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Study the Histological and Physiological Changes that Induced by Hydrogen Peroxide for Liver in Mice and the Role of Camel's Milk to Treatment

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ABSTRACT

The purpose of this study was to evaluate the effect of camel's milk on the mice liver after induction by hydrogen peroxide. Twenty albino mice were randomly divided into five groups A, B, C, D and E. (each group consisted of 4 mice), the first group was control, administrated only normal diet and water. The second group was administrated with 1% of H2O2. The third group was administrated with 1.5% H2O2. The fourth group was administrated 1% H2O2 and treated with camel milk for 15 days and the fifth group was administrated 1.5% H2O2 and treated with camel milk for 15 days. The Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels were increased and showed high significant changes ($P < 0.01$) in groups that administrated H2O2 only compared with control group and also the liver tissue showed different lesion including necrosis, degeneration of hepatocytes, infiltration of lymphocytes, sclerosing bile ducts and fibrosis, but when these groups treated with camel milk, the ALT and AST levels return to the normal ranges and the liver tissue became normal and repaired completely. It was concluded from this study that the camel milk has amply good effect on the liver enzymes and tissue repair.

دراسة التغيرات النسيجية والفسلجية باستخدام بيروكسيد الهيدروجين في كبد الفئران ومعالجتها بحليب النوق

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الكلمات المفتاحية:
التغيرات النسيجية ، الفسلجية ، بيروكسيد الهيدروجين ، كبد الفئران ، حليب النوق.

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الخلاصـــة

كان الغرض من هذه الدراسة هو لتقييم تأثير حليب الإبل على آفات كبد الفئران البيض المحدثة بواسطة بيروكسيد الهيدروجين H2O2. تم استخدام عشرون فأر ابيض وقسمت عشوائيا إلى خمس مجموعات أ، ب، ج، د، هـ. (كل مجموعة تتكون من 4 فئران)، المجموعة الأولى هي مجموعة السيطرة والتي جرعت بحمية غذائية وماء فقط،، المجموعة الثانية جرعت 0.1٪ H2O2، المجموعة الثالثة التي جرعت 0.15٪ H2O2، المجموعة الرابعة التي جرعت 0.1٪ H2O2 وتم معالجتها مع بحليب الإبل لمدة 15 يوما، المجموعة الخامسة التي جرعت 0.15٪ H2O2 وتم معالجتها بحليب الإبل لمدة 15 يوما. ارتفعت مستويات GOT و GPT وأظهر تغييرات عالية المعنوية ($P <0.01$) في المجاميع التي جرعت H2O2فقط مقارنة مع مجموعة السيطرة وكذلك أظهرت أنسجة الكبد افات مختلفة والتي تضمنت تنخر، تتكس الخلايا الكبدية، ارتشاح الخلايا اللمفية وتصلب القنوات الصفراوية ، ولكن عندما تم معالجة هذه المجاميع بحليب الإبل، اظهرت مستويات GOT و GPT حدود طبيعية وأنسجة الكبد تم إصلاحها بشكل شبه كامل. يستنتج من هذه الدراسة أن لحليب الإبل تأثير جيد بما فيه الكفاية على انزيمات والأنسجة الكبد.

Introduction:

Camel milk has been used for several of medical problems; especially it's showed some medicinal potential. Camel's milk is different from other ruminant milk; it is low in cholesterol, protein and sugar but high in minerals (sodium, potassium, magnesium, copper, zinc and iron), vitamins A, B2, C and E, and contains a high concentration of insulin [1, 2, & 3]. It has no allergic properties and can be consumed by lactase-deficient individuals and those with a weakened immune system. A series of autoimmune diseases are successfully being treated with camel's milk. Furthermore in India, camel's milk is used therapeutically to treat jaundice, dropsy, spleen problems, tuberculosis, anemia, asthma, piles and diabetes [4]. Camel milk contains good amounts of lysozyme, lactoferrin, Lactoperoxidase, immunoglobulin G and secretory immunoglobulin A [5], these antimicrobial factors were present at significantly greater concentrations in camel milk and were more heat stable compared with those in cow and buffalo milks [6]. Camel milk has been antitoxic effects and its may be used to protect against toxic effects of chemical agents in liver [7]. Therefore, the present study deigned to show the protective effects of camel's milk against hydrogen peroxide H_2O_2 toxicity in the liver.

Materials & methods:

Animal model

twenty adult albino mice (*Mus musculus*), (wt 25-28 g) obtained from the Public company of medicines manufacture and requirements medicals - Samara, Iraq, and kept on standard pellet diet and water.

Collect of milk sample

Milk samples were collected from camels (from Al-Hadam region, in the Tikrit city), there camels were in third month from period of milk production. The age, color, type of food and period of milk production for camel were reported. Milk was collected from the camels by hand milking as normally practiced by the farmers. The samples were collected in sterile screw bottles and kept in cool boxes until transported to the laboratory.

Experimental design

In this study, twenty albino mice were used and divided for five groups (each group consisted of four mice) as follow:

1. **Group A**: control group administrated with normal saline only for seven days, then killed, all were euthanized at eighth day.
2. **Group B**: administrated with 0.1% H_2O_2 for seven days, then killed, all were euthanized at eighth day.
3. **Group C**: administrated with 0.15% H_2O_2 for seven days, then killed, all were euthanized at eighth day.
4. **Group D**: administrated with 0.1% H_2O_2 for seven days. After that, treated with 1ml camel milk for 15 days, then killed, all were euthanized at sixteenth day.
5. **Group E**: administrated with 0.15% H_2O_2 for seven days. After that, treated with 1ml camel milk for 15 days, then killed, all were euthanized at sixteenth day.

Prepare of blood solution

Subjected mice under anesthesia (by using chloroform) then later took heart blood and put in test tubes that contain EDTA. This solution was mixed with phosphate buffer, centrifugation 2000 cycle/min for 10 min. Supernated was taken and 1 ml distal water added for it. ALT, AST in blood extract were analaysed with least significant difference in $p< 0.01$.

Histological study

Fresh pieces of liver from each mice was cut out rapidly, fixed in 10% formalin and then dehydrated with ascending grades of ethanol. Dehydration was then followed by clearing then tissue samples in two changes of xylene before being impregnated with three changes of melted paraffin wax, embedded and blocked out. Tissue sections thickness (5 um) were stained with haematoxylin-eosin [8].

Microscopic study and microscopic photograph

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

Statistical analysis

Data were analyzed statistically using a statistical Minitab program under SPSS and Microsoft Excel XP system. The data were presented in simple measure of mean ± SD (standard deviation), minimum and maximum values. Results were analyzed statistically using Analysis of Variance (ANOVA) test, in order to evaluate the significance of variability between treated and control groups. Means of data were compared using Duncan's Multiple Range test. Probability levels of more than 0.01 were regarded as statistically non-significant, whereas values less than 0.01 were considered as significant as follows:
$P< 0.01$ highly significant [9].

Results :

Biochemical tests

ALT tests

The results of the present study showed significant changes ($P>0.01$) in level of ALT between groups. As shown in chart (1), the group B that administrated 0.1% H_2O_2 showed significant change compared with control group. Also, group C that administrated 0.15% H_2O_2 showed significant change compared with control group. But in the groups D and E, (groups that administrated with H_2O_2 for seven days and treated with camel milk), showed non-significant change compared with control group.

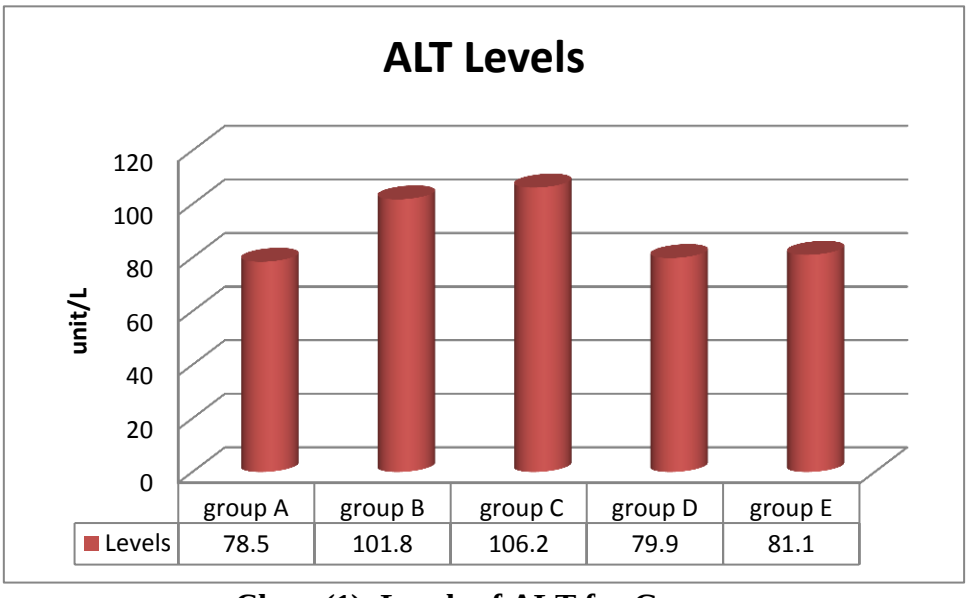

Chart (1): Levels of ALT for Groups

AST tests

The results of the present study showed significant changes (P>0.01) in level of AST between groups. As shown in chart (2), the group B that administrated 0.1% H_2O_2 showed significant change compared with control group. Also, group C that administrated 0.15% H_2O_2 showed significant change compared with control group. But in the groups D and E, (groups that administrated with H_2O_2 for seven days and treated with camel milk), showed non-significant change compared with control group.

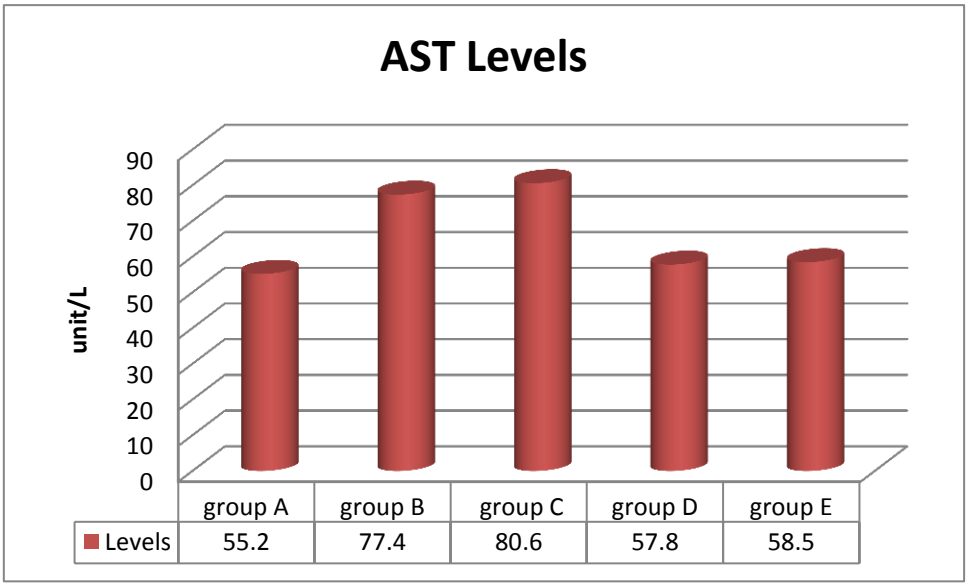

Chart (2): Levels of AST for Groups

Histological examination

1. Control group (A)

The microscope examination showed normal structure of liver and demonstrated normal central vein. The present of hepatic lobules, each lobule is formed by cord of hepatocytes arranged in the form of radial pattern toward the central vein, kupffer cells were present in the sinusoids. The portal area was present in between the lobules and containing branch of portal veins, hepatic artery, bile duct and lymph vessels (Fig.1).

2. Group (B) administrated 0.1% H_2O_2

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 of some hepatocytes (Fig. 2).

3. Group (C) administrated 0.1% H_2O_2

The histological examination showed thickening the wall of central vein, degeneration and necrosis of most hepatocytes and infiltration of lymphocytes with present the collagen fibers and fibroblasts (Fig.4).

4. Group (D) administrated 0.15% H_2O_2 and treated with 1ml of camel milk

The liver from this group showed degeneration of most hepatocytes and sclerosing of bile ducts that appeared surrounded by fibrocytes and infiltration of lymphocytes (Fig. 3).

5. Group (E) administrated 0.15% H_2O_2 and treated with 1ml of camel milk

The histological examination showed more recovery for hepatocytes and normal central vein without degeneration of hepatocytes or any changes in its nuclei. Also, the kupffer cells and sinusoids appear normal (Fig.5).

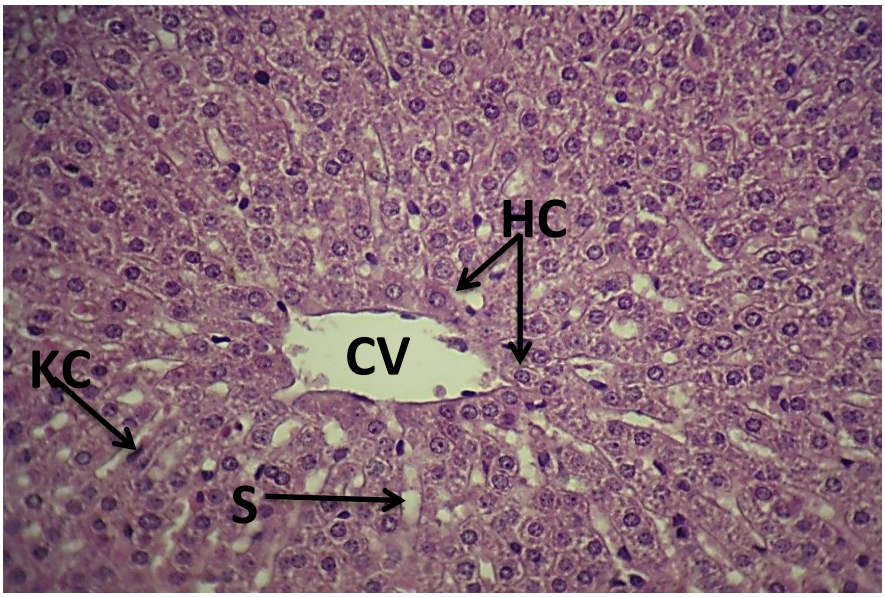

Figure (1): Liver of control group showed central vein (CV), hepatocytes (HC), sinusoids(S) and kupffur cells (KC) H&E 400X.

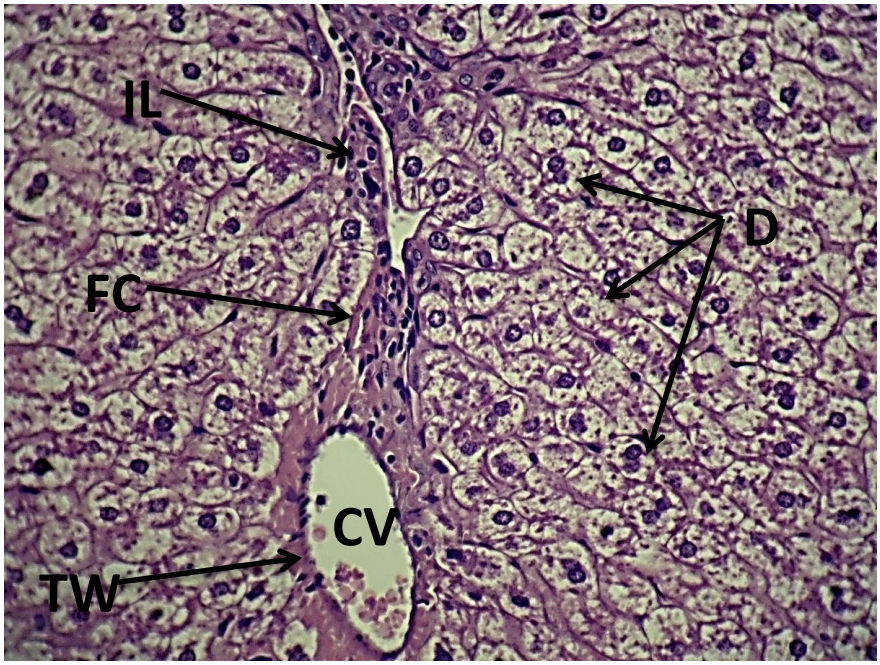

Figure (2): Liver of 0.1% H2O2 group showed thickening wall (TW) of central vein, degeneration (D) of liver cells and lymphocytes infiltration (IL) around sinusoids and fibrocytes (FC) H&E 400X.

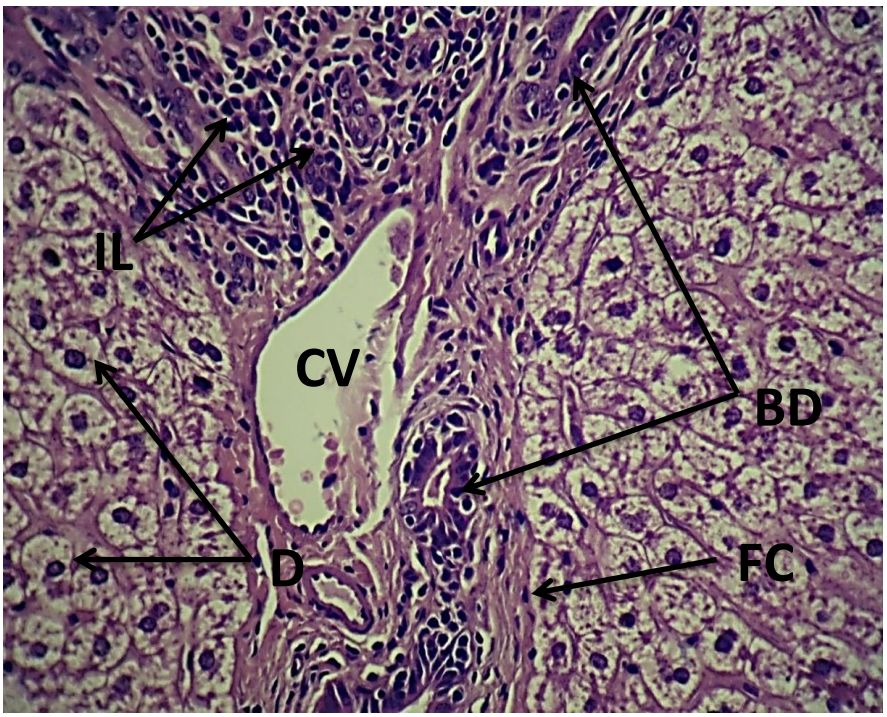

Figure (3): Liver of 0.15% H2O2 group showed degeneration (D) of hepatocytes and sclerosing bile ducts (BD) with lymphocytes infiltration (IL) and fibrocytes (FC) H&E 400X.

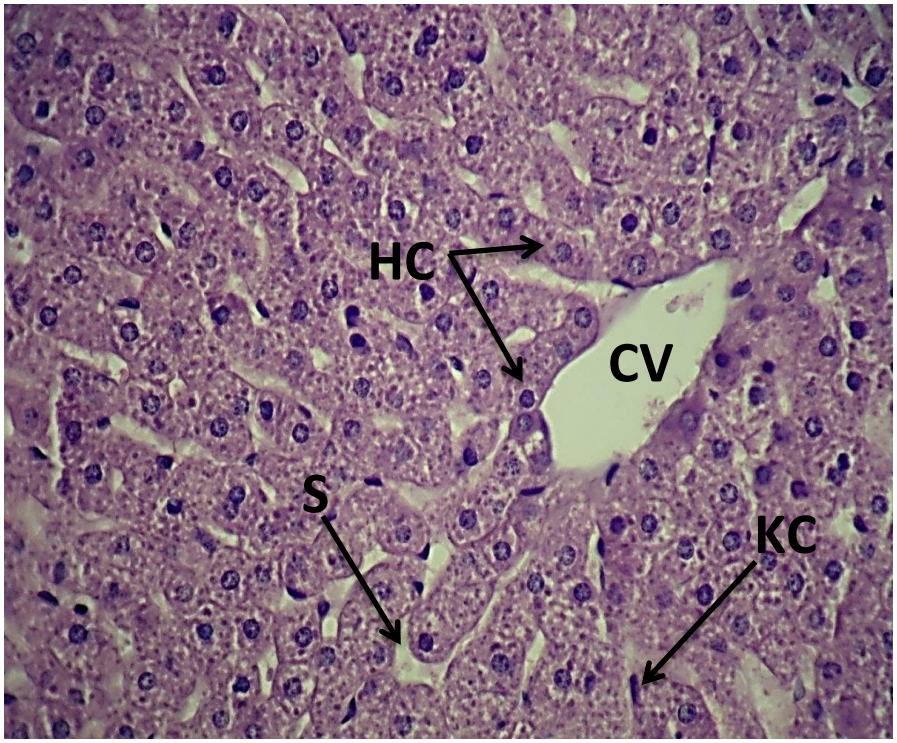

Figure (4): Liver of 0.1% H2O2 and treated with camel milk group showed central vein (CV), hepatocytes (HC), sinusoids(S) and kupffur cells (KC) H&E 400X.

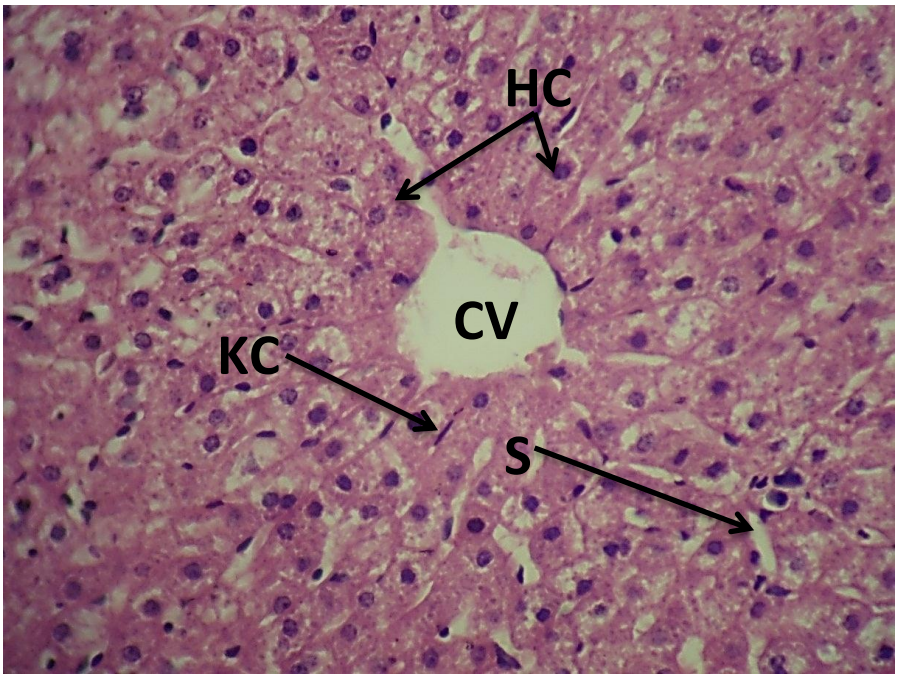

Figure (5): Liver of 0.15% H2O2 and treated with camel milk group showed central vein (CV), hepatocytes (HC), sinusoids(S) and kupffur cells (KC) H&E 400X.

Discussion :

Khudiar (2010) stated that the serum ALT and AST was more than normal range in the animals (rabbits) after administrated hydroxide peroxide, this was found in the results of the present study, especially in the hydroxide peroxide groups compared with the control groups.

Rahim et al. (2014) referred that the rat treated with H2O2 (0.5%), in drinking water, showed increased in serum ALT and AST levels in the animals (rats) after administrated hydroxide peroxide. Also, histopathological alterations present which including infiltration of lymphocytes, and degeneration of hepatocytes (necrosis), in comparison with control that is in agreement with the results of the present study.

Aoki and Tani. (1972) referred that the mice treated with H2O2 in drinking water, showed histopathological alterations including congestion, infiltration and degenerative changes the liver cells that is in agreement with the results of the present study.

In study carried by *Althnaian et al (2013)* to show the role of the camel milk in healing the liver lesion caused by carbontetrachloride. They found elevation the ALT and AST levels in the groups that administrated carbontetrachloride and also liver showed different lesions including degenerative changes, infiltration of lymphocytes but when these treated with camel milk the liver enzymes and tissue back to normal that is in agreement with the results of the present study. Otherwise, Darwish et al (2012) referred that the ethanol lead to elevation the ALT and AST levels in the rat groups that administrated ethanol and also liver showed different lesions including degenerative changes, infiltration of lymphocytes With fibroblastic cells proliferation, but when these treated with camel milk the liver enzymes and tissues were to normal that is in agreement with the results of the present study.

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