The Bacterial Effect on the kidney Histology caused by Fecal Coliform Bacteria as a biomarker for water pollution

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Abstract: This study was conducted to analyze well waters effect in Mughynia site, which lies along the valleys that discharge in the Red Sea in the Western Region of Saudi Arabia. This is to evaluate and determine whether the water of these wells is suitable for drinking and agricultural use via tested the effects of drinking well water contaminated with bacteria on the anatomic structure of mice kidney. Forty male White Swiss Albino mice were given drinking water with fecal coliform bacteria at rates of (control, 490, 1100 and 2400 colonies/100L) for 90 days. Up to 490 colonies/100L didn't affect the kidney structure. But at bacterial concentration of 1100 colonies/100L there was an increase in size of the endothelial cells of the portal and distal tubules in the cortex area, and enlargement of the glomerulus with increase of its nuclei number. A damage of the endothelial cells of the portal tubules, and swollen cells with small dark nuclei could be seen. At the highest bacterial concentration of 2400 colonies/100L the kidney cells suffered necrosis of endothelial cells of the tubules and thickening of Henle Loop, nuclear margination, leakage of blood cells, appearance of debris inside the urinary tubules, unstained cytoplasm and strong blood tubules congestion between the kidney tubules. These results suggest that indicators of fecal pollution should be used in drinking water microbiological analysis and that microbiological control of drinking water should be the norm everywhere. Therefore, the continue to discharge untreated water into well water should be limited and water resources should be treated to eliminate the pollutants.
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1. Introduction

The kidneys are a pair of organs located in the back of the abdomen. Each kidney is about 4 or 5 inches long about the size of a fist. The kidneys' functions are to filter the blood. The renal cortex is where several of the distal convoluted tubules merge and form a collecting duct, which passes into the renal mudalla forming a large tube called papilla. The mudalla is composed of conical masses of tissue called renal pyramids, and their apex form the renal papillae. The renal pelvis is located inside the renal sinus, which is the hollow chamber inside the kidney. The entrance to this sinus is termed the hilum, and through it passes various blood vessels, nerves, lymphatic vessels, and the ureter.

Research workers always tend to depend on the biochemical tests in kidney infection. Pathologists carefully examine renal specimens for histological features of infection. Infection of kidney normally causes alterations in the renal cell structures. Finlay. (1988) treated mice with Salmonella choleraesuis bacteria and found an increase in paracellular permeability penetrating through the epithelial barriers of Madin-Darby canine kidney. Symptoms on kidney cells due to bacterial infections represent diffuse lymphositic inflammation of the cortex and inflammation of glomeruli and sever tubular atrophy (Majumdar et al. 2000). Silveira (2010) working on toxicity of kidney by bacteria noticed granulomatous inflammation with cytoplasm containing numerous phagolysosomes, and Wu et al. (2005) observed characteristic intra-cytoplasmic bodies. Renal infection by bacteria most commonly E. coli caused nuclear enlargement of tubular epithelial cells with prominent nucleoli, and these findings are in the cortex, medulla and also can be more prominent in the tubules and collecting ducts of the deep cortex and medulla (Griffin et al. 1989). And also they observed swelling of the tubular epithelial cells, and lysis with intraluminal cellular debris. The following symptoms

were detected in kidney infected with bacteria, variable degrees of interstitial fibrosis and signs of tubular atrophy including thickening of the basement membrane and simplification of the tubular epithelial cells (Brown et al. 1997). On the other hand Bracamonte et al. (2007) noted on infected renal structures necrosis of tubular cells, detachment of renal tubular epithelial cells from the basement membrane, and sloughing of cells into the tubular lumen, with loss of brush border in proximal tubular segments. Renal structures infected with bacteria revealed formation of tubular casts derived from sloughed cells, tubular debris and protein, and tubular regeneration and tubular cells with hyper chromatic nuclei (Haas, 2010). The infection of kidney with bacteria causes cell vacuolization, peritubular accumulation of leucocytes in the interstitium and congestion of the peritubular capillaries in the outer medullary region (Laffavi et al., 2010). Bacterial infection of renal cells caused swelling of endothelial cells, disruption of glycocalyx and endothelial monolayer, migration of some leucocytes through the endothelial cells into the interstitial compartment which in turn expanded with great number of inflammatory cells (Sutton and Molitoris 1993). Also due to bacterial infection renal structures suffer cell and debris release into the lumen, proximal tubules (PT) lose brush border and filled with cellular debris (Brown et al. 1997).

This study aimed at testing whether drinking water contaminated with fecal coliform bacteria (FCB) would affect and cause injury to the kidney histological structure. A number of 40 white Swiss Webster Albino mice were given well drinking water with different concentrations of FCB (control, 490, 1100 and 2400 colonies/100L) for 90 days. Microscopic transverse sections of the kidney were prepared and examined for bacterial effects on the kidney histology.

2. Material and Methods

In this study mice were administered fecal coliform bacteria (FCB) with different concentrations to investigate its effect on the mice liver structures. Eighty (80) male Swiss Webster Albino mice (MFI Strain) were used. Similar in weight (28-30-gm) and age (8-10 weeks). Fed on Pillsbury diet and kept in cages at 18-25 C□ (Green berg, 1972), and light was controlled by a timer (12 D / 12 L).

Methods: The animals were grouped into 4 groups each 20 (N=80) mice were supplied with the treated water for 90 days. Group I = control given pure distilled water, group II = given drinking water with low dose (LD) of FCB 490 colony/100L, group III = given medium dose (MD) of FCB of artesian well water containing 1100colonies/100L, group IIII = given high dose (HD) of FCB of artesian well water containing 2400colonies/ 100L.

Preparation of liver histological sections: After animal treatment with contaminated drinking water for 90 days liver sections were prepared using method of Culling, (1974) for microscopic study of changes in the histology of the liver tissues due to bacterial infection. The sections were put in buffered formalin (From Acros Organic Belgium) for 24 hours, washed with water and dehydrated in ascending ethyl alcohol (70, 80, 90 and 100%) from B.D.H.Limited (England), and then passed and cleaned in a mixture of ethyl alcohol and xylene at a rate of 1:1 for 10 minutes. Sections were then put in pure xylene for 20 minutes, and taken to paraffin wax dissolved in xylol for 15 minutes in an oven at 60 □C and left in pure dissolved paraffin inside the oven for one hour for completion of infiltration inside the tissues. The sections were then embedded with dissolved wax in special metallic frames, and after wax freezing, the frames were subjected to trimming and fixed on wood stand. Sections 5 microns were made using microtone. The sections were placed on clean glass slides, dried at 40 □C, passed in pure xylene for wax removal, and passed in a descending methyl alcohol concentrations (100, 90, 80 and 70%). Then stained with Mayer Hematoxy Line from Acros Organic Co. (Belgium) for 2-3 minutes, washed with distilled water, taken to Eosin Y dye for 2-3 minutes, and passed into ascending ethyl alcohol from 70 to 100% for 3 minutes in each concentration. The sections were then passed into pure xylene for 10 minutes, taken out and drops of the substance (D.P.X.) were put on the slides. The sections were covered with glass covers and dried on hot plate to dry out the D.P.X. and they are now ready for test.

3. Results

The normal histological structure of the kidney in the control group.

The first group (control):

The microscopic sections of the kidney of the mice given normal distilled water free from FCB for 90 days (Fig.1) shows part of the cortex containing urinary corpuscles and is formed from Bowman,s capsole (BC) surrounding the glomerulus (G), and separated by urinary space (US). Also the proximal tubule (PT) and the distal tubule (DT) are clear, and nuclei (N) of the endothelial cells are clear.

The second group (Low concentration):

The transverse section of the kidney is showing absence of clear variations in the tissue structure after giving the animals drinking water containing 490 colonies/100L of FCB for 90 days. The section illustrates the normal tissue structure of part of the cortex and is showing that part of Bowman,s capsule (BC) is surrounding the glomerulus (G), together with the proximal tubule (PT) and distal tubule (DT) and the nuclei of the endothelial cells (Fig. 3). Also section (4) is showing the renal normal tissue structure of part of the pelvis containing the thick limb of Henle Loop (T) and the collecting tubules (CT).

The third group (medium concentration):

The transverse section (Fig.5) of the animal kidney given drinking water containing 1100 colonies/100L of the FCB for 90 days shows increase in cell size and cell regeneration of the endothelial cells of the portal PT) and distal tubules (DT), in addition to enlargement of the size of the glomerulus (G) and increase in number of its nuclei which caused narrowness in the urinary space. On the other hand figure (6) shows part of the glomerulus and damaged endothelial cells of the proximal tubules, and empty looking cytoplasm. Also appearance of swollen cells, and small dark nuclei which indicate apoptosis (death of cells), and chromatin margination and congestion of blood vessels.

The fourth group (High FCB concentration):

Sections (7 and 8) are showing the microscopic tests of the kidney sections at the cortex area of the animals treated with drinking water containing high concentration (2400 colonies/100L) of FCB for 90 days. Section (7) shows necroses (Ne) of the endothelial cells of the tubules, and the cytoplasm appears unstained (double arrow), the nuclei are small and dark colored i.e. nuclear pyknosis (→), with nuclear margination (→). Leaked blood cells outside the blood vessels is clear (thin→), and these similar changes happened in the thick limb of Hendle Loop (K) and the collecting tubule (CT) (H & E; X40). On the other hand section (8) shows Hyaline cast (HC) inside urinary tubules cavities, the endothelial cells are showing enlarged nuclei (E) and nuclear margination (→). There is also nuclear pyknosis and vascular congestion of the blood vessels between the renal tubules which appear to have damage unstained cells (double arrow head). Some karyolysis cells also appear, together with nuclei of other cells dark and unstained, and empty looking cytoplasm (double arrow head). The same changes are clear in the endothelial cells of the limbs of Henle Loop (T) and the collecting tubes (H & E; X100).

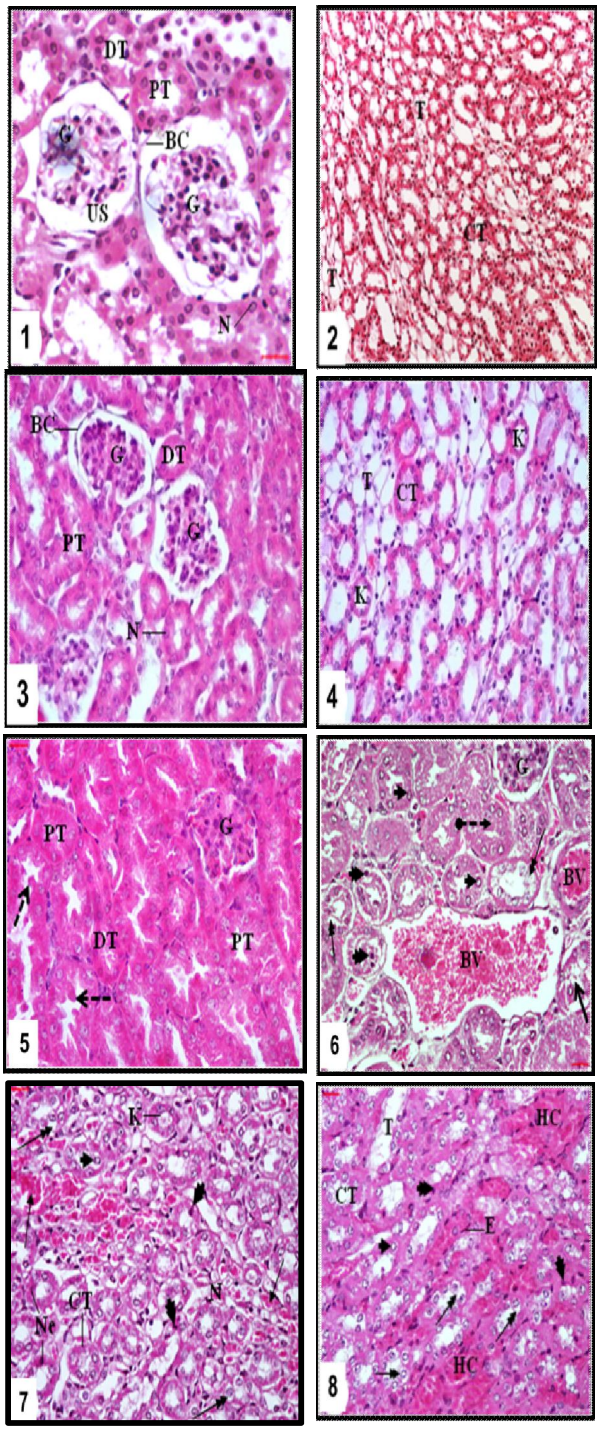

Figure 1. Kidney sections stained with H & E (1–8); (1,2) sections of the control group (X40). (3,4) sections of the low rate (490colonies/100L) (X100); (5,6) sections of the moderate rate (1100 colonies/100L) (X100); (7,8) sections of the high rate (2400 colonies/100L) (X40, 100 respectively).

4. Discussions

Regarding effects of drinking water contaminated with different concentrations of FCB on the anatomical structure of mice kidney, the microscopic sections showed absence of clear tissue changes when the animals were given drinking water with low bacteria concentration (490 colonies/100L). When the bacterial concentration was increased to moderate level (1100 colonies/100L) there was cell regeneration and cell enlargement of the proximal tubules (PT) and distal tubules (DT) in the cortex area, and there was enlargement of the size of the glomerulus (G) and increase in its cell nuclei. These findings agree with the results of (Griffin et al. 1989) who treated mice with E. coli bacteria and found that it caused nuclear enlargement of tubular epithelial cells with prominent nucleoli in the cortex, medulla and also more prominent in the tubules and collecting ducts of the deep cortex and medulla. Also the damage of the endothelial cells of the portal tubules was clear, and the cells were seen swollen, and the nuclei were seen small and dark in color with nuclear margination which is sign of cell death. This is in agreement with (Sutton and Molitoris 1993), who found that renal infection of mice by bacteria caused swelling of endothelial cells, disruption of glycocalyx and endothelial monolayer, migration of some leucocytes through the endothelial cells into the interstitial compartment which in turn expanded with great number of inflammatory cells. And agrees with the findings of Laffavi et al. (2010) who treated mice with bacteria and found peritubular accumulation of leucocytes in the interstitum, and also with (Bruno, 1986) during his study on effect of bacteria on the Atlantic salmon. When mice were given drinking water with high concentration of FCB up to 2400 colonies/100L necrosis of endothelial cells of the tubules appeared clearly, with nuclear margination, unstained cytoplasm, leaked blood cells outside the blood vessels, and nuclei are small and dark in color. These same changes happened in the endothelial cells of the thick limb of Henle Loop, with very strong congestion in the blood vessels between the urinary tubules. These results agree with the findings of some other researchers, Bracamonte et al. (2007) noted on infected renal structures necrosis of tubular cells, detachment of renal tubular epithelial cells from the basement membrane, and sloughing of cells into the tubular lumen.

5. Conclusion

Giving the male Swiss Webster Albino mice well drinking water with low FCB concentration (490 colonies/100L) for 90 day, didn't affect the kidney structure. But when the dose was increased to 1100 colonies/100L the size of the endothelial cells of the portal and distal tubules in the cortex area increased, accompanied with enlargement of the glomerulus and increase of its nuclei number. Also a damage of the endothelial cells of the portal tubules, and swollen cells with small dark nuclei appeared. Under the highest bacterial concentration dose of 2400 colonies/100L the kidney structure changes included necrosis of endothelial cells of the tubules and thickening of Henle Loop, nuclear margination, leakage of blood cells, appearance of debris inside the urinary tubules, unstained cytoplasm and strong blood tubules congestion between the kidney tubules. Thus these wells are not suitable for drinking and agricultural use at the highest concentration. This study concludes that indicators of fecal pollution should be used in drinking water microbiological analysis and considered preliminary as there are few published studies dealing with bacteria effect on renal toxicity.

Declaration

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