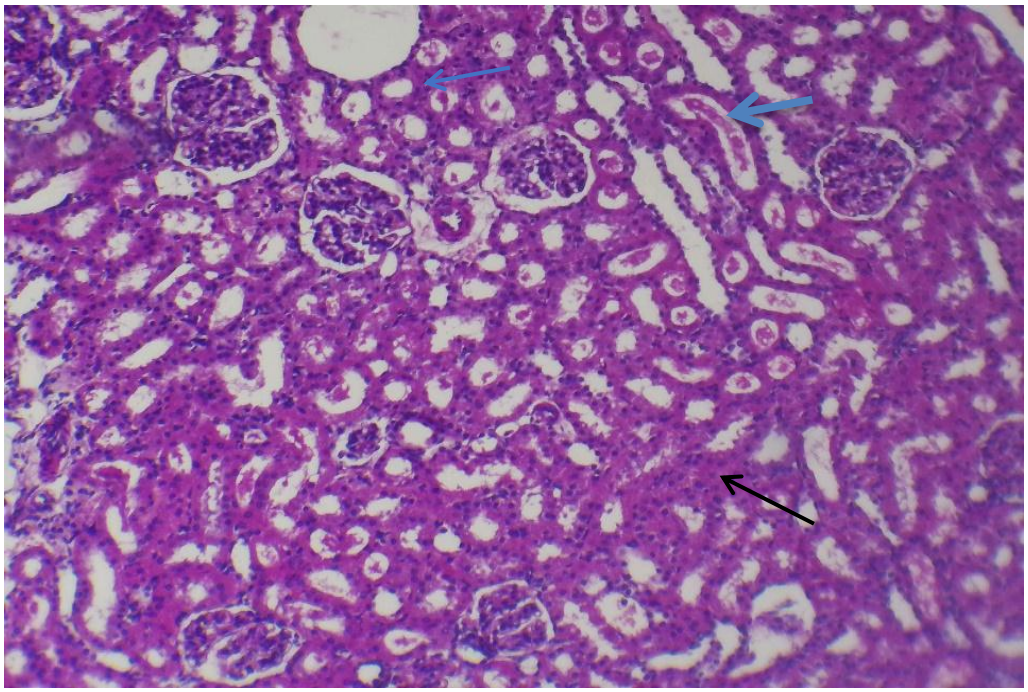

Figure 1-14 :Histopathological section of Rat kidney tissue from (G3) following 7-21 days showing vaculation ,protonatious material (red arrow)with infiltration of inflammatory cells and cystic dilatation(black arrow) H&E 10 &40X).

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