Study the morphological changes and histological lesions that induced by *shigella flexneri* for liver in mice and the role of camel's milk and antibiotic to treatment

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Abstract

The present study demonstrated the morphological changes and histological lesions in the liver of albino mice *Mus musculus* induced per oral infection of (*Shigella flexneri*). The aim of the study is to assess treatment by used camel milk and antibiotic Ciprofloxacin and the effect of camel milk and antibiotic on infected organs. Also study the inhibition efficacy of milk in vivo. The study tested sensitive of *Shigella flexneri* toward group from antibiotics, the results showed that the bacteria sensitive for (Ciprofloxacin, Neomycin, Amikacin) and resistance for (Ampicillin, Cefixin, Clindamycin, Vancomycin, Tetracyclin, Erythomycin, Ceftriaxone). Also test the inhibition efficacy of camel milk toward this bacteria and obvious that this bacteria very sensitive for camel milk. The present study used 32 mice that divide randomly to eight groups (each group consist 4 mice), the first group was control group administrated only normal diet and water, the second group administrated lethal dose for 50% of animals, the third group administrated infected dose from bacteria, but (3, 4, 5, 6, 7) groups administrated infected dose from bacteria and treated with camel milk with doses (0.25, 0.5, 1 ml and with ad libitum), the eighth group administrated infected dose from bacteria and treated with antibiotic (concentration 0.125 mg/ kg).
The microscopic examination showed many lesions in liver for groups that treated with lethal and infected dose from *Shigella flexneri*. This lesion appeared as vacuolated and focal necrosis of hepatocytes, hemorrhage and congestion was appeared, infiltration of inflammatory cells between hepatocytes. This lesions appeared more sever in the groups administrated with lethal dose. The histological lesions in groups that administrated with bacteria and treatment with camel milk presented enhancement in the arrangement of hepatocytes and less necrosis, degeneration, hemorrhage and infiltration of inflammatory cells especially in the groups which was treated with open dose of camel milk. The group which was treated with antibiotic alone, microscopic examination showed enhancement in liver compared with groups that administrated with lethal and infected dose but that less efficiency compared with camel milk.

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الملخص

هدفت الدراسة الحالية الى دراسة التغيرات المظهرية والآفات النسجية في كبد الفئران السويسرية ***Mus Musculus*** التي تظهر عند اصابتها ببكتريا ***Shigellia flexneri*** عـن طريـــق الفم مختبريا ومحاولة العلاج باستخدام حليب الناقة والمضاد الحيوي **Ciprofloxacin** وتأثيراتها على الكبد . ودراسة الفعالية التثبيطية لحليب الناقة خارج جسم الكائـن الحي. تم اختبار حساسية البكتريا ***Shigellia flexneri*** تـجاه مجمـوعة مــن المضادات الحيوية وتبين من النتائج بأنها كانت حساسـة لكل من (Ciprofloxacin,Neomycin, Amikacin) ومقاومة لكل من (Ampicillin, Cefixin, Clindamycin, Vancomycin, Tetracyclin, Erythomycin, Ceftriaxone). كما وتم اختبار الفعالية التثبيطية لحليب الناقة تجاه هذه البكتريا وتبين بانها كانت حساسة وبدرجة عالية له. تم استخدام 32 فأرة في الدراسة الحالية وقسمت بصورة عشوائية الى 8 مجاميع كل مجموعة شملت اربع فئران ، المجموعة الاولى كانت مجموعة السيطرة المجرعة بالمحلول الفسلجي. أما المجموعة الثانية فكانت المجموعة المجرعة بالجرعة المميتة الوسطية أما المجموعة الثالثة فكانت المجموعة المجرعة بالجرعة الممرضة من البكتريا. أما المجاميع (3،4،5،6،7) فكانت مجاميع الفئران المجرعة بالجرعة الممرضة من البكتريا والمعالجة بحليب الناقة وبالجرعات (0.25، 0.5، 1 ml ، وبالجرعة المفتوحة ad libitum)، على التوالي، اما المجموعة 8 فهي مجموعة المجرعة بالجرعة الممرضة والمعالجة بالمضاد الحيوي وبتركيز

0.125ملغم/كغم. اما الفحص النسجي فلوحظ حصول العديد من الآفات النسجية في كبد مجموعتي الفئران المجرعة بالجرعة المميتة والممرضة من البكتريا *Shigellia flexneri* تمثلت بحصول التنخر البؤري والفجوي لخلايا الكبد وحدوث نزف واحتقان دموي، وارتشاح الخلايا الالتهابية بين خلايا الكبدية، الا ان هذه الآفات كانت اكثر شدة في الجرعة المميتة. أما الآفات النسجية في المجاميع المجرعة بالبكتريا والمعالجة بحليب الناقة أظهرت تحسنا ملحوظا من حيث ترتيب الاشرطة الكبدية وقلة حالات النخر والتحلل والنزف الدموي وارتشاح الخلايا الالتهابية وكانت افضل تحسن بالجرعة المفتوحة حيث كانت مقاربة لمجموعة السيطرة. أما مجموعة الفئران المعالجة بالمضاد الحيوي اظهر الفحص النسجي تحسنا ملحوظا في كل من كبد هذه المجموعة مقارنة بالجرعتين المميتة والوسطية الانه انه كان اقل كفاءة من حليب الناقة.

Introduction

The camel milk has many immune characters due to content high concentration from inhibition and kill compounds for different types from infected bacteria and some virus, whereas camel milk content many enzyme such as Lysozyme, Lactoperoxidase, Immunoglobulins, Lactoferrin, Lactoalbumin and Amylase (1). Also, camel milk content high levels from proteins that similar insulin that could be cross through the stomach and adsorbed in the intestine (2, 3). Camel milk content high amounts from vitamins especially vitamin C that increased about three times from vitamin C in cows milk and about two time in human milk (4). Today we see in world us diffusing many diseases and increased the cases of death, also the side effects of drugs and disability of antibiotics for treatment diseases due to appearance a new bacteria dynasties that resistance to antibiotics. So that people return to the medicine populist to treatment diseases (5). Whereas, from sun of Abas (reported that the messenger of allah) said: allah messenger (peace and blessings of allah be upon him) said: ((in camel milk and its urine recovering for person who suffering diarrhea)) (6). Diarrhea was happen consequence to infect by different types from microorganism that effect on the small and large intestinal that lead to damage in the gastrointestinal tract. Because there were high percentage from infection by ***Shigella flexneri,*** so that this study aim to studied the histological lesions in liver of mice that infected by ***Shigella flexneri*** and study the inhibition effect of camel milk in the growth of ***Shigella flexneri*** and to determining the potential of camel milk to treated the damages of tissues that occurred by ***Shigella flexneri*** and its toxins and compared with ability of Ciprofloxacin to treated this damages.

Material & method

1. *Shigella* culture

Shigella culture was obtained from Mousle University/ college of science/ department of biology.

2. Test sensitive of antibiotic

The study tested sensitive of *Shigella flexneri* toward (Ciprofloxacin, Neomycin, Amikacin, Ampicillin, Cefixin, Clindamycin, Vancomycin, Tetracyclin, Erythomycin, Ceftriaxone) according to method of *Kirby et al (1966)*

3. Collect of milk sample

Milk samples were collected from two different camels (from Al-Hadam regin, in the Tikrit city), one of this camels was in third month from period of milk production and the other in the fifth month from period of milk production. Also the age, color, type of food and period of milk production for camel was reported. After that the samples of milk took to laboratory and test the inhibition efficacy of milk toward ***Shigella flexneri,*** according to the inhibition efficacy, chosen milk that obtained from camel that product of milk in third month.

4. Experimental infection in mice

Infected of mice was happened by Depended on method of *Olivier et al. (2009)* with some modified in this method, in this study used 32 mice and divided for eight groups (each group consist four mice) that obtained from the Public company of medicines manufacture and requirements medicals - Samara, Iraq, Streptomycin antibiotic was add to water that given to mice for 4 days, after that begin to administrated mice with ***Shigella flexneri,*** and determining the lethal dose for 50% from animals and the infected dose according to *Reed& Muench, (1938).* The group divided as following: the first group, control group administrated only normal diet and water, the second group administrated lethal dose for 50% of animals, the third group administrated infected dose from bacteria, but (3, 4, 5, 6, 7) groups administrated infected dose from bacteria and treated with camel milk with doses (0.25,

0.5, 1 ml and with ad libitum), the eighth group administrated infected dose from bacteria and treated with Ciprofloxacin antibiotic (concentration 0.125 mg/ kg). The method of induce with infectious for as mentioned previously groups performed orally, and then anesthesia and dissected the animals of groups (1, 2, 3) in the eleventh day. The other groups leave for 4 days to induced the disease and to present symptom and in the fourth day perform treated with camel milk for groups (4, 5, 6, 7) and with antibiotic for group (8), after that the animals as mentioned previously groups anesthesia and dissected after 7 days from treatment and taken liver to study the effect of different treatments on the structures of the organs.

Histological study

Fresh portions of liver from each mice was cut out rapidly, fixed in 10% neutral buffered formalin and then dehydrated with ascending grades of ethanol (70, 80, 90, 100 and 100%). Dehydration was then followed by clearing then tissue samples in 2 changes of xylene before being impregnated with 3 changes of melted paraffin wax, embedded and blocked out. Tissue sections thickness (7 um) were stained with haematoxylin-eosin (10).

Microscopic study and microscopic photograph

The microscopic investigation of organs sections involved the morphemetric and the descriptive histology. A light microscope (Motic microscope) was used to perform the microscopic investigations of this study. Microscopic photograph was made using (Optica\ SN 212973\Italy) microscope supplied with a special camera prepared for this purpose.

Results

1. Diagnosis of *Shigella* and determining species of these bacteria occurred according to following steps:

A. Diagnosis of bacteria

Diagnosed bacteria by microscopic examination for slides that prepared from bacteria colonies that grow after 24 hours with temperature 37C°. Whereas all the isolated bacteria were negative for gram stain and the shape were Coccobacilli.

B. Biochemistry diagnosis

Studied the ability of bacteria to fermentation of glucose and other chemical characters such as has no ability to produce H2S, no motile, non product urease, non product andole, don't fermented sucrose, lactose and xylose. In addition, performed catalos test, the result for this test was positive, also oxidase test was performed; the result for this test was negative.

2. Test sensitive *of Shigella flexneri* toward group from antibiotics

Test sensitive of *Shigella flexneri* was performed toward 10 different types from antibiotics, the results showed that the *Shigella flexneri* was very sensitive toward (Ciprofloxacin, Amikacin, Neomycin) and resistance to (Ampicillin, Cefixim, Clindamycin, Ceftriaxone, ErythomycinK, Tetracycline, Vancomycin) (Fig. 1).

3. Results of inhibition efficacy for camel milk toward *Shigella flexneri*

The results of inhibition efficacy for camel milk toward *Shigella flexneri* showed very high inhibition efficacy for camel milk and clear, which reached to 40 ml (Fig. 2).

4. Contents of camel milk

Camel milk was composed from 3.5% protein, 4.9% lipid, 5% lactose, 88% water and 9.28% solid materials.

5. Experimental infection for mice by *Shigella flexneri*

A. deterrnining lethal dose for 50% from animals This present study showed that the 10^{-1} dilution lead to killed 50% from mice that administrated with *Shigella flexneri*, so that lethal dose for 50% from animals was 6×10^2 cell/ml.

B. determining infected dose

This present study showed that the 10^{-4} dilution lead to present clinical symptoms on mice that administrated with *Shigella flexneri*, so that infected dose for animals was 4×10^5 cell/ml.

6. Morphological malformation

The present study showed many morphological changes in liver when compared with liver of control group (Fig. 3). These changes appeared as redness or darkness color of liver due to congestion or hemorrhage of liver in mice that administrated with lethal and infected dose (Fig. 4), but the groups that administrated with infected dose from bacteria and treated with

camel milk by (0.5, 1 ml and with open dose) showed presented enhancement, where that similar to the control group (Fig. 5, 6).

7. Histological observation

A. Control group

The microscope examination showed normal structure of liver and demonstrated normal central vein, normal arrangement of hepatocytes and sinusoids (Fig.7).

B. group administrated with LD-50%

The microscope examination showed extensive degeneration and necrosis of hepatocytes and karyolysis of its nuclei, also auxes of nuclei of some cells, there was hemorrhage and infiltration of lymphocytes and vacuolated (Fig. 8).

C. **group administrated with infected dose**

There was desquamation of endothelium of central vein, extensive degeneration and necrosis of hepatocytes, also there was pyknotic nuclei for some cells, vacuolated and swelling sinusoids (Fig.9).

D. group administrated infected dose and treated with 0.25ml from milk

The microscope examination showed recovery in tissue of liver, whereas arrangement of hepatocytes around central vein and normal size of sinusoids was appeared, but also there was desquamation of endothelium of central vein and some nuclei of hepatocytes were swelling and hypertrophy of kupffer cells (Fig. 10).

E. group administrated infected dose and treated with 0.5ml from milk

This group showed more recovery when compared with previously groups, whereas arrangement of hepatocytes around central vein and normal size of sinusoids was clear and appeared, but there was hemorrhage and some hepatocytes were swelling (Fig.l1).

F. group administrated infected dose and treated with 1ml from milk

The microscope examination showed more recovery for hepatocytes and normal central vein without any desquamation of endothelium that associated with hypertrophy of kupffer cells that play important defend role to removal injure cells, and there were some cells and its nuclei that may prepared to divide to produce a new cells that replace with damage cells (Fig. 12).

G. group administrated infected dose and treated with open dose from milk

The results showed more recovery in liver of this group, whereas arrangement of hepatocytes around central vein was very clear and arrangement as radial plates and the normal shape and size of sinusoids that similar to liver of control group, also increased in the number of kupffer cells and swelling hepatocytes to inter mitosis phases to product a new cells (Fig. 13).

H. group administrated infected dose and treated with Ciprofloxacin antibiotic

The microscope examination showed recovery in liver of this group when compared with liver of infected group, but this recovery was less when compared with liver of groups that treated with camel milk, whereas arrangement of hepatocytes was not clear, also there was degeneration and necrosis of hepatocytes and auxes of its nuclei (Fig. 14).

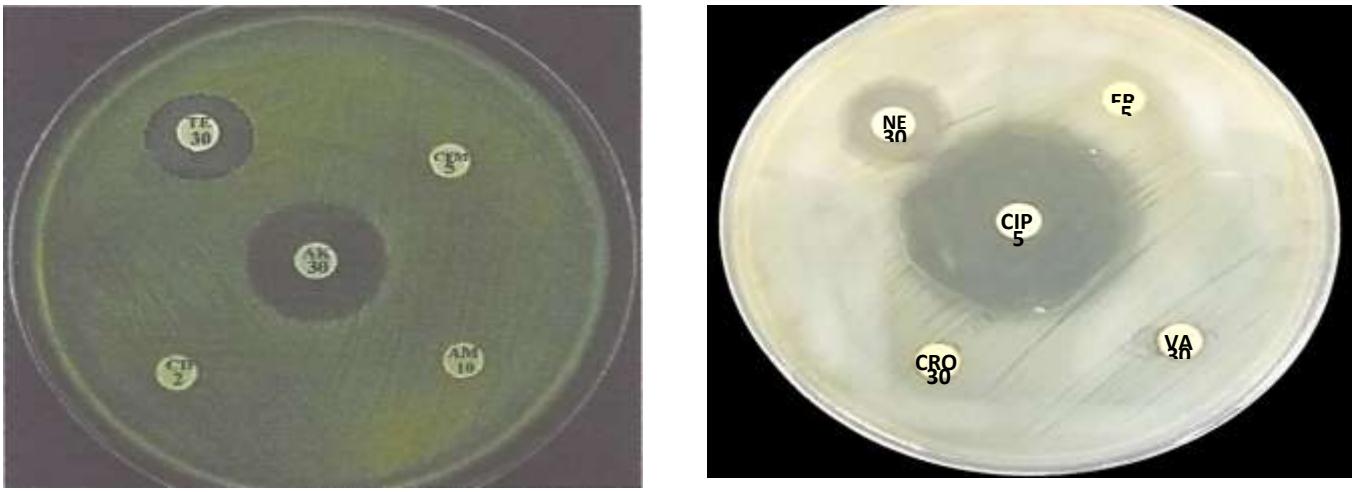

Fig.(1): Test sensitive of *Shigella flexneri* toward group from antibiotics. Ampicillin: AM, Amikacin: AK, Ciprofloxacin: CRO, Ceftriaxon; CIP, Cefixime: CEM, Clindamycin: CD,

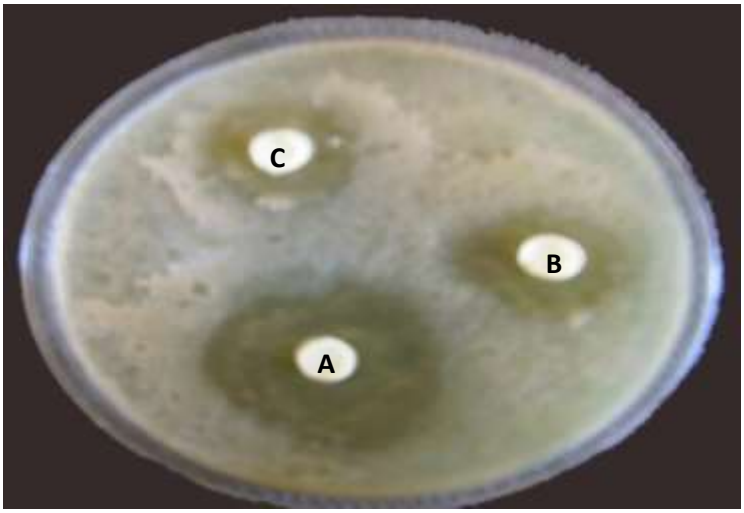

Fig.(2): Inhibition efficacy for camel milk toward *Shigella flexneri.* A: Milk was taken from camel in third month from period of milk production. B: Milk was taken from camel in fifth month from period

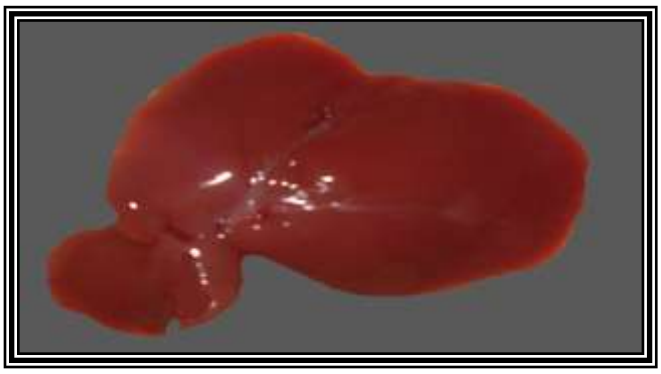

Fig.(3): Liver of Control Group

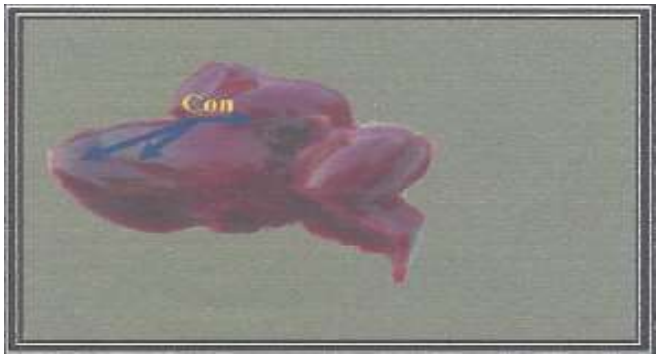

Fig. (4): Congestion liver of group administrated with LD-50%.

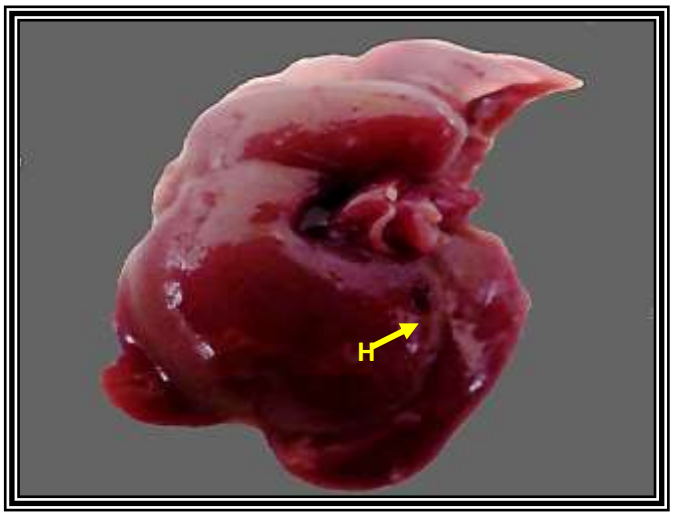

Fig. (5): Hemorrhage in liver of group administrated with 1ml from milk.

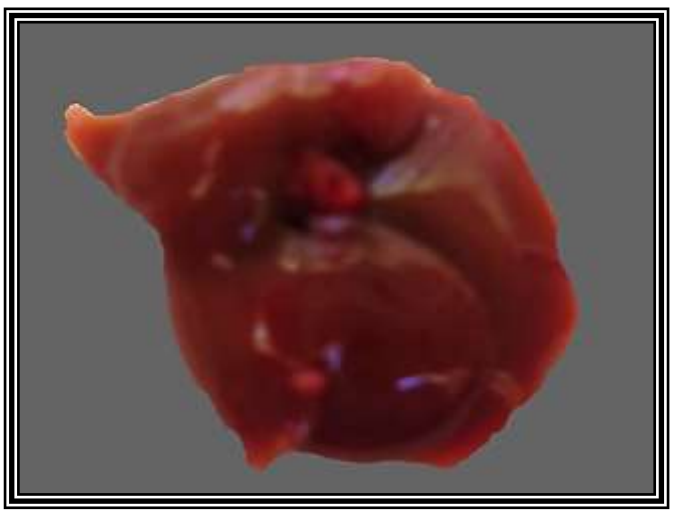

Fig.(6): Liver of group administrated with open dose from milk.

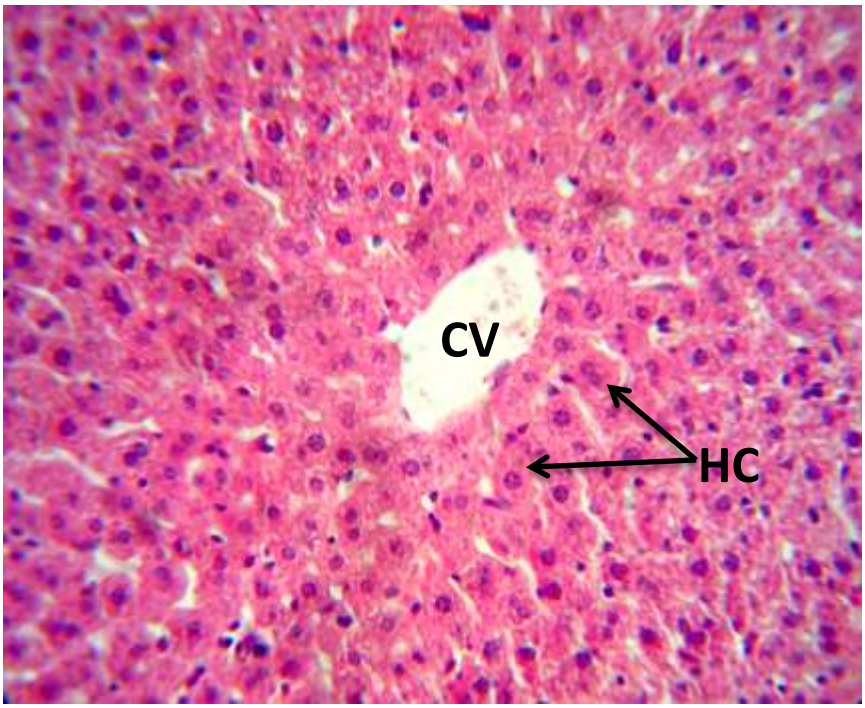

Fig. (7): Liver of control group showed normal central vein and normal arrangement of hepatocytes

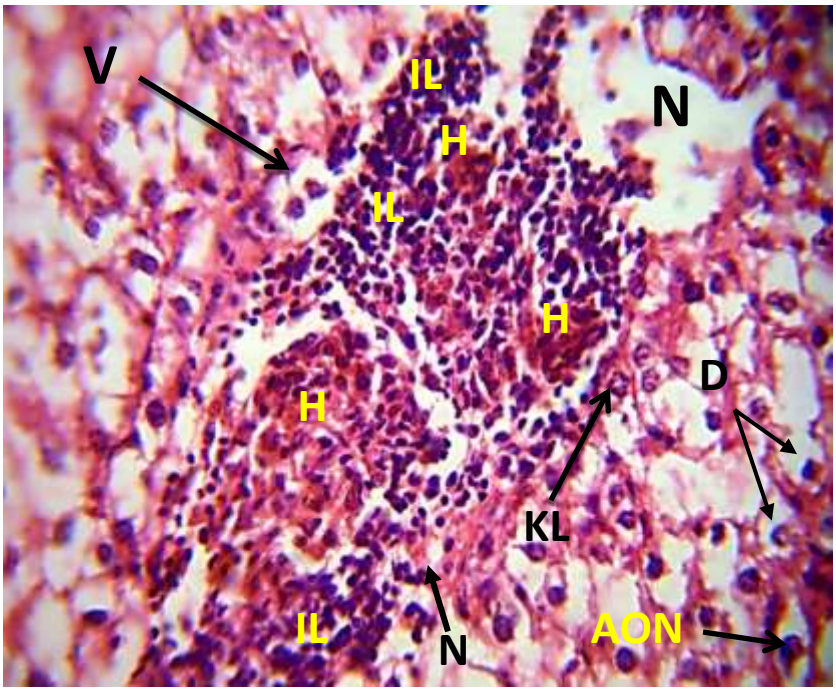

Fig. (8): Liver of mice administrated with LD-50% showed Degeneration (D) and Necrosis (N) of hepatocytes and Karyolysis (KL), Auxes of Nuclei (AON), there was hemorrhage (H) and Infiltration of Lymphocytes (IL) and Vacuolated Necrosis (VN) (H&E X400).

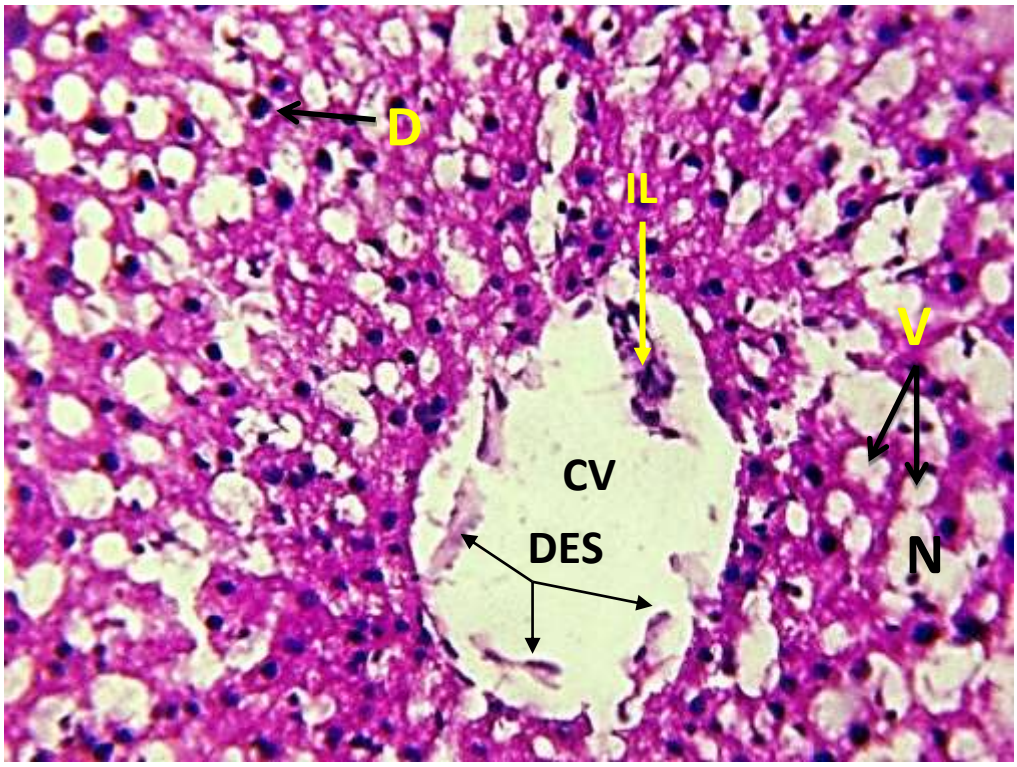

Fig. (9): Liver of mice administrated with infected dose showed Degeneration (D) and Necrosis (N) of hepatocytes and Desquamation of endothelium (DES), Infiltration of Lymphocytes (IL) and Vacuolated (V) (H&E X400).

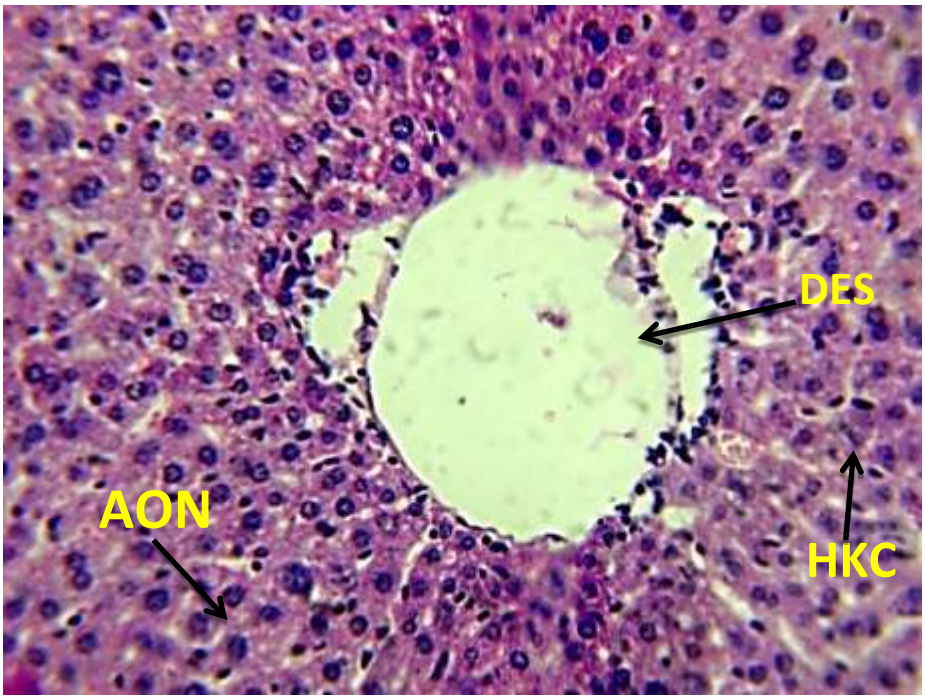

Fig. (10): Liver of mice administrated with 0.25ml from milk showed Desquamation of endothelium (DES), Auxes of Nuclei (AON) and Hypertrophy of Kupffer cells (HKC) (H&E X400).

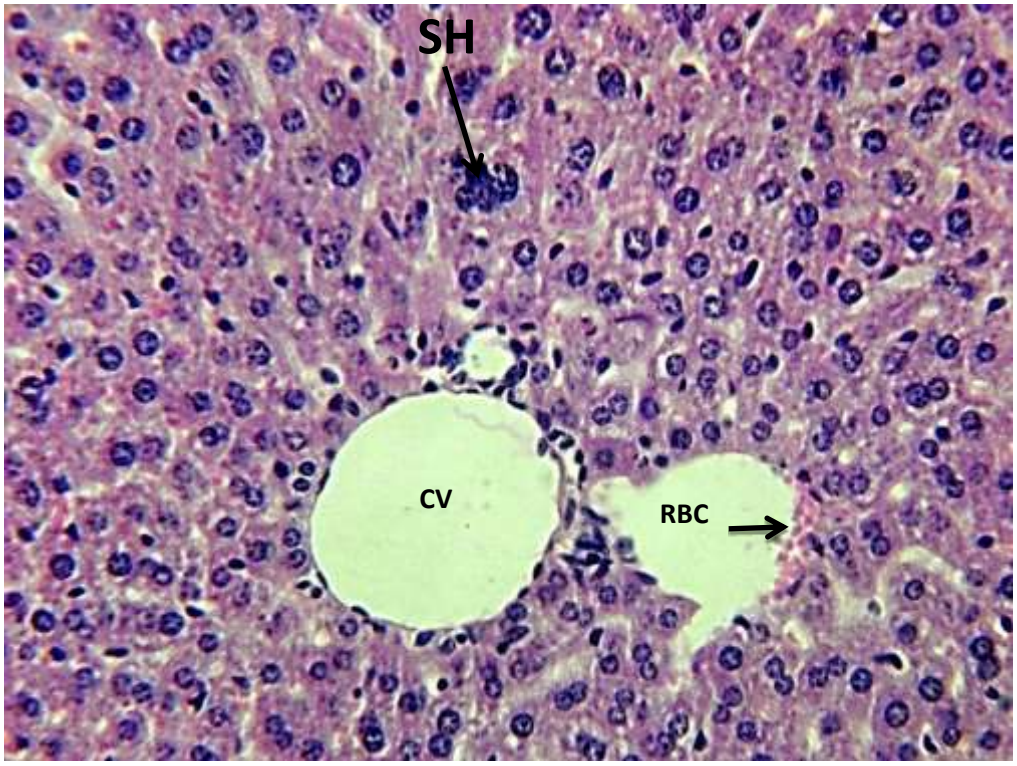

Fig. (11): Liver of mice administrated with 0.5ml from milk showed arrangement hepatocytes around Central Vein (CV), there was Red Blood Cells (RBC) and Swelling Hepatocytes (SH) (H&E X400).

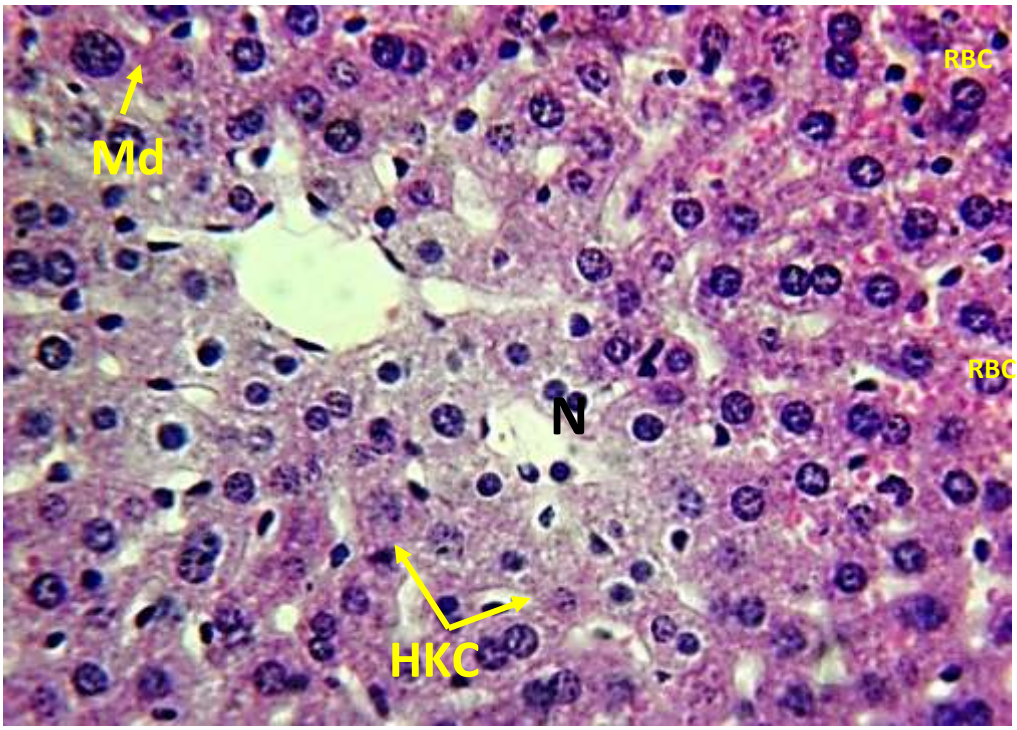

Fig. (12): Liver of mice administrated with 1ml from milk showed Necrosis (N) of hepatocytes, Hypertrophy of Kupffer cells (HKC) and there was increased in size of hepatocytes that prepared to inter mitosis phases (Md) (H&E X400).

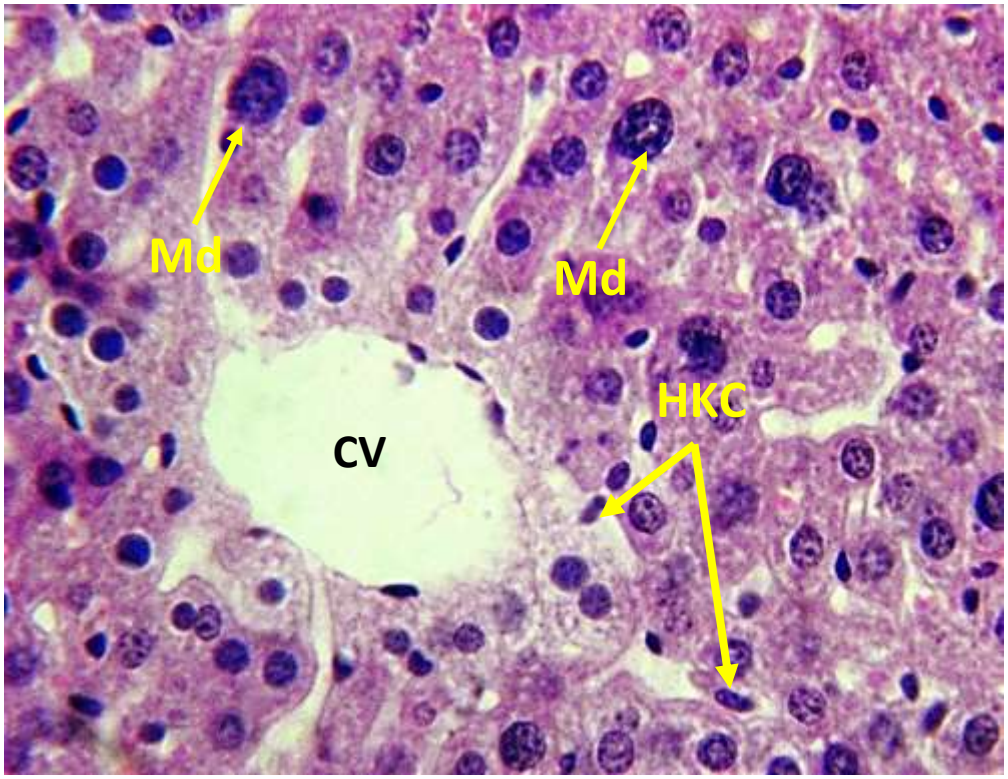

Fig. (13): Liver of mice administrated with open dose from milk showed normal shape of Central Vein (CV), Hypertrophy of Kupffer cells (HKC) and there was increased in size of hepatocytes that prepared to inter mitosis phases (Md) (H&E X400).

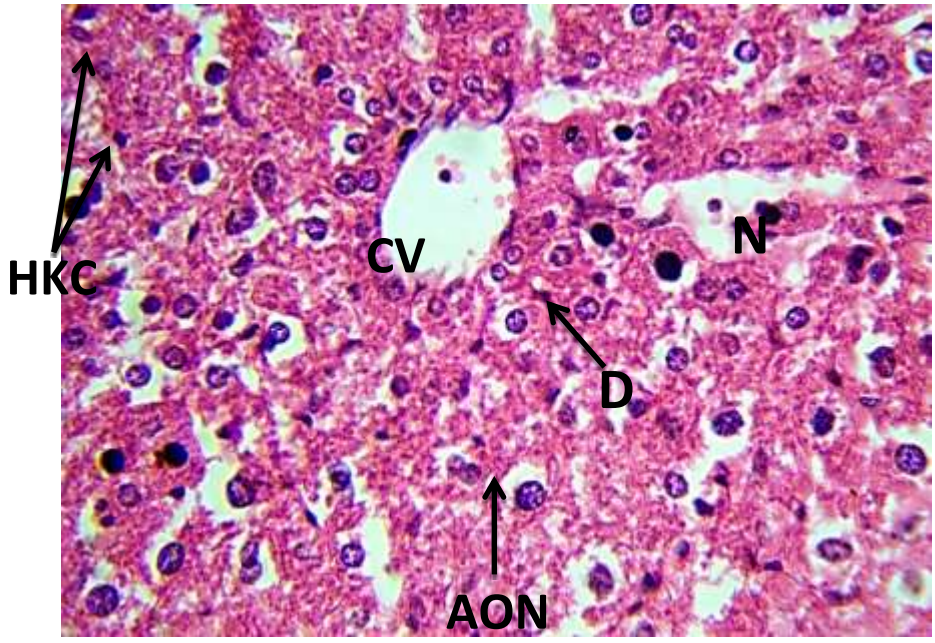

Fig.(14): Liver of mice administrated with Ciprofloxacin Degeneration (D) and Necrosis (N) of hepatocytes, Auxes of Nuclei (AON) and Hypertrophy of Kupffer cells (HKC) (H&E X400).

Discussion

1. Diagnosis of bacteria

Diagnosis of *Shigella* and determining species of these bacteria depended on several steps such as culture characters, microscopic examination and biochemistry diagnosis, so these characteristics that present in this study was in agreement with *Quinn (2006)*

2. Test sensitive *of Shigella flexneri* toward group from antibiotics

This study showed that *Shigella* was very resistance for some antibiotics, the reason may return to wrong use or randomly use of these antibiotics without test sensitive of bacteria toward antibiotics, so that lead to appear dynasties that resistance to many antibiotics by modified in permeability of membrane or modified the target site for antibody or produce some compounds that competing the function of antibody (12). Also this bacteria content beta-lactamase enzyme, which destroy the antibody by break down Amide bond that lead to transferred antibody to a new compound without any activism (13).

3. Results of inhibition efficacy for camel milk toward *Shigella flexneri*

The results showed very high inhibition efficacy for camel milk toward *Shigella flexneri,* that return to a high levels of proteins in milk such as Lysozyme, Lactoperoxidase, Immunoglobulins, Lactoferrin, Lactoalbumin and Amylase (1). *Al-Amin et al. (2006)* referred to the levels of proteins increased especially in the first months from milk production; so that this result was in agree with result of this study, whereas the samples of milk that used in this study was collected in the third month from milk production. *Kennedy et al. (2000)* referred to that lactoperoxidase was bactericidal or inhibition of growth depended on type of bacteria dynasty, the high inhibition efficacy for milk return to the role of lactoferrin which inhibition for gram negative bacteria and lysozyme which inhibition grow of gram positive bacteria, whereas *Richard et al (1991)* assured that the lactoferrin and lysozyme play important role to inhibition of growth according to type of bacteria, and referred that lactoferrin make to induce modified in the first defend line for gram negative bacteria.

4. Contents of camel milk

The results showed that the level of proteins in milk was 3.5% that is in agreement with *Al Taee (1989)* and *Park & Gorago (2006).* High levels of proteins in camel milk may return to nature of food, whereas camel was feed on the desert plants that consist antimicrobials materials (19). The percentage of lipid was 4.9%, this percentage was more when compared with results of *Turki et al. (2008); Shuiep et al. (2008)* and *Hanne (2001).* Also this percentage was less when compared with results of *EL-Naggar (1998)* and *Schwartz (1992). Knoess et al. (1986)* referred that the percentage of lipid in camel milk depends on temperature, nature of food and number of dairymaid times in day. Also *Khan & Iqbal (2001)* referred to the percentage of lipid was increased in hot months and decreased in cold months. The percentage of fructose was 5% that was in agreement with *Wernery (2006)* and more when compared with results of *EL-Agamy (1998)* and less when compared with results of *Eberlein (2006).* The percentage of solid materials was 9.28% that approached to result of *Khaskheli et al. (2005)* and *Shuiep et al. (2008).* Also percentage of water was 88% that was in agreement with *Al Taee (1989). Faye et al. (2008)* referred to the difference in percentage of milk contents return to several factors such as environment conditions, physiology factors or genetic factors.

5. Histological observation

A. microscopic examination for liver of mice that infected by *Shigella flexneri*

The effect of *Shigella* on morphological of liver was appeared as redness and darkness color that caused by hemorrhage or congestion of blood vessels. The group that administrated LD-50% from bacteria showed many lesions and more when compared with other groups. *Jubb (1985)* referred to these damages and lesions in liver return to the liver is the organ that responsible

for detoxification that lead to destroy the foreign materials to assist body to eliminated its by the kidney. The lesions in the lethal and infected dose was appeared as extensive degeneration and necrosis of hepatocytes and karyolysis of its nuclei, also auxes of nuclei of some cells, desquamation of endothelium of central vein and swelling sinusoids, there was hemorrhage and infiltration of lymphocytes. *Hinton & Girgis (1993)* evidence that the toxins lead to change the permeability of membranes by effect on the structure of membrane associated with increased or reduced the materials in cell that lead to necrosis and degeneration of hepatocytes. This result was in agreement with *Lomnitski et al. (2000)* which referred to the toxins of bacteria lead to hemorrhage and congestion of blood vessels and infiltration of lymphocytes. *Jaeschke et al. (1998)* referred to the swelling sinusoids return to atrophy of hepatocytes and degeneration it, while infiltration of lymphocytes due to degenerative changes in hepatocytes that lead to secreted chemical factors from hepatocytes to attract the inflammatory cells to injure site (36).

B. microscopic examination for liver of mice that infected by *Shigella flexneri* and treated with camel milk

The microscope examination showed recovery in tissue of liver, whereas hepatocytes were arrangement around central vein as radial plates and could be recognized kupffer cells and sinusoids. This recovery return to the milk contents and its characters, milk has rich with high concentration of vitamins, immune proteins and minerals, these characters make it different from other types of animals milk, whereas it was rich with antioxidant (vitamin A, B2, C and E). *AL- Hashem (2009)* referred in his study on the white rat that administrated with aluminum chloride and treated with camel milk do not happen much damages to hepatocytes compared with rat administrated with aluminum chloride, also regenerating of hepatocytes was present, so suggested that this regenerating of hepatocytes return to antioxidant that found in milk that lead to destroy free radical. *Also Abdel-Magjeed (2005)* referred to the ability of camel milk to inhibition grow of malignant cells and the ability to regenerating of hepatocytes and return to the normal size and function, also suggested that the regenerating of hepatocytes return to antioxidant. The microscope examination for sections that prepared from groups administrated with (1ml and open dose) from camel milk appeared as increased in size of hepatocytes that return to prepared to cell division by mitosis, this prepared for division return to role of zinc that found in milk to stimulation hepatocytes to division and to duplication and replication DNA (39,40). Camel milk has rich with lactoperoxidase (1), so zinc assists to increase the efficacy of this enzyme to removal free radical from cells that lead to protect it from the damage of free radical. *Quita & Kurdi (2010)* referred to the milk has rich with high levels from Linoleic acid, whereas *Badr EL-in & Omaye (2007)* evidence that Linoleic acid was antioxidant and has biological efficacy for blocked the diseases.

C. group administrated infected dose and treated with Ciprofloxacin antibiotic

Ciprofloxacin was used to treatment many diseases such as inflammations of kidney, prostate, bladder, stomach, intestine, bone and skin that caused by different types from microorganism (43). The microscope examination showed recovery in liver of group administrated infected dose and treated with Ciprofloxacin, but in the same time the lesions continuously appeared such as degeneration and necrosis of hepatocytes, karyolysis and other lesions. The recovery of liver return to the ability of antibiotic to killed bacteria, Ciprofloxacin assist to inhibition bacterial enzymes that important to duplication and replication DNA of bacteria, so the results was in agreement with *Tetz et al. (1994)* who studied the effect of some antibiotics on *Shigella* and *Kcoli*, they observed revambecin and chloromvnicol changed adherence factors. So when compared the results that obtained from this study between groups administrated infected dose and treated with camel milk and group administrated infected dose and treated with Ciprofloxacin, so we can say the results that obtained from this study between groups administrated uifected dose and treated with camel milk was very best that refer to efficiency of milk to treatment the tissues and assisted it to return to normal structure because

milk was very rich with different types from advantageous materials.

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