The Bacterial Effect on the Liver Histology caused by Fecal Coliform Bacteria

ZakiAl-Hasawi[1], Esam Al-Wesabi[1,4,*], Hassan Al-Harbi[2],Reem Al-Hasawi[3]

[1]Department of Biological Sciences, Faculty of Science, King Abdul Aziz University, Jeddah 21551, Saudi Arabia.
[2]Poison Control and Medical Forensic Toxicology Center, Jeddah 21551, Saudi Arabia.
[3]Department of Chemistry Sciences, Faculty of Science and arts, King Abdul Aziz University, Rabigh21911, Saudi Arabia.
[4]Al-Hodeidah University, Al-Hodeidah, Republic of Yemen.
* Corresponding authors: Esam O. Al-Wesabi; E-mail: esam.omer@yahoo.com

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.Administration of White Swiss Webster Albino mice with drinking well water containing Fecal Coliform Bacteria (FCB) at a concentration up to 2400 colonies/100L for 90 days, caused liver injury. There were alterations in the liver cells structures, swelling of cells, the cells became large in size and changed in shape, and most of the sinusoids disappeared. There was an increase in the percentage of cytoplasmic vacuolization and granulation, and dissolution of nuclear material of most of the cells, together with the destruction of some cells. Chromatin inside most of the nuclei was lost, and the cytoplasmic vacuolization was congested with many blood cells and death of some cells. 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.
[Al-Hasawi Z, Al-Wesabi E, Al-Harbi H, Al-Hasawi R. **The Bacterial Effect on the Liver Histology caused by Fecal Coliform Bacteria .***Life Sci J* 2017;14(10):55-58]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com.8. doi:10.7537/marslsj141017.08.

Keywords: Well water pollution; fecal coliform bacteria; Histological; Liver

1. Introduction

Liver is the largest organ in the body, and its most important task is to filter toxic substances from the body. Liver provides residence to a large and heterogeneous population of immune cells (like kupffer cells) each with specific function of protection , tolerance or/and inflammation (Gakuhei Son et al., 2010) .Its normal function is to metabolize, synthesize and/or degrade both absorbed and circulating products, and this places it in direct contact with the gut-driven bacteria (Moura and Mendes, 1999) . Previous studies by (Lichtman et al., 1991) suggested that intestinal bacterial overgrowth or infection with helicobacter alone contributed to hepatic pathology disorders recruiting inflammatory cells. Studies showed connection between bacteria and resident hepatic immune cells. Gakuhei Son et al. (2010) said that bacteria when delivered from the gut to the liver contributes significantly to various acute and chronic liver diseases through activation of both innate and adaptive immune responses and wound healing processes. Absence of gut bacteria significantly reduced liver injury, and reduced early IFN and IL4 production and hepatocellular apoptosis (Gakuhei Son et al., 2010). Anderson and Hans (1960) studied changes in hepatic structure due to infection, and noticed most of nuclei enlarged up to 20µ, the nuclear border appeared sharp and refractive, cell margination, regeneration of liver cells with giant nuclei and cytoplasmic vacuolization. Monga and Sharm (2013) in their study on effects on liver cells noticed congestion in the central vein and sinusoids, proliferation of kupffer cells and nodular hyperplasia, degradation of liver cells, extensive cytoplasmic vacuolization encircling the nucleus, and the sinuses were greatly dilated with kupffer cells of hepatocytes. Fetouh and Ibrahim (2013) studied histology of an infected liver and reported extensive vacuolization and variable areas of fatty degeneration, and the nucleus irregular with nuclei membrane.

In this study aimed at testing whether drinking water contaminated with FCB would affect and cause injury to the structure of the liver. A number of 80 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 liver sections were prepared for examination of the bacterial effects on the liver histology.

2. Material and Methods

In this study mice were administered 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^0 (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, and 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 ^OC 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 ^0C, passed in pure xylene for wax removal, and passed in descending methyl alcohol concentrations (100, 90, 80 and 70%). Then stained with Mayer Hematoxyline 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 anatomic structure of the liver

1. Histological structure of the liver under the control treatment:

Sections (1 and 2) represent the liver tissue of the control group. In section (1) the hepatocytes (H) are arranged in rows separated by liver pockets called sinusoids (S) that contain some Kupffer cells (K). The central vein (CV) and a branch of the portal vein (PV) are present (H&E; x40). Section (2) shows the normal arrangement of hepatocytes (H) in the portal area .Each cell is containing a big and circular nucleus (N) and homogeneous cytoplasm. The hepatocytes are separated by sinusoids that contain kupffer cells. Branch of the portal vein (PV) and branch of the bile duct (BD) are clearly seen (H&E; X100).

2. Histological structure of liver under low rate (490colonies/100L) of bacterial concentration:

Sections (3 and 4) are representing structure of liver of mice treated with 490colonies/100L. In section (3) some of the hepatocytes are suffering cytoplasmic vacuolization (CV) especially in the portal area, and cytoplasmic granulation (CG), in addition to gathering of the nucleic matter behind the margin of the nucleus (arrow head). The normal structure of the sinusoids that contain kupffler cells is clear, and also the normal structure of the branch of the portal vein. Section (4) illustrating normal hepatocyte structure but some of them are suffering cytoplasmic vacuolization (CV) with presence of nuclei in normal appearance, and the presence of many Kupffer cells (K) with dark staining inside the sinusoids (S) together with lymphatic cells. Two branches of the bile duct (BD) can also be seen.

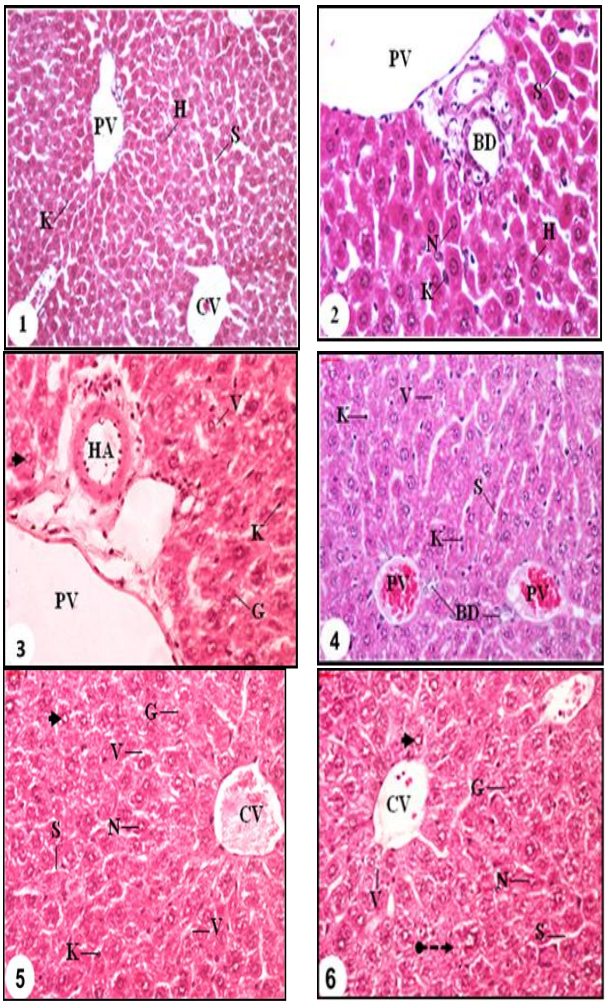
3. Histological structure of liver under moderate rate (1100 colonies/100L) of bacterial concentration:

Sections (5 and 6) illustrating alterations in hepatocyte structures resulting from the effects of the bacterial dose 1100 colonies/100 L. section (5) is showing hepatocytes with little swelling which result of narrowing of the sinusoids (S) .Also there is cytoplasmic vacuolization (CV) and cytoplasmic granulation (CG) with circular nuclei (N) some of it suffer chromatin margination (→) . Also appear Kupffler cells (K), and central vein (CV). Section (6) is illustrating some swollen hepatocytes (---→) and cytoplasmic vacuolization (CV) and cytoplasmic granulation (CG). Also chromatin margination (→), and central vein CV) is clear.

4. Histological structure of liver under high rate (2400 colonies/100L) of bacterial concentration:

Sections (7& 8) illustrate the changes in hepatocyte structures due to the high bacterial concentration of 2400 colonies/100L. In section (7) very high swelling can be seen in hepatocytes which led to alterations in most of the cell structures, and disappearance of sinusoids (S). Hepatocytes suffer cytoplasmic vacuolization (CV), and presence of scattered stainlessareas inside the cytoplasm, and loss of the chromatinoid substance inside most of the nuclei (→). Some cell nuclei appear small, dark in color with hepatocyte atrophy or apoptosis (double arrow). Damaged hepatocytes are clear (→), and also

activeKupffer cells (double arrow), and phagocytes (2 arrows), and conjunction of the central vein (CV) with many blood cells. Section (8) on the other hand illustrates cytoplasmic vacuolization (CV) and cytoplasmic granulation (CG) in most of the hepatocytes, in addition to karyolysis (---→) in most of the hepatocyte nuclei with nuclear margination (→) in other cells. Nuclei of some cells appear large in size in what is called koryomegaly. A branch of portal vein is full of blood cells, and a branch of the bile duct (BD) appears surrounded with epithelial cells suffering from dissolution of the nuclei material.



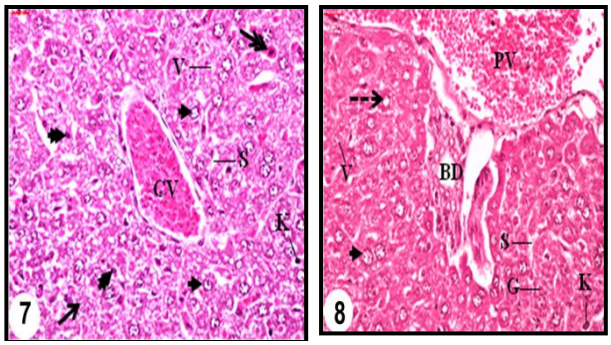


Figure1. Liver sections stained with H&E (1–8); (1,2) sections of the control group (X40,100; respectively). (3,4) sections of the low rate (490colonies/100L) (X100,40; respectively); (5,6) sections of the moderate rate (1100colonies/100L) (X40); (7,8) sections of the high rate (2400 colonies/100L) (X40).

4. Discussions

The microscopic sections did not show major distortions in the liver tissues of mice given drinking water with low FCB concentration up to 490 colonies/100L, so the liver cells are looking normally arranged with naturally looking nuclei, and Kupffer cells appear dark in color inside the sinusoids, also the portal veins and two branches of the bile duct are clear. But some cells suffer cytoplasmic vacuolization especially in the portal area with little cytoplasmic granulation and chromatin granulation behind cell wall. Raising the concentration of the bacteria in the drinking water to 1100 colonies/100L caused some distortions in the liver cells little cell swollen, cytoplasmic vacuolization and granulation, some cells suffering chromatic margination. The liver cells were more affected under the effect of the high bacteria concentration of 2400 colonies/100L, cells shape changed, cells with great swollen appeared, most of the sinusoids disappeared, the percentage of cytoplasmic vacuolization and granulation increased, most of the hepatocite nuclei dissolved and some cells were destructed .Chromatin was lost from most of the nuclei and cytoplasmic vacuolization was congested with many blood cells with death of some cells. These results are in agreement with the results of Mastroeni et al. (2001) and Rishi et al. (2006) who treated mice with Salmonella bacteria and noticed appearance of the central vein (CV) n and the sinusoids (S) and Kupffer cells and spaces between the cells, in addition to distortion of the hepatic lobules and regeneration in the sinusoids and enlargement of the blood vessels and necrosis of some cells of the hepatic lobules. Foster et al. (1982) observed swollen in liver cells and distribution of cellular spaces when they treated mice with bacteria. Gakuhei Son et al. (2010) reported that when bacteria is delivered from the gut to the liver it contribute significantly to various acute and chronic

liver diseases , through activation of both innate and adaptive immune responses and wound healing processes. Lichtman et al. (1991) treated rats with bacteria and found that bacteria caused liver injury, portal tract inflammation and bile duct proliferation and destruction. Monga and Sharm (2013) registered congestion in the central vein and sinusoids, proliferation of kupffer cells and nodular hyperplasia, and degradation of infected liver cells. Fetouh and Ibrahim (2013) studied histology of an infected liver and reported extensive vacuolization and variable areas of fatty degeneration, and the nucleus irregular with nuclei membrane.

5. Conclusion

Giving White Swiss Webster Albino mice well drinking water contaminated with FCB at a concentration up to 2400 colonies/100L for 90 days, caused alterations in the liver cells structures, thus these wells are not suitable for drinking and agricultural use. 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 hepatotoxicity.

Declaration

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23/10/2017