Outcomes of Albino Mice Liver Infected with Contaminated Camel Milk

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Original Article

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ABSTRACT

Background: Camel milk is an excellent source of nutrients and has medicinal importance for human in many countries all over the world especially in nomadic societies of Arab countries. *Staphylococcus aureus* (*S. aureus*) is instinctively present in milk and dairy products, and associated with many outbreaks. Milk is a good substrate for *S. aureus* rise and toxin production. When transmitted to human body this pathogen may affect skin, brain, kidney, liver and several other vital organs. Therefore, the pathological effects of *S. aureus* have been the focus of several recent research works.

Aim: The purpose of this study was to better understand the histopathological effects of *S. aureus* isolated from contaminated raw camel milk on the mice livers.

Materials and Methods: *S. aureus* were isolated from specimens of lactating she-camels (*Camelus dromedarius*), weighing 300-540 kg, in Libya and identified using microbiological and molecular techniques. Twenty healthy male Swiss albino mice, weighing 22-25 gm, were divided into two groups; Group I (control) and Group II (orally administrated mice with a single dose of aqueous solutions of the isolated *S. aureus* at a concentration of 5x108/0.1ml). Animals sacrificed after three days of infection with bacteria and their livers were dissected out for macro- and microscopic inspection.

Results: Our study reported that oral administration with *S. aureus* collected from camel milk causes outer liquid-filled liver abscesses. Furthermore, various histopathological changes could be detected.

Conclusion: This study may highlight the potential risk of consuming raw she-camel milk, especially upon lack of strict hygienic and preventative measures to avoid the presence of *S. aureus* in milk.

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INTRODUCTION

Camel milk is considered an excellent source of nutrients for human and has medicinal properties[1]. However, it provides a suitable medium for microbial growth and metabolism. Bacteria, in raw milk, can affect the quality, safety and consumer acceptance of dairy products. Nonpathogenic bacteria may affect milk quality[2]. Thus, different regions have milk quality regulations, including limits on the bacterial count in raw milk, to ensure the quality and safety of the final product. The microbial load in milk, immediately after milking, are concerned by several factors including animal health, apparatus cleanliness and time[3].

Staphylococcus spp are microorganisms that are commonly present in milk and they are often associated with food-borne outbreaks due to the ability of some strains to produce thermostable enterotoxins. In addition, they are from the most important pathogen due to the rise in antibiotic resistance[4] *S. aureus* is a widespread Gram-positive coccus and is considered a prominent cause of human infections globally in health care and community settings. It can be commensal but also a dangerous pathogen[5,6]. It can cause skin abscesses, wound infections, pneumonia and septic arthritis[7]. Milk is a good substrate for *S. aureus* and toxin production. The bacterium is also capable of producing several pathological conditions in human[8].

There are numerous animal models of staphylococcal infection have contributed to the knowledge of virulence genes involved in disease. Many researchers reported that *S. aureus* causes osteomyelitis,[9,10] pneumonia,[11] also it causes interaction with the nasal mucosa[12].

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In fact, camel milk production is important source of high-quality nutrient in nomadic societies. Therefore, the current research was designed to better characterize the histopathological actions of *S. aureus* isolated from raw camel milk from various areas in Libya on the mice liver

MATERIALS AND METHODS

Collection of samples

220 milk samples of apparently healthy lactating she-camels (*Camelus dromedarius*) were aggregated from various regions in Libya. These dromedaries weigh between 300 and 540 kg. The udders of she-camels were flushed with tap water and mopped to dry with clean cloth. The milkers' hands were carefully washed prior to collection of samples, discarding the early surge of milk. Nearly, 30 ml of specimen from each portion were separately collected in hygienic tubes. Collected milk samples were kept in an ice box and then transported to bacteriology laboratory at the Department of Microbiology, Faculty of Veterinary Medicine, University of Omar-Al Muktar, Libya.

Bacterial strain

Bacterial isolates showed β-hemolysis on enriched media and showed clusters of Gram-positive cocci upon staining. Mannitol fermentation with the color change of mannitol salt agar (MSA) and production of small yellow colonies. Catalase and coagulase trials revealed positive isolates. Assembly of oxygen was detected by bubble formation. Isolates were identified as *S. aureus* by coagulase test and confirmed by formation of curd like clotting. The microbe was then centrifuged and the residue was washed with sterile phosphate buffer saline, measured at 620 nm by Schimadzu™ UV-1800 spectrophotometer. The milk sample containing bacterial load $1.0X10^9$ was approximately adjusted to correspond to an optical density of 1.6[13]. Milk sample diluted with phosphate buffer saline was performed to get $5X10^8$/0.1ml of bacterial suspension as high bacterial load to examine histopathological variations.

Qualitative Molecular detection

A specific 23s rRNA primer for 23s rRNA gene was used for genetic identification of *S. aureus* (23S rRNA Primer) F: 5'ACG GAG TTA AGGCAA AGG ACG AC3′, and (tst primer): F: 5'ATG GCA GCA TCA GCT TGA TA3' as classical toxin gene of *S. aureus* according to[14].

Animals and infection

Twenty healthy male albino mice, weighing 22-25 gm, were purchased from the animal house of Faculty of Medicine, Ain Sham University. The animals were resided in plastic cages at 25 °C and 12 h alternative cycles. Mice were fed with standard diet ad libitum and water. Animals were divided into two groups (n=10/group) and were assigned at random to one of the following treatments. The first group was left uninfected and served as control group. The second group were infected with identified *S. aureus* by oral administration of a single dose (0.1ml) of milk containing bacterial suspension at a concentration of $5X10^8$ colony forming unit /0.1 mL. Mice of control group received the vehicle in a similar manner. All animals were sacrificed after three days of infection. All animal experiments were approved by the Ain Shams University Research Ethics Committee.

Gross morphology

During the experiment, mice were monitored to determine the effect of *S. aureus* on their gross morphology.

Sample collection

Mice were fasted overnight, sacrificed by cervical dislocation, and dissected. The livers were excised, washed in phosphate buffered saline, weighed wet, and photographed. Then, the livers weight and relative weight was recorded then processed for tissue assessments.

Histological Procedures

Pieces of the left lateral lobe of liver were settled in neutral buffer formalin for 24 hours, dehydrated using ascending grades of ethanol, cleared in terpineol, embedded in paraffin wax, and serially sectioned at 5 µm thick. By using hematoxylin and eosin, paraffin sections were stained. At the Regional Center for Mycology and Biotechnology, Al-Azhar University, liver sections were photographed using a camera attached to a Leica DM LS2 microscope (Leica Microsystems, Wetzlar, Germany).

Ultrastructural Procedures

Slices of the right lobe of the liver were diced into 1 mm cubes, immersed in 2.5%glutaraldehyde in 0.1 M phosphate buffer at pH 7.0. Samples were dipped in the buffer and post-fixed in 1% osmium tetroxide (OSO4), dehydrated, inserted in epoxy 812 resin. Semi-thin sections with toluidine blue were processed and stained. Ultrathin sections were collected on grids and stained in uranyl acetate and lead citrate. In the Regional Center for Mycology and Biotechnology in Al azhar University, ultrathin sections were examined using Jeol 100S transmission electron microscope.

Morphometry

By using the image analysis software, Leica Q 500 (Leica Imaging Systems Ltd., Cambridge, UK), the diameter and numerical density (i.e., the number of organelles in the unit hepatocyte volume) of mitochondria, in addition to the diameter of both central and portal veins were also measured. Stereological procedures for calculating the numbers and volumes of the mitochondria were similar to those described by Weibel[15].

Statistical analysis

Data are collected as means and standard deviation and processed using GraphPad Prism software (version 5.0). $P<0.05$ was deemed statistically significant.

RESULTS

Isolation and purification of microbial isolates

Five coagulate positive *S. aureus* were collected from 220 milk samples. The pure cultures displayed β-hemolysis on blood agar media. Isolates were positive for catalase and coagulase tests.

Molecular detection of specific gene of *S. aureus* isolated from she-camel milk samples

Among the 220 camel milk samples investigated, five samples were found to be positive for *S. aureus*. The species identity of all 5 strains could be confirmed by PCR amplification of the *S. aureus*-specific chromosomal DNA fragment, the *S. aureus*-specific part of 23s rRNA. The amplification of staphylococcal coagulase encoding 23s rRNA gene yielded a uniform amplicon size of approximately 1251 bp for all six *S. aureus* (Figure 1). The ability to synthesize classical enterotoxins (tst gene) in one isolate (16.7%) and the amplicon size of the tst gene ≈ 260 bp (Figure 2). The milk sample containing this isolate was used for infection of mice and other experiments.

Body weight and relative liver weight

After completing of the test, there was body mass and liver density gain in the control group, as in the infected group. Statistical analysis depicts that infection with *S. aureus* lead to increase the body and liver weights of mice (Table 1).

Liver gross morphology

Macroscopic testing for the gross morphology of the liver of control group exposed that the liver had normal color, smooth surface, and moderately bright appearance (Figure 3a). On the other hand, the liver of *S. aureus*-infected mice was relatively more or less similar to normal, but contained certain abscesses on its surface between liver lobes. These abscesses were large and full of liquid (Figure 3b).

Histological observations

The liver tissues of control mice after hematoxylin and eosin, as well as semithin sections, revealed normal polygonal hepatocytes with granular cytoplasm and round nuclei with heterochromatin adjacent to the nuclear border in addition to Normal sinusoids with Kupffer cells (Figures 4 a,b, 5a). Liver sections of *S. aureus*-infected mice revealed histological changes in the form of highly degenerated hepatocytic areas comprising necrotic and disintegrated hepatocytes (Figures 4c,5b) with pyknotic nuclei (Figure 4c). Mononuclear cellular infiltration was seen in the areas surrounding the blood sinusoids, central, and portal veins. These structures were also associated with dark areas indicating colonies of *S. aureus* (Figure 4c). The Kupffer cells were hypertrophied (Figure 5b). The central and portal veins were highly congested especially in the degenerated hepatocytic areas (Figures 4 c,d, 5b). The central vein significantly decreased in the diameter, while the portal vein was significantly dilated compared to the control group (Figures 4 c,d, Table 2). The hepatocytes neighboring to the portal triad were hydropic, swollen, and possessed severe ballooning degeneration with only wisps of cytoplasm around the nucleus. Hyperplasia of the bile duct and accumulation of cell debris in the duct lumen were also seen (Figure 4d).

Ultrastructural observations

Ultrathin sections of control mice liver reveal normal polygonal hepatocytes bounded by a well-defined plasma membrane (Figure 6). The hepatocytic cytoplasm contained several mitochondria of varying shapes and sizes, well-developed rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER) and glycogen rosettes. All these fine structures were situated around the centrally located rounded nuclei. Chromatin was arranged as peripheral chromatin, chromatin islands and nucleoli-associated chromatin (Figure 6).

The ultrastructural observation of mice liver treated with *S. aureus* collected from camel milk revealed hypertrophied, and vacuolated hepatocytes (Figure 7a), containing a number of swollen mitochondria. This swelling was insignificant, but they were significantly decreased in the number, had impaired internal structure with cristolysis (Figure 7b, Table 2). A certain number of these mitochondria were circled by small parts of vesiculated RER. Some hepatocytic areas contained highly dispersed SER, and some lysosomes and vacuoles (Figure 7b).

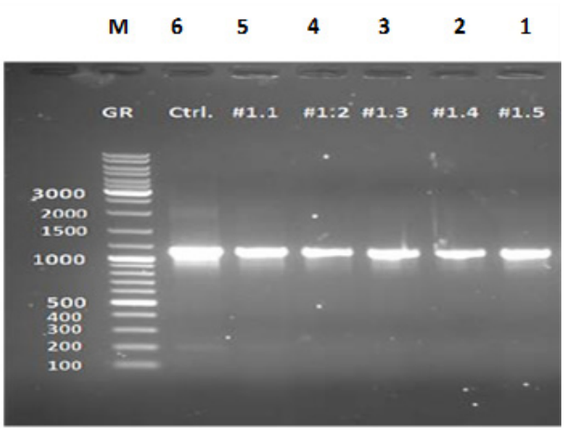

Fig. 1: Agarose gel electrophoresis patterns showing PCR-amplified products of qualitative PCR for identifying *S. aureus* 23s rRNA gene in five strain samples using the specific primer of 23s rRNA gene. Bands in lanes 1 to 6 are amplicon sizes of specific segment of the target gene with a uniform molecular weight 1251 bp. Lane M represents DNA molecular weight 100bp DNA marker Sizes are marked in base pairs on the left.

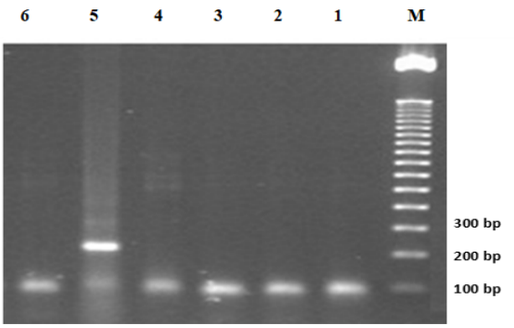

Fig. 2: Agarose gel electrophoresis patterns showing PCR-amplified products of qualitative PCR for identifying tst enterotoxin gene of *S. aureus*. Only one positive bacterial sample of group 5 at primer tst (lane 5) is seen with a molecular weight ≈ 260 bp. Lane M represents DNA molecular weight PCR marker. Sizes are marked in base pairs on the right.

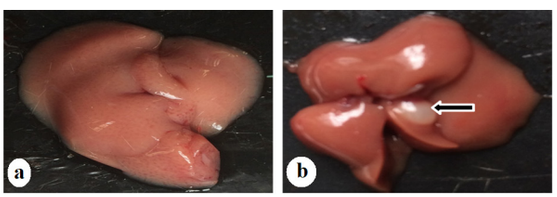

Fig. 3: photomicrograph showing gross morphology of liver. a) From control mouse liver. b) From mouse orally administered with *S. aureus* collected from camel milk Note the large abscess (arrow).

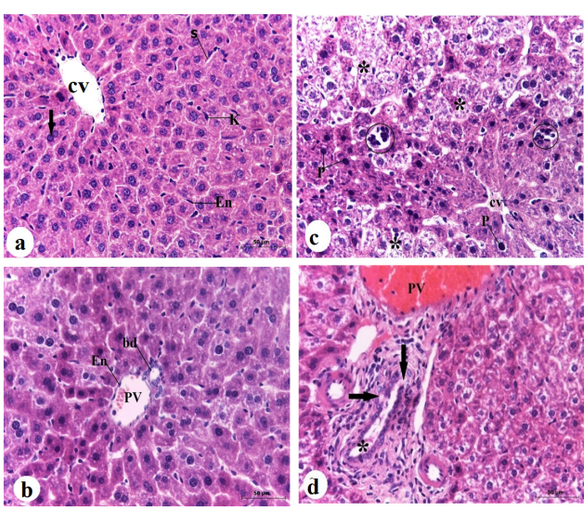

Fig. 4: photomicrograph of liver sections stained with Hx & E. a) and b) From control mouse liver. c) and d) From mouse orally administered with *S. aureus* collected from camel milk a) Showing a central vein (cv) surrounded by normal hepatocytes. Each hepatocyte has one nucleus or two nuclei (arrow heads). Note the blood sinusoids (s) between the hepatic strands, lined by endothelial cells (En). Kupffer cells (K) are located at the corner of sinusoids. b) Showing a branch of the portal vein (PV) lined with endothelial cells (En) and facing small branch of bile duct (bd). c) Showing necrotic and highly degenerated hydropic and swollen hepatocytic areas (*), as well as aggregated inflammatory cells associating small colonies of *S. aureus* (circles). P, pyknotic nuclei; cv, central vein. d) Showing a remarkable dilated and congested portal vein (PV), and blebbing of the bile duct associated with focal injury of cholangiocytes (arrows). Bile duct stenosis and accumulation of cell debris in the duct lumen are also seen (*).

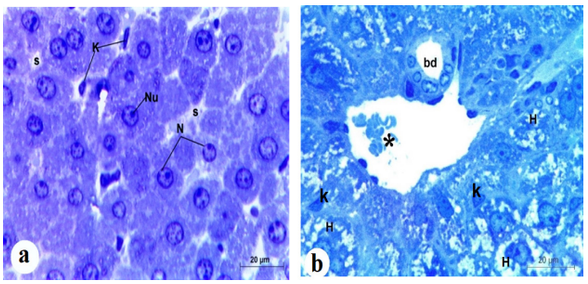

Fig. 5: Photomicrograph of semithin sections of liver stained with toluidine blue. a) From control mouse liver. b) From mouse orally administered with *S. aureus* collected from camel milk. a) Showing normal hepatic architecture. The hepatocytes contain centrally located nuclei (N) with prominent nucleoli (nu). Notice the blood sinusoids (s) between the hepatic strands and Kupffer cells in their corners (K). b) Showing congested portal vein with RBCs (*). The hepatocytes are hypertrophied, highly degenerated and vacuolated (H). Kupffer cells (K) are hypertrophied. bd, bile duct.

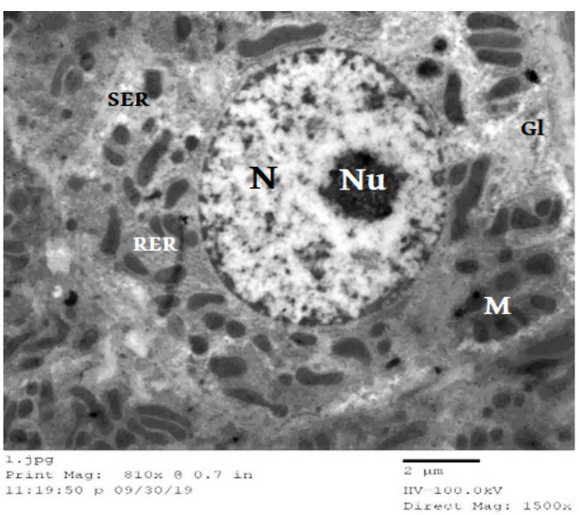

Fig. 6: Electron micrograph of liver section from control mouse showing a hepatocyte with a round nucleus (N), one distinct nucleolus (Nu), several numbers of mitochondria (M) exhibiting rounded or elongated shapes, rough endoplasmic reticula (RER), smooth endoplasmic reticula (SER), and abundant glycogen particles (Gl) scattered in the ground cytoplasm.

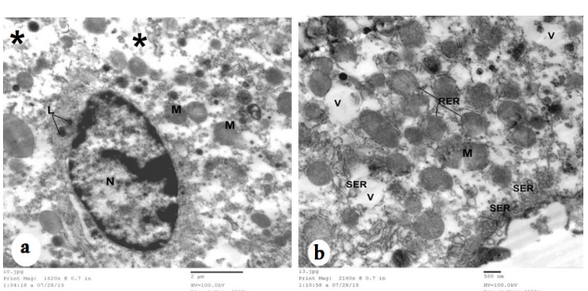

Fig. 7: Electron micrograph of liver sections from mouse orally administered with *S. aureus* collected from camel milk a) With low magnification. b) With high magnification. a) Showing a necrotic hepatocyte. N, nucleus; M, mitochondria; L, lysosome; *, necrotic area. b) Showing slightly swollen mitochondria with cristolysis (M). Small vesiculated parts of RER and dispersed SER were also seen. Note the vacuoles (v).

Table 1: Body and liver relative weights of the control and *S. aureus*-infected groups

	Body weight (g)	Liver weight (g)	Relative liver weight
Control mice	25.56± 2.44	1.35±0.10	5.29±0.23
Infected mice	32.24±2.17	1.82±0.11	5.68±0.37

Data are expressed as mean ± SEM. Values depict slightly increase of body & liver weight of infected mice from that of the control group: $P<0.06$

Table 2: Hepatic changes in the control and *S. aureus*-infected groups

	Control mice	Infected mice
Central vein diameter (µm)	38.65± 1.5	34.04±3.21*
Portal vein diameter (µm)	48.85±1.56	67.5±7.29*
Mitochondrial diameter (nm)	621.43±46.11	702.38±20.19**
Mitochondrial number	41.71±1.19	33.86±0.46*

Data are means ± SEM.
* indicates the significant difference of infected group vs. the control group * $p<0.05$, ** $p<0.001$.

DISCUSSION

Camel milk is a very rich source for the growth of lot of different species of bacteria that have various effects. Many investigators highlight the beneficial effects of these isolated bacteria including antimicrobial effects probiotic candidates[16,17]. However, a limited data is available at present regarding potential of *S. aureus* living in camel milk in Libya and the proposed role of bacterial enterotoxins isolated from raw camel milk.

S. aureus can be commensal and dangerous pathogen affects the bloodstream, skin, soft tissues and lower respiratory tracts[18]. It leads to severe deep-seated injuries as septic arthritis, endocarditis and osteomyelitis[19]. In addition, it is often responsible for toxin-mediated symptoms, such as scalded skin syndrome and staphylococcal food borne diseases. In this report we investigated histological changes of liver upon infection with *S. aureus* collected from camel milk producing of classical enterotoxin. One of the major causes of hospital- and community-acquired infections is *S. aureus* which can have severe implications[20]. It has ability to cause life-threatening infections and remarkable potential to develop antimicrobial resistance[18].

In the present report, the macroscopic examinations of liver of mice infected with *S. aureus* isolated from contaminated raw camel milk revealed more than one large pyogenic abscesses, full in liquid, on its surface. Kobayashi *et al*. stated that abscesses are a frequent manifestation of *S. aureus* skin and soft tissue infections[21,22]. Worth mentioning, although pyogenic hepatic abscess is rare, potentially as serious disease caused by *S. aureus*[23]. The pyogenic abscess begins as a localized host immune response to bacterial infection that is a lethal bacterial infection of the hepatic parenchyma, with a 5.6% mortality rate[24]. Gong *et al*. (2014) suggested that exotoxins produced by *S. aureus* are important for the infection[25-27]. Different kinds of exotoxins required to cause infection can be expressed by *S. aureus*, especially α-toxin which is the essential exotoxin of *S. aureus*[28]. Moreover, Gong *et al*. showed that injecting 5µl bead suspension of *S. aureus* (10^6 CFU) into the brain of mice caused intracranial abscesses growth[25].

In the present work, the histological examinations of *S. aureus*-infected liver revealed mononuclear cellular infiltration, especially in association with dark areas which are thought to be colonies of *S. aureus*. Most likely, the mild diffuse inflammation that occurred in liver may be due to the endotoxin of bacteria, which leads to cellular morphological changes[28]. In this work, the affected liver sections revealed degenerated, vacuolated, necrotic, and swollen hepatocytes. Pyknosis of nuclei and congestion of blood sinusoids, central and portal veins were also seen. Most of these histological remarks came in accordance with a study on the pathogenesis of experimental infection by *S. aureus* in rabbits, which revealed congestion of the liver, hydropic degeneration and necrosis of hepatocytes and obstruction of bile sinusoid with bile pigment in the livers of infected animals[29].

These pathological changes emphasize the role of bacterial toxins as a pivotal stimulus for these changes. The infection leads to effectiveness of neutrophils and macrophages, the primary edge of defense against infection[29]. The neutrophil secretes chemotaxis which kill bacteria, lyses infected host hepatocyte, that were detected in the present infiltration of hepatic tissue with neutrophils that caused liquefaction and lyses of hepatocytes (necrotic cells)[30]. *S. aureus* stashes compounds that downregulate activation and chemotaxis or that lyses neutrophils, neutralize antimicrobial defenses peptide and its cell boundaries is altered to reduce their effectiveness[31].

Cellular swelling including hydropic change, vacuolar degeneration, or cellular edema were detected in the liver of infected mice. These reversible changes are most likely resulting as a response to non-lethal injuries[32], or to the intracytoplasmic water accumulation due to inability of the cells to maintain the ionic and fluid homeostasis. It is clearly detected in parenchymal organs, such as liver with hepatitis and hypoxia, kidney with shock, and myocardium with hypoxia, and it may be local or diffuse, affecting the whole organ[32].

Otto reported that many *S. aureus* hemolysins and leukotoxins destruct cellular membranes, coax cell death, degenerate neutrophils after gulp, to eradicate bacteria by humoral host defense[33]. Taking these attributes collectively, the vacuolated and hydropic hepatocytes recorded in the infected groups of this report are most likely due to the cytoplasmic membrane is the target of a

wide range of bacterial toxins, comprising several that are produced by *S. aureus*. Consistent with these attributes, the histopathological cross examination of the liver of treated rats with *E. coli*, *S. typhi*, and *S. aureus* revealed swollen hepatocytes with lessen sinusoidal spaces and extensively dispersed necrotic foci[34].

In this study, branches of the portal vein in the liver of *S. aureus*-treated groups were greatly expanded and congested. Effect of *S. aureus* is prominent on the vascular bed, especially the portal vein. This effect may be developed during infection or as a result of direct action of bacteria on the vessel wall. These areas also possessed periductal fibrosis associated with inflammatory cells infiltration surrounding the bile ducts. Hyperplasia of the bile duct and accumulation of cell debris in the duct lumen were also seen. The bile duct injury is always associated with liver abscess and may cause obliteration of adjacent portal branches and liver parenchymal atrophy[35].

This explanation is most likely steady with the appearance of hepatocytes in the vicinity of the central vein, as around the portal triad, with vacuolar cytoplasm and pyknotic nuclei. Taking these attributes collectively, it is simply to tell that these cells are both structurally and functionally dead.

The ultrastructural observations reported in the current work may support the aforementioned histological findings dealing with the action of *S. aureus* on the mice liver. In these studies, the mitochondria were the most affected organelles in the hepatocytes of *S. aureus*-infected mice. The mitochondrial damage was detected in the form of swelling, impairment of the internal structure, and cristolysis (loss of cristae). The observed mitochondrial swelling, probably to compensate for their decreased number. These mitochondrial pathological changes may reflect alterations in the hepatocytes osmolarity leading to salt influx to mitochondrial matrix and affecting the integrity of the inner mitochondrial membranous structures[36].

Mitochondrial cristae play an essential step in their function[37,38]. In fact, cristolysis may affects the mitochondrial potentiality in the process of oxidative phosphorylation and electron transport system, and subsequent ATP production[38].

Ultrastructural remarks of the hepatocytes of *S. aureus*-treated group revealed losing of array, vesiculation, and stack shortening of RER. In fact, detrimental factors affecting the cell and its microenvironment, for example, are the transitions in the redox milieu or acidosis in ER[39]. Therefore, these variations in the RER architecture are most likely due to the enterotoxin of *S. aureus* which has probably affected the integrity of the hepatocyte redox system in the ER membranes, as in the mitochondria. Fine-tuning of these redox and ion signaling pathways might be difficult in unhealthy cells[40]. In addition, the pronounced dilatation of ER lumen is a typical ultrastructural transition to ER stress. This pathology would indicate that *S. aureus* would decrease the surface density of the ER that may negatively impact protein and lipid biosynthesis by the affected hepatocytes. Additionally, in the current work, the elevation in the dispersed SER amount reveals the increase in the detoxification demand that resulted from the pathogen toxin.

In the current study, lysosomal granules were most commonly seen in the hepatocytes and Kupffer cells in the liver of infected mice. Cytoplasmic vacuoles with granular structures were located in the infected hepatocytes. These granules are most likely the enterotoxins of *S. aureus*. Literally, lysosomes are special organelles that contain hydrolytic enzymes that can break down many kinds of biomolecules[41]. Lysosomes act as the waste disposal system of the cell by digesting obsolete or unused materials in the cytoplasm, from both inside and outside the cell[42].

The present work demonstrated Kupffer cell hyperplasia and hypertrophy due to toxin exposure. Kupffer cells are hepatic macrophages activated by hepatotoxins and always demonstrated with liver injuries[43]. The activation and enlargement of these cells are always related to stress induced in the hepatic tissues by some drugs and environmental pollutants. Furthermore, the main function of Kupffer cells was reported as the defense of the liver against various microbes, they also phagocytose and partially destroy bacterial antigens before passing them on to the hepatocytes for excretion into the bile[44].

Thus, histological and ultrastructural studies, undertaken in this study, reveal that oral administration of mice with *S. aureus* isolated from contaminated raw camel milk most likely causes the development of outer liquid-filled abscesses and various histological and fine structural changes in the mice liver which may highlight the potential risk of consuming raw she-camel milk, especially upon lack of strict hygienic and preventive measures to avoid the existence of *S. aureus* isolates in milk

Many scientists reported that *S. aureus* can be present and disseminate to gut. Also, the liver as largest internal organ receiving blood flow from circulatory system and as primary organ for filtering bacterial pathogens to keep blood sterility[45]. Therefore, our report highlights the possibility of some species *S. aureus* isolated from camel milk to cause adverse effects at high loads in liver in certain conditions. Moreover, public health and food hygiene practices during milking, transportation, storage and consuming should be implemented to reduce the risk of *S. aureus* related camel milk poisoning.

STATEMENT OF ETHICS

All animal experiments were approved by the Ain Shams University Research Ethics Committee.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Abrhaley A and Leta S: Medicinal value of camel milk and meat. J App Anim Res. (2018) 46(1): 552-558.
2. Samaržija D, Zamberlin S and Pogačić T: Psychrotrophic bacteria and milk and dairy products quality. Mljekarstvo. (2012) 62(2): 77-79.
3. Swai E and Schoonman L: Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. Asian Pac J Trop Biomed. (2011) 1(3): 217-222.
4. Gabriela N, Paula M, Anderson K and Luís A. Enumeration of coagulase and thermonuclease-positive Staphylococcus spp. in raw milk and fresh soft cheese: An evaluation of Baird-Parker agar, Rabbit Plasma Fibrinogen agar and the Petrifilm™ Staph Express count system. Food Microbiol. (2009) 7(4): 447-452.
5. Wertheim H, Melles D, Vos M, van Leeuwen W, van Belkum A, Verbrugh H and Nouwen J: The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis. (2005) 5: 751e762, 2005.
6. Musser J and DeLeo F: Molecular Pathogenesis Lessons from the World of Infectious Diseases Research. Am J Pathol. (2015) 185(6): 1-10.
7. Capparelli R, Paralato M, Borriello G, Salvatore P and Iannelli D: Experimental phage therapy against Staphylococcus aureus in mice. Antimicrob Agents Chemother. (2007) 51(8): 2765-2773.
8. Ahmed A, Abdelrahman A, Bernard F, Lucie B and Sallam A: Assessment of Quality of Camel Milk and Gariss, North Kordofan State, Sudan Res J Anim Veterin Sci. (2010) 5(1): 18-22.
9. Sottnik JL, U'Ren LW, Thamm DH, Withrow SJ and Dow SW: Chronic bacterial osteomyelitis suppression of tumor growth requires innate immune responses. Cancer Immunol Immunother. (2010) 59(3): 367-378.
10. Funao H, Ishii K, Nagai S, Sasaki A, Hoshikawa T, Aizawa M, Okada Y, Chiba K, Koyasu S, Toyama Y and Matsumoto M: Establishment of a real-time, quantitative, and reproducible mouse model of Staphylococcus osteomyelitis using bioluminescence imaging. Infect Immun. (2010) 80(2): 733-741.
11. Rubinstein E, Kollef MH and Nathwani D: Pneumonia caused by methicillin-resistant Staphylococcus aureus. Clin Infect Dis. (2008) 46(5): S378-S385.
12. Krismer B, Weidenmaier C, Zipperer A and Peschel A: The commensal lifestyle of Staphylococcus aureus and its interactions with the nasal microbiota. Nature Rev. Microbiol. (2017) 15(11): 675-687.
13. El Zubeir IE and Ahmed MI: The hygienic quality of raw milk produced by some dairy farms in Khartoum State, Sudan Res J Microbiol. (2007) 2(2): 988-991.
14. Meka VG, Pillai SK, Sakoulas G, Wennersten C, Venkataraman L, DeGirolami PC, Eliopoulos GM, Moellering Jr RC and Gold HS. Linezolid resistance in sequential Staphylococcus aureus isolates associated with a T2500A mutation in the 23S rRNA gene and loss of a single copy of rRNA. J Infect Dis. (2004) 190(2): 311-317.
15. Mahmoud YI and Hegazy HG. Ginger and alpha lipoic acid ameliorate age-related ultrastructural changes in rat liver. Biotech Histochem. (2016) 91(2): 86-95.
16. Soliman MM, Hassan MY, Mostafa SA, Ali HA and Saleh OM: Protective effects of camel milk against pathogenicity induced by Escherichia coli and Staphylococcus aureus in Wistar rats. Mol Med Rep. (2015) 12(6): 8306-8312.
17. Vimont A, Fernandez B, Hammami R, Ababsa A, Daba H and Fliss I: Bacteriocin-Producing Enterococcus faecium LCW 44: A High Potential Probiotic Candidate from Raw Camel Milk. Front Microbiol. (2017) 8: 865.
18. Plata K, Rosato AE and Wegrzyn G: Staphylococcus aureus as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity. Acta Biochim Pol. (2009) 56(4): 597.
19. Schito GC: The importance of the development of antibiotic resistance in Staphylococcus aureus. Clin Microbiol Infect. (2006) 12(1): 3-8.
20. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN and Beach M: Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, the Western Pacific region for the SENTRY Antimicrobial Surveillance Program 1997–1999. Clin Infect Dis. (2001) 32(2): S114-S132.
21. Kobayashi S, Malachowa N and DeLeo F. Pathogenesis of Staphylococcus aureus abscesses. Am J Pathol. (2015) 185: 1518e1527.
22. Zhang R, Wang X, Zhang X and Yang Y: An experimental model for Staphylococcus aureus hepatic abscess in Bama minipig. Genet Mol Res. (2014) 13(3): 7113-7122.
23. Krishna S and Miller L: Innate and adaptive immune responses against Staphylococcus aureus skin infections. Semin Immunopathol. (2012) 34(2): 261-280.
24. Jepsen P, Vilstrup H, Schonheyder H and Sorensen H: A nationwide study of the incidence and 30-day mortality rate of pyogenic liver abscess in Denmark. Alim Pharmacol Therap. (2005) 21(10): 1185-1188.
25. Gong J, Dongzhi Li, Yan J, Liu Y, Li D, Dong J, Gao Y, Sunb T and Yang G: The accessory gene regulator (agr) controls Staphylococcus aureus virulence in a murine intracranial abscesses model. Brazil J Infect Dis. (2014) 18(5): 501-506.

26. Yarwood J and Schlievert P: Quorum sensing in Staphylococcus infections. J Clin Invest. (2003) 112: 1620-1625.

27. Yarwood J, Bartels D, Volper E and Greenberg E: Quorum sensing in Staphylococcus aureus biofilms. J Bacteriol. (2004) 186: 1838-1850.

28. Mathurin P, Deng QG, Keshavarzian A, Choudhary S, Holmes EW and Tsukamoto H. Exacerbation of alcoholic liver injury by enteral endotoxin in rats. Hepatology. (2000) 32(5): 1008-1017.

29. AL-Nakeeb N: The pathogenesis of experimental infections by Staphylococcus aureus in rabbit. Kufa J Vet Med Sci. (2011) 2(2): 1-10.

30. de Haas CJ, Veldkamp KE, Peschel A, Weerkamp F, Van Wamel WJ, Heezius EC, Poppelier MJ, Van Kessel KP Van Strijp JA. Chemotaxis inhibitory protein of Staphylococcus aureus, a bacterial antiinflammatory agent. J Experimen Med. (2004) 199(5): 687-695.

31. Foster TJ: .Immune evasion by Staphylococci. Nat rev microbial. (2005) 3(12): 948.

32. Kumar V, Abbas A and Aster J: Robbins Basic Pathology. 10th ed., Elsevier, USA. 2017

33. Otto M. Staphylococcus aureus toxins. Curr Opin Microbiol. (2014) 2: 32-37.

34. Osman G, Menshawy M and Farrag A: Effect of Escherichia coli, Salmonella spp., and Staphylococcus aureus isolated from polluted drainage water on rats. J Arab Soc Med Res. (2016) 11: 1-8.

35. Yu J, Kim K, Jeong M, Lee D, Park M and Yoon S: Predisposing factors of bile duct injury after transcatheter arterial chemoembolization (TACE) for hepatic malignancy. Cardiovasc Intervent Radiol. (2002) 25: 270-274.

36. Almansour M, Sajti L, Melhim W and Jarrar BM: Ultrastructural hepatocytic alterations induced by silver nanoparticle toxicity. Ultrastruct pathol. (2016) 40(2): 92-100.

37. El-Shenawy NS: Oxidative stress responses of rats exposed to roundup and its active ingredient glyphosate. Environ Toxicol Pharmacol. (2009) 8:379-385.

38. Singh A, Bhat T and Sharma OP: Clinical biochemistry and hepatotoxicity. J Clin Toxicol. (2011) 4: 11-18.

39. Schoenthal AH: Endoplasmic reticulum stress its role in disease and novel prospects for therapy. Scientifica. 2012

40. Yoboue E, Sitia R and Simmen T: Redox crosstalk at endoplasmic reticulum (ER) membrane contact sites (MCS) uses toxic waste to deliver messages. Cell Death Dis. (2018) 9(3): 331.

41. Mescher AL: Junquerira's Basic Histology: Text and Atlas. 14th ed., McGraw Hill, USA. (2015).

42. Underwood, Emily. When the brain's waste disposal system fails. Knowable Magazine (2018).

43. Kegel V, Pfeiffer E, Burkhardt B, Liu JL, Zeilinger K, Nüssler AK and Damm G: Subtoxic concentrations of hepatotoxic drugs lead to Kupffer cell activation in a human in *vitro* liver model: An approach to study DILI. Mediat Inflamm. (2015).

44. Li P, He K, Li J, Liu Z and Gong J. The role of Kupffer cells in hepatic diseases. Mol Immunol. (2017) 1(85): 222-229.

45. Wollina U: Microbiome in atopic dermatitis". Clin Cosmet Investig Dermatol. (2017) 10: 51-56.

الملخص العربى

تأثيرات الإصابة بحليب الإبل الملوث على كبد الفئران

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خلفية: يعتبر حليب الإبل مصدرا جيدا للمغذيات وله أهمية طبية للإنسان في العديد من البلدان في جميع أنحاء العالم وخاصة في المجتمعات البدوية في البلدان العربية. تتواجد المكورات العنقودية (*S. aureus*) بشكل ملحوظ في الحليب ومنتجات الألبان، حيث يعتبرالحليب وسط جيد لهذه المكورات والسموم التى تنتجها. فعندما تنتقل هذه المكورات إلى جسم الإنسان فأنها قد تؤثر على الجلد والدماغ والكلى والكبد والعديد من الأعضاء الحيوية الأخرى. لذلك، كانت الآثار المرضية لـ *S. aureus* محور العديد من الأعمال البحثية الحديثة.

الهدف: صممت هذه الدراسة لمعرفة التأثير المرضى للمكورات العنقودية على التركيب النسيجي لكبد الفئران بعد فصلها من حليب الإبل الخام الملوث بهذه المكورات.

المواد والطرق: تم عزل *S. aureus* من عينات من الإبل المرضعة (*Camelus dromedarius*) والتى كانت تزن 300-540 كجم، في ليبيا وتم تحديد نسبتها فى الحليب بإستخدام التقنيات الميكروبيولوجية والجزيئية. تم تقسيم 20 فأر ألبينو من الذكور الأصحاء السويسرية ، زنت 22-25 جرامًا ، إلى مجموعتين: المجموعة الأولى وهى المجموعة الضابطة والمجموعة الثانية وهى فئران تم إعطاؤها عن طريق الفم جرعة واحدة من المحلول المائي من المكورات العنقودية المعزولة بتركيز $5/x10^8$(0.1ml) . تم ذبح الحيوانات بعد ثلاثة أيام من العدوى بالمحلول البكتيرى وتم أخذ أكبادها للفحص الظاهري والمجهري.

النتائج: أوضحت النتائج ظهور خراجات على الأسطح الخارجية لأكباد الفئران التى أعطيت المكورات العنقودية المجمعة من حليب الإبل عن طريق الفم، بالإضافة إلى ظهور تغيرات نسيجية متنوعة ذات دلالات مرضية واضحة.

الخلاصة: سلطت هذه الدراسة الضوء على المخاطر المحتملة لإستهلاك حليب الإبل الخام، خاصةً فى غياب التدابير الصحية والوقائية الصارمة لتجنب وجود المكورات العنقودية في الحليب.