Research Article

EVALUATION OF GENTAMICIN INDUCED NEPHROTOXICITY IN CANINE: CLINICAL, HEMATOLOGICAL, BIOCHEMICAL, ULTRASONOGRAPHIC AND HISTOPATHOLOGICAL FINDINGS

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ABSTRACT: The present study was planned to evaluate the clinical, hematological, biochemical, ultrasonographic, and histopathological alterations in kidneys and other organs in dogs injected with gentamicin. Six mongrel dogs were experimentally injected I/M with gentamicin by 20 mg/kg BW daily till the appearance of renal failure signs. Blood samples were collected before and after induction (on day 9 and day 12). Hematological results revealed a non-significant decrease in hemoglobin and RBC’s, a significant decrease in hematocrit values in both days, a non-significant increase in TLC at day 12 but a non-significant decrease at day 9, a significant increase in non-segmented neutrophil at day 9 but non-significant decrease at day 12, non-significant decrease in lymphocyte at day 9 but non-significant increase at day 12, a significant increase in monocyte at day 9 but non-significant decrease at day 12. Biochemical results revealed significant increase in urea and creatinine at day 12, non-significant increase at day 9. Urinalysis results indicated an increase in leukocyte, albumin, glucose, pus cells and RBCs. Urine culture revealed isolates of E.coli, Proteus and Staphylococcus bacteria. Ultrasonographic results showed increased echogenicity of renal cortex with loss of corticomedullary junction at day 9. At day 12 ultrasonographic results revealed intensive hyper-echogenicity of renal cortex, increased echogenicity of the right renal cortex as compared with spleen, and the renal cortex of right kidney appeared more hyperechoic when compared with liver. Histopathological examination of kidneys showed different degenerative changes, hemorrhages, nephritis, and other organs revealed degeneration, inflammation and hemorrhages. The findings indicted that despite the therapeutic effect of gentamicin in treating the kidney infection, it may have an adverse effect on kidneys and other organs especially with massive doses.

Key words: Gentamicin, Dog, Nephrotoxicity, Ultrasonography, Hematobiochemical, Histopathology.

INTRODUCTION

Kidneys represent a major target for toxic xenobiotics due to the role in the control of body fluid and electrolyte homeostasis, high blood perfusion rate (20% of cardiac output), and ability to extract, metabolize, secrete and concentrate toxic compounds make the kidneys extremely vulnerable to a wide variety of toxic materials (Jennings et al. 2003). Gentamicin is an aminoglycoside drug used to treat bacterial infection (mainly Gram-negative bacteria). Although its potency against bacterial infection, its clinical use is limited due to its nephrotoxic effect (Abd-Elhalim et al. 2021).

Acute renal injury is a form of kidney damage that occurs within a brief period of time may be a few hours or days, with signs of abdominal pain, polyuria, oliguria, and other symptoms that occur in the case of acute renal damage (Fauzi et al. 2020). Acute kidney injury is characterized by a sudden decrease in renal filtration, as well as elevated serum creatinine, acute uremia, and an increase in urine volume (Legatti et al. 2018). Gentamicin-induced nephrotoxicity is characterized by direct proximal tubular necrosis (Josiah et al. 2020). In addition, gentamicin may induce vascular, glomerular, and tubular damage due to its accumulation in the proximal renal tubules in the cortex (Dajem et al. 2020). The renal cortex, specifically glomerules and proximal tubules, are targeted in gentamicin induced renal damage (Stojilkovic et al. 2018). Diagnosis of gentamicin-induced renal failure based on the clinicopathological symptoms of renal dysfunction (polyuria, azotemia, and

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enzymuria) and history of nephrotoxic drug administration (Papich 2005), hematological, biochemical analysis (uremia), ultrasonography, and histopathological examination of deceased dogs (Balakrishnan et al. 2013). Proteinuria, elevated serum urea and creatinine levels, and a decreased glomerular filtration rate are all symptoms of gentamicin induced nephrotoxicity (Balakumar et al. 2010). They all contribute to acute kidney failure (Stojiljkoviæ et al. 2018).

The present study was conducted to estimate the possible renal damage caused by gentamicin administration in dogs and also the effect of this drug on other body organs by evaluating the hematological, biochemical, urinalysis, ultrasonographic parameters in kidneys, and also histopathological alterations in kidneys and other body organs.

MATERIALS AND METHODS
Chemicals
Kits for estimation of creatinine, urea and uric acid were purchased from the Biodiagnostic company (Dokki, Giza, Egypt).

Animals
Six apparently healthy mongrel dogs aged between 8 -12 months and weighed from 15-25 kg of different sex were used. These dogs were housed in the clinical hospital of the Faculty of Veterinary Medicine-Cairo University. Dogs were feed commercial dry food in addition to fresh food with free access to water. In addition, all dogs were exposed to complete and comprehensive clinical examination including temperature, pulse rate, respiratory rate, they treated with deworming (Praferan®), anti-flea (Dectomax®, Pfizer) and vaccinated against rabies (Defensor®, Pfizer Company) before the beginning of the experiment.

Experimental design
Experimental induction of renal failure in dogs using gentamicin sulphate 156.25 (Gentacure®, Pharma Swede) was done two weeks after the initial accommodation. Animal handling and treatment procedures were conducted according to the guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and approved by a research ethics committee of the Faculty of Veterinary Medicine-Cairo University (VetCU10102019094). Dogs were injected with gentamicin I/M by a dose of 20 mg/kg BW daily till the appearance of renal failure signs.

Clinical signs and samples collection
Clinical signs were observed and recorded daily during the experimental period. Blood samples were collected before (at day zero) and after the treatment (at day 9 and at day 12) for assessment of red blood corpuscles (RBC’s), hemoglobin (Hb), hematocrit (PCV), total and differential leukocytic counts. Serum was separated and used for the detection of urea, creatinine, and uric acid. At necropsy, kidneys, urinary bladder, spleen, colon, liver, and heart were removed, washed with physiological saline, collected, and fixed in 10% buffered neutral formalin solution for histopathological examination.

Table 1. Hematological findings of dogs at day 9 and 12 of the experiment (Mean ± SEM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>At day 0</th>
<th>At day 9</th>
<th>At day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (million/c.mm)</td>
<td>6.45±0.250 a</td>
<td>6.61±0.240 a</td>
<td>6.45±0.372 a</td>
</tr>
<tr>
<td>HB (gm)</td>
<td>14.08±0.546 a</td>
<td>13.67±0.669 a</td>
<td>13.08±0.792 a</td>
</tr>
<tr>
<td>PCV%</td>
<td>48.17±2.30 a</td>
<td>42.67±1.42 b</td>
<td>43.00±2.11 b</td>
</tr>
<tr>
<td>Platelets (1000/c.mm)</td>
<td>250.67±13.41 a</td>
<td>224.83±10.54 a</td>
<td>261.67±12.38 a</td>
</tr>
<tr>
<td>MCV(fl)</td>
<td>79.03±0.574 a</td>
<td>64.55±0.973 b</td>
<td>66.76±0.696 b</td>
</tr>
<tr>
<td>MCH(pg)</td>
<td>21.80±0.273 a</td>
<td>20.57±0.317 a</td>
<td>28.03±0.223 a</td>
</tr>
<tr>
<td>MCHC</td>
<td>29.26±0.518 a</td>
<td>31.93±0.825 b</td>
<td>30.30±0.404 b</td>
</tr>
<tr>
<td>WBCs (1000/c.mm)</td>
<td>15.63±1.13 ab</td>
<td>13.66±2.28 b</td>
<td>19.03±1.43 a</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>2.33±0.210 a</td>
<td>3.33±0.666 a</td>
<td>2.67±0.666 a</td>
</tr>
<tr>
<td>Segmented %</td>
<td>55.50±6.08 a</td>
<td>57.17±1.47 a</td>
<td>46.00±4.84 a</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>33.83±6.55 ab</td>
<td>20.00±1.03 b</td>
<td>44.00±5.39 a</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>5.17±0.792 b</td>
<td>9.50±1.31 a</td>
<td>4.83±0.872 b</td>
</tr>
</tbody>
</table>

Data with different letters in the same row are significantly different at p< 0.05.
Hematological and biochemical analysis

Red blood corpuscles (RBC’s), hemoglobin (Hb), hematocrit (PCV), total leukocytic counts (TLC), and differential leukocytic counts (DLC) were determined according to (Schalm 1986) using a veterinary hematology analyzer. Urea, creatinine, and uric acid were measured with commercial kits by a spectrophotometer (Apple 302, USA) (Willard et al. 2011).

Urinalysis

Urinalysis was performed via urine dipstick using samples that were collected through catheterization under complete aseptic conditions for microbiological estimation. Combi-10 SGL urine strip test was used for a rapid urinalysis and microscopic examination of urine sediment to detect abnormal crystals and inflammatory cells. Urine sample cultured principally in nutrient broth at 37°C for 18-24 hr, then sub-cultured onto MacConkey and Mannitol salt agar by streak plate method to detect the colony morphology.

Ultrasonography

The ultrasonographic examination before (at day zero) and after the injection (at day 9 and at day 12) were done using Esaote my lab one vet. Ultrasound a 6.5 to 10 MHz micro convex probe. The hair at the ventral abdominal was clipped to the level of the pubic bone, and the ultrasonic gel was applied to the skin. A trans-abdominal approach with the animal in dorsal recumbency was preferred.

Histopathological examination

Necropsy was performed immediately after death at day 12 for all experimental dogs and postmortem examination was done. Specimens from kidneys, urinary

Table 2. Kidney function of dogs at day 9 and 12 of the experiment (Mean ± SEM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>At day 0</th>
<th>At day 9</th>
<th>At day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>16.33 ±0.912 b</td>
<td>20.83 ±2.53 b</td>
<td>95.33 ±3.68 a</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.78 ±0.059 b</td>
<td>0.81 ±0.118 b</td>
<td>6.34 ±1.23 a</td>
</tr>
<tr>
<td>Uric acid (mmol/l)</td>
<td>0.67 ±0.078 a</td>
<td>1.61 ±0.638 a</td>
<td>1.64 ±0.298 a</td>
</tr>
</tbody>
</table>

Data with different letters in the same row are significantly different at p< 0.05.

Table 3. Urinalysis in dogs at day 9 and 12 of the experiment (Mean ± SEM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>At day 0</th>
<th>At day 9</th>
<th>At day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.0±0.516 a</td>
<td>6.33±0.21 a</td>
<td>6.66±0.221 a</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1030±3.87 b</td>
<td>1032±2.50 b</td>
<td>1022±0.846 a</td>
</tr>
<tr>
<td>Leukocyte</td>
<td>Nil b</td>
<td>+ a</td>
<td>+++ b</td>
</tr>
<tr>
<td>Nitrite</td>
<td>Nil b</td>
<td>Nil a</td>
<td>+ b</td>
</tr>
<tr>
<td>Protein</td>
<td>Nil b</td>
<td>+ b</td>
<td>+++ a</td>
</tr>
<tr>
<td>Glucose</td>
<td>Nil b</td>
<td>4 b</td>
<td>+++ a</td>
</tr>
<tr>
<td>Ketones</td>
<td>nil</td>
<td>Nil b</td>
<td>nil b</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>nil</td>
<td>Nil b</td>
<td>nil b</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>nil</td>
<td>Nil b</td>
<td>nil b</td>
</tr>
<tr>
<td>Blood</td>
<td>+ a</td>
<td>+++ b</td>
<td>+++ b</td>
</tr>
</tbody>
</table>

Data with different letters in the same row are significantly different at p< 0.05.

Fig. 1. Ultrasonographic findings of dogs kidneys.
[(a) Enlarged kidney with slight hyperechoic renal cortex and loss of corticomedullary junction (day 9). b) Sub capsular edema, enlarged kidney with hyperechoic cortex suggesting acute nephritis accompanied with ascites (day 12). c) Mild increase in renal cortex echogenicity, slight hyperechoic than liver suggesting acute cystitis (day 12)].
bladder, spleen, colon, liver and heart were collected, fixed in neutral buffered formalin 10%, washed, dehydrated, cleared and embedded in paraffin. The paraffin embedded blocks were sectioned at 5 micron thickness and stained with Hematoxylin and Eosin (H&E), alizarin red stain, periodic acid Schiff (PAS), Prussian blue and Masson’s trichrome stain (MTC) for histopathological examination. The sections were examined by a light microscope (Olympus BX50, Japan) (Bancroft et al. 2012).

**Statistical analysis**

For statistical analysis, the SPSS computer program was used. The statistical analysis was carried out by one-way ANOVA setting the probability level to p<0.05, post hoc analysis of group differences was performed by LSD test. The treated groups were compared both with each other and with untreated control group. Data was expressed as mean ±SEM.

**RESULTS AND DISCUSSION**

Gentamicin is usually used as a therapeutic agent against infections. But long-term exposure of gentamicin may induce hepatorenal toxicity (Jannat et al. 2018). In our study, six mongrel dogs were used, they were injected with gentamicin I/M by a dose of 20 mg/kg BW daily. Clinical signs, hematology, biochemistry, sonography and histopathology were evaluated.

**Clinical signs**

There were general non-specific signs at day 9 of treatment as, inappetance, lethargy, arched back and hematuria. At day 12, rapid deterioration and multisystem dysfunction occur including anorexia, vomiting, polyuria/polydypsia, ulcers in mouth and tongue, edematous swelling in limbs and joints, uremia, ascites, nervous signs, these observations are in line with Tripathi and Mehta (2010). Other clinical signs including hematochezia, ocular discharge, generalized lymphadenopathy, dyspnea, tenesmus, ataxia, and otitis were represented by one dog and these signs were in compliance with (Dunaevich et al. 2020). During experiment, there was no improvement and dogs was worthen then died, and this is in agreement with (Jannat et al. 2018). Edema and ascites mainly secondary to hypoalbumenemia and sodium retension as recorded by Tilley and Smith (2015).

**Hematological findings**

At day 9, there was significant decrease in hematocrit, significant increase in non-segmented neutrophils and monocytes and non-significant decrease in TLC and lymphocytes. At day 12, there was significant decrease in hematocrite, non-significant increase in TLC and lymphocytes and non-significant decrease in non-segmented neutrophil and monocyte (Table 1) (Kelahan et al. 2019, Allaam et al. 2012). Increase in TLC mainly related to multi bacterial infections that may be present at urinary tract, some of these bacteria may respond to gentamicin treatment leading to decrease in TLC at day 9. But other bacteria such as *Staphylococcus* spp. doesn’t respond to treatment which may be due to resistance and further caused the increase in TLC at day 12 (Methicillin resistant *Staphylococcus aureus*). Significant decrease in hematocrite at day 9 and day 12 were in agreement with that recorded by Allaam et al. (2012). Long-term high-dose gentamicin exposure affects bone marrow hemopoietic cells and reduces erythrocyte production (Jannat et al. 2018).

**Biochemical findings**

At day 9, there was non-significant increase in urea and creatinine and this result was in agreement with Gberindyer et al. (2015). Cortical echogenicity has increased in dogs with normal serum creatinine level, suggesting that this change may occur early in the course of renal diseases in dogs (Perondi et al. 2020). Early diagnosis of renal injury may be difficult to be detected as animals were clinically apparent healthy. Renal dysfunction tends to have advanced kidney disease, by the time clinical or biochemical changes were noted (McGrotty 2008). At day 12, there was significant increase in urea and creatinine levels (Table 2), the increased urea and creatinine levels reflect reduction of glomerular filtration rate (Azouz and Korany 2021, Madkour et al. 2021), and this result was in agreement with Abd-Elhalim et al. (2021) and Maqtoof et al. (2020). Gentamicin related nephrotoxicity produces obvious and outwardly irreversible renal injury prior to increase in either blood urea nitrogen or serum creatinine. The activity of a diversity of urinary enzymes is elevated before, or at the same time as, the morphologic appearance of cell damage (Spangler et al. 1980).

**Urinalysis findings**

Urinalysis revealed presence of leukocyte, albumin, nitrite, glucose, pus cells and RBCs in urine at day 9 and day 12 with significant decrease in specific gravity, significant increase in pH between day 9 and day 12 and significant increase in leukocyte, glucose, nitrite and protein along with presence of amorphous urate and phosphate were observed. Urine culture on MacConkey
Fig. 2. Photomicrograph of dog kidneys, intoxicated with gentamicin showing.
(a) Coagulative necrosis of tubular lining epithelium (arrows) (H&E, X200). b) Calcium salt precipitation on necrosed tubular epithelium (arrows) (H&E, X200). c) Alizarin red stains calcium salts with red color (arrow) (Alizarin red, X100). d) Renal tubules showing cystic dilatation with presence of esinophilic protein casts inside tubular lumen (arrow) (H&E, X200). e) Cystic dilatation of most renal tubules in cortex (arrows) with presence of esinophilic protein casts inside tubular lumen (H&E, X100). f) Absence of brush border staining in PAS-stained sections (arrow) (PAS, X200).
Fig. 3. Photomicrograph of dog kidneys, intoxicated with gentamicin showing.

[(a) Thickening of glomerular tuft basement membrane (arrow) (H&E, X200). b) Thickening of glomerular tuft basement membrane by PAS (arrow) (PAS, X200). c) Thickening of Bowman’s capsule by PAS (arrow) (PAS, X200). d) Presence of golden brown pigment in interstitial tissue of renal cortex (arrow) (H&E, X200). e) Presence of blue hemosiderin granules in renal cortex by Prussian blue reaction (arrow) (Prussian blue, X200). f) Hyalinosis of cortical interstitial tissue with infiltration of mononuclear inflammatory cells (arrow) (H&E, X200)].

agar revealed presence of isolates of *E. coli* Lactose fermenting bacteria that appeared as pink colonies (acidic pH) while *Proteus* sp. non-lactose fermenting bacteria appeared as pale colonies. *Staphylococcus* sp. appeared as yellow colonies with yellow zones on Mannitol Salt Agar (Table 3).

Ultrasonographic findings

At day 9, ultrasonographic results revealed increased the echogenicity of renal cortex with loss of corticomedullary junction (Fig. 1a) (Allaam et al. 2012). At day 12, there was intensive hyper echogenicity of renal cortex, increased echogenicity of the renal cortex as compared with spleen, and the renal cortex of right kidney appears
Fig. 4. Photomicrograph of dog intoxicated with gentamicin.

[(a) Urinary bladder showing vacuolation and karyopyknosis of some urothelium covering (black arrow), sub epithelial connective tissue is showing dilatation and congestion of their blood capillaries (white arrow) (H&E, X 200). b) Urinary bladder, extravasations of large number of RBC’s in-between sub epithelial connective tissue fibers (arrow) (H&E, X 200). c) Spleen is showing lymphoid depletion in white pulp (arrow) (H&E, X100). d) Spleen, splenic hemorrhage with presence of hemosiderin-laden macrophages (arrows) (H&E, X400). e) Spleen, presence of large number of blue-stained hemosiderin granules by Prussian blue reaction (arrow) (Prussian blue, X 200)].
more hyperechoic when compared with liver (Fig. 1b and 1c) (Burti et al. 2020).

**Postmortem findings**

Gross picture of kidneys of treated dogs showed small sized kidney with loss of cortico-medullary junction. Other examined organs showed degeneration, congestion and hemorrhages (Sharma et al. 2016).

**Histopathological findings**

Kidney

Histopathological examination of kidneys revealed presence of thickening of renal capsule with fibrosis, there was degeneration and coagulative necrosis of considerable number of lining epithelium of renal tubules especially in cortex (Fig. 2a) and this result was in accordance to Abd-Elhalim et al. (2021), moreover there was a calcium salts precipitation on degenerated renal tubules (Fig. 2b) which confirmed by alizarin red staining (Fig. 2c) (Randjelovic et al. 2017). The mechanism of gentamicin-induced nephrotoxicity is unknown. Drug accumulation in epithelial tubular cells triggers a variety of actions, including tubular cytotoxicity. Gentamicin has a long-half life in the renal proximal tubular cells, causing renal damage. Other gentamicin effects such as phospholipidosis, oxidative stress, activation of extracellular calcium sensing receptor, and energetic catastrophe have also been discussed with cell death. The primary retention of gentamicin in proximal tubular cells after the formation of oxygen-associated metabolites and free radicals has been demonstrated to occur before gentamicin-induced nephrotoxicity. Furthermore, gentamicin’s indirect effects, such as decreased renal blood flow and inflammation, can lead to or exacerbate its cytotoxicity. (Fartashvand et al. 2014), some renal tubular lumen were filled with esinophilic protein cast

![Fig. 5. Photomicrograph of dog colon, intoxicated with gentamicin showing.](image-url)
Fig. 6. Photomicrograph of dog liver, intoxicated with gentamicin showing.

[(a) Vacuolar degeneration of some hepatocytes (arrow) (H&E, X400). b) Sinusoidal dilatation and congestion (arrow) (H&E, X400). c) Presence of blue-stained hemosiderin granules between hepatocytes (arrow) (Prussian blue, X400). d) Portal fibrosis (black arrow), hyperplasia of bile duct (white arrow) and congestion of portal blood vessels (C) (H&E, X200). e) MTC-stained liver section is showing proliferation of portal connective tissue (arrow) (MTC, X200)].
Fig. 7. Photomicrograph of dog heart, intoxicated with gentamicin showing.
[(a) Thickening of pericardium by fibrin exudate (f) and neutrophils infiltration (arrow) (H&E, X400). b) Presence of golden brown hemosiderin granules in pericardium (arrow) (H&E, X200). c) Infiltration of inflammatory cells between myocytes (black arrow), note also hemorrhage in-between myocytes (white arrow) (H&E, X200). d) Presence of blue-stained hemosiderin granules between myocytes by Prussian blue reaction (arrow) (Prussian blue, X200). e) Zenker’s necrosis of some myocyte (black arrow), fluid exudation (E) and inflammatory cells infiltration between myocytes (white arrow) (H&E, X200). f) PAS- positively stained section of myocardium indicating glycogen infiltration inside myocytes (arrow) (PAS, X200)].
or even free RBC’s (Fig. 2d) and other revealed cystic dilatation (Fig. 2e) (Dhanarasu et al. 2018), also there was no brush border staining of most renal tubules in PAS- stained sections (Fig. 2f), there was a thickening and/ or congestion of interstitial blood vessels. Renal corpuscles revealed a thickening of their glomerular tuft basement membrane and Bowman’s capsule (Fig. 3a), glomerular and capsular thickening were confirmed by PAS staining that gave positive staining (Fig. 3b and 3c) (Nafiu et al. 2019). The relative PAS staining intensity of proximal tubular brush borders may represent a useful early indicator of nephrotoxicity in renal biopsy material (Spangler et al. 1980). Some renal corpuscles showed atrophied glomerular tuft. Some sections revealed presence of renal tubular regenerating nodules. There was a golden brown granule in some section suggesting presence of hemosiderin pigment (Fig. 3d) which confirmed by Prussian blue stain (Fig. 3e). Interstitial tissue showed hyalinosis with infiltration of mononuclear inflammatory cells (Fig. 3f) (Fauzi et al. 2020, Josiah et al. 2020).

**Urinary bladder**

Some urothelium showed vacuolar degeneration with karyopyknosis in some (Fig. 4a), subepithelial connective tissue showed vascular congestion and severe hemorrhage in between connective tissue fibers (Fig. 4b).

**Spleen**

Histopathological examination of spleen revealed presence of lymphoid depletion in white pulp (Fig. 4c), there were also splenic hemorrhage, hemosiderosis and hemosiderin laden macrophages (Fig. 4d). Hemosiderosis was confirmed by Prussian blue stain (Fig. 4e). Azotaemia causes hemolysis in patients with renal failure. The increased fragility of erythrocytes is considered to be due to the uremic toxins associated with marked elevation of BUN. Furthermore, extensive hemosiderin deposition in the spleen of gentamicin-treated animals supports the shortened life span of erythrocytes in azotaemic conditions (Nagano et al. 1990).

**Colon**

Mucosa revealed focal areas of necrosis infiltrated with mononuclear inflammatory cells (Fig. 5a), there was hyperplasia of goblet cells confirmed by PAS staining (Fig. 5b), there was congestion of proprial blood vessels. Submucosa showed presence of large number of free RBC’s and hemosiderin pigment (Fig. 5c) which is confirmed by Prussian blue stain (Fig. 5d).

**Liver**

Hepatocytes revealed presence of vacuolar degeneration in considerable number of hepatocytes (Fig. 6a), there was a great sinusoidal dilatation (Fig. 6b), hemorrhage and hemosiderosis were evident (Fig. 6c). Concerning portal areas, there were few mononuclear inflammatory cells infiltration, bile duct hyperplasia and congestion of portal blood vessels, also there was a portal fibrosis (Fig. 6d) which confirmed by MTC staining (Fig. 6e). and this result was compatible with that discussed by Jannat et al. (2018). The use of gentamicin causes an increase in the production of reactive oxygen species (ROS) as well as an increase in lipid peroxidation in cell membranes and tissues. Lipid peroxidation is an oxidative stress, and increased production of reactive oxygen species (ROS) decreased antioxidants, resulting in an imbalance between oxidant and antioxidant status and, eventually, cellular damage (Jannat et al. 2018).

**Heart**

Pericardium showed great thickening, infiltration of fibrin threads, large number of inflammatory cells mostly neutrophils were present (Fig. 7a), and also there was a golden brown granules of hemosiderin pigment (Fig. 7b). There was infiltration of mononuclear inflammatory cells, hemorrhage and hemosiderin pigment in between muscle fibers of myocardium (Fig. 7c), and hemosiderin pigment confirmed by Prussian blue staining (Fig. 7d). Some myocytes showed degeneration and Zenker’s necrosis (Fig. 7e), by PAS some myocytes showed positive staining suggesting presence of glycogen infiltration inside myocytes (Fig. 7f). This result was compatible with that discussed before by Ali et al. (2020). This may be in some point due to impaired free-radical defense system by gentamicin in the heart similar to that of the kidneys (Ali et al. 2020).

**CONCLUSION**

In spite of the therapeutic effect of gentamicin in the treatment of kidney infection, it may have adverse hematological, biochemical, ultrasonographic, and histopathological effect on kidneys and other body organs. Gentamicin application for clinical use should be cautiously used to avoid nephrotoxicity especially in high-risk cases, and renal function should be checked frequently during the treatment.

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