Evaluation of the Activity of Camel's Milk and Its Purified Immunoglobulins on Some Microorganisms

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Abstract:

Camel milk nowadays is of great importance either for its nutritional value or for its therapeutic effects, and many studies were done on it in the last decade. In this study the antibacterial and antifungal effect of camel milk and its purified immunoglobulins were evaluated against some microorganisms, so milk samples were collected from apparently healthy animals and were tested against some microorganisms by well diffusion test. The antibacterial activity of camel milk and its purified immunoglobulins was tested against *Staph. aureus* and *E. coli* and camel milk showed marked inhibitory effect against both bacteria while camel milk purified immunoglobulins showed no inhibitory effect against both of them. The antifungal activity of camel milk and its purified immunoglobulins was tested against *Candida albicans* and *Aspergillus fumigatus*, but camel milk and its purified immunoglobulins didn't show inhibitory activity against both of them. Overall, the results indicated that camel milk has antibacterial activity and didn't show antifungal activity, and camel milk purified immunoglobulins didn't show either antibacterial or antifungal activity.

Keywords: camel milk; purified immunoglobulins; antibacterial; antifungal

Introduction:

Camel milk is of a great value. It is considered "The white gold of the desert". It has a high nutritive value due to its higher content of Na, K, Fe, Cu, Zn, Mg and Vitamin C. It is lower in cholesterol and sugar. It is very rich in protective proteins (lactoferrin, lactoperoxidase, lysozyme and

immunoglobulins, so it seems to be the most similar milk to human milk than any other milk. ***(Kumar et al., 2015)***.

Its composition may vary depending on some factors such as the physiological status, the feeding, geographical origin, seasonal variation, health status and genetic status of the camel ***(Konuspayeva et.al., 2009).***

Camel milk constitutes of 87% water, 4.4% lactose, 3.5% fat, 3.4% protein, and 0.79% ash ***(Al Haj and Al Kanhal, 2010).***

Camel milk was known for a long time as a potent therapeutic agent for some diseases ***(Asresie and Yusuf, 2014)***. As it is characterized by several therapeutic activities such as antibacterial, antiviral, antifungal, anti-hepatitis, anti-arthritis and anti-oxidative activity, it is also used in the treatment of autoimmune diseases, para-tuberculosis ***(Abdullahi, 2019)***, and drug resistant tuberculosis ***(Devendra et al., 2016)***. Camel milk was found to be the most suitable type of milk for the lactose intolerant individuals as it lacks β-lactoglobulin. Its insulin is found to be more effective in controlling the blood glucose level in diabetic patients. It is found to be useful for children having autism by decreasing its symptoms. Lactoferrin in camel milk also inhibits cancer cell proliferation ***(Abdullahi., 2019).***

Camel milk has effect on several bacterial species due to the presence of the high amounts of antimicrobial peptides and protective proteins such as short peptidoglycan recognition protein, lactoperoxidase and N-Acetyl-D-glucosaminidase ***(Cardoso et al., 2013).***

Camel milk lactoferrin inhibits microbial growth and invading pathogens ***(Hosam et al., 2013).*** It has antioxidant properties. Camel milk contains more lactoferrin than other ruminants' milk (0.22 mg/mL) ***(Abbas et al., 2013). There is marked in vitro and in vivo anti-candidal effect of camel lactoferrin.*** The treatment of mice by camel lactoferrin for 7 days before infection protected the liver of these mice from the severity of infection compared to untreated infected mice. So it can be considered as a potent and safe treatment for invasive candidiasis ***(Mohamed and Emam, 2017).***

The highest concentration of Peptidoglycan Recognition Protein (PGRP) in the milk of camel has marked controlling effect of metastasis in breast cancer patient as it stimulates the immune system ***(Gizachew et al., 2014),*** **also it has potent antimicrobial activity.** ***(Gul et al., 2015).***

Camel milk lysozyme has more antibacterial effect against gram

positive bacteria than human and cow milk because of its higher concentration. Camel milk lactoperoxidase is bactericidal on gram negative bacteria (*E. coli, Pseudomonas and Salmonella*), it has growth promoting and antitumor effect. **(Abbas *et al.,* 2013)**. Lactoperoxidase is a biosimilar (71%) to human thyroid peroxidase, which is important for the thyroid hormones formation ***(Mullaicharam, 2014).***

Camel Immunoglobulins are very small in size, so they penetrate tissues and cells that couldn't be penetrated by human Igs, pass the blood brain barrier, and easily absorbed into the general circulation ***(Abdel Gader et al., 2016).***

Camel milk lactoferrin, lysozyme, lactoperoxidase, IgG and secretory immunoglobulins have a bacteriostatic effect on gram positive bacteria while bactericidal effect on gram negative bacteria. Immunoglobulins have a very little bacterial inhibitory effect. ***(Kula, 2016).***

Camel milk, in addition to its antimicrobial and antioxidant, antidiabetic and anticholesterol properties, it has angiotensin converting enzyme inhibitory peptides. ***(Swelum et al., 2021).***

Camel milk fat contains low carotene ***(Stahl et al., 2006).*** Camel milk contains several fatty acids (capric, caproic, caprylic, butyric, lauric, oleic, linoleic, myristic, myristoleic, stearic, arachidic, palmitic and palmitoleic) ***(Panwar and Rohit, 2015).*** It also has a unique fatty acid profile as it contains less short chain fatty acids (by 6-8 times) compared to other ruminants' milk ***(Gizachew et al., 2014).***

Camel milk fat globule is small in size and easily digested and its membrane has antibacterial and anti-adhesion characters, this makes camel milk suitable for individuals suffering from cows' milk allergy. As camel milk contains less cholesterol, less saturated fatty acids and more essential fatty acids than cow milk this enhances the lipid profile ***(Bakry et al., 2021).***

Lactose in camel milk is easily digested by human without any signs of "lactose intolerance" ***(Abbas et al., 2013),*** so it is suitable for lactose-intolerant patients ***(Mullaicharam, 2014),*** as it is rich in L-lactate while cow milk mainly contains D-lactate ***(Baubekova et al., 2015),*** also it contains few oligosaccharides that prevents microbial infection in infants, and improves the nervous system development ***(Park and Haenlein, 2013).***

Camel milk is useful in case of iron deficiency anemia because of it is high Fe content. Lactoferrin antimicrobial activity in camel milk is more than that of cow milk

because of its low citrate content ***(Park and Haenlein, 2013).***
There are high levels of vitamin C, B1 and B2 **(Ereifej et al., 2011)**. vitamin C is 3-5 times that of cow milk ***(Zhao et al., 2015; Kamal and Karoui, 2017).*** This gives it more antiradical and antioxidant effect ***(Izadi et al., 2019),*** and also help in the absorption and deposition of calcium in the bones of osteoporosis patients ***(Levy et al., 2013).***

The aim of this work was to assess the antimicrobial effect of camel milk and its purified immunoglobulins on some microorganisms (bacteria and fungi).

Material and methods:

1. Collection of camels' milk samples:

Ten healthy she camels (apparently healthy she camels without any clinical manifestations or any observed abnormal signs) were used for milk samples collection. The milk samples were collected according to **Omer and Eltinay (2009)**

2. Purification of camels' immunoglobulin from milk:

It was done according to **Ofurum (2004),** The supernatant was discarded and the residue was re-suspended in 0.1% Triflouroacetic acid (TFA) or in PBS.

3. Preparation of Inoculum:

Bacterial inoculum (e.g. *Staph aureus, E. coli*) and inoculum of *Candida albicans* was prepared for the culture, density of inoculum was standardized using 0.5 McFarland's concentration (1.5-3×10^8 bacteria/ml), the inoculum was prepared according to **Islam *et al.*, (2008)**. *Aspergillus fumigatus* inoculum was prepared according to **CLSI guidelines (2008, 2010)**. These microorganisms was chosen for the antimicrobial testing as they are the most common models for Gram positive bacteria, Gram negative bacteria, yeast and fungus.

4. Examination of camel milk and its purified immunoglobulins inhibitory Activity against bacteria and fungi:

a. antibacterial activity:

It was done on Mulleur Hinton agar by well diffusion method according to **(Valgas *et al.*, 2007)** as well diffusion method is the most commonly used for evaluation of antimicrobial activity of any extract or substance **(Magaldi *et al.*, 2004; Valgas *et al.*, 2007).** Manufacturer's instructions were followed for Mulleur Hinton agar preparation**.** The agar was poured to the plates and the plates were streaked with bacteria according to **(Health Protection Agency "HPA", 2008).** The wells were done in the medium then milk, and purified

protein samples were put in the wells to test their antibacterial effect.

b. Antifungal activity:

It was done on sabouraud dextrose agar by well diffusion method according to **(Magaldi *et al.*, 2004)**. Manufacturer's instructions were followed for sabouraud dextrose agar preparation. The agar was poured to the plates and the plates were streaked with yeast and fungi according to **(Health Protection Agency "HPA", 2008).** The wells were done in the medium then milk, and purified protein samples were put in the wells to test their antifungal effect.

Results:

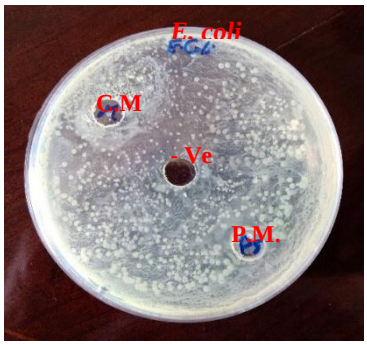
I. Antibacterial activity testing by Well diffusion assay:

The well diffusion assay was done to evaluate the antibacterial effect of camel milk and camel milk purified protein against the following microorganisms (*Staph.aureus*, *E. coli*). Each plate had three wells one well for camel milk, the second well for camel milk purified protein, the third well for saline (as a negative control). In case of *E. coli* camel milk showed agglutination zone while camel milk purified protein showed no effect, in case of Staph aureus camel milk showed a marked zone of agglutination, while camel milk purified protein showed no effect. **(Figure.1)**.

II. Antifungal activity testing:

A) Well diffusion assay:

The well diffusion assay was done to evaluate the antifungal effect of camel milk and camel milk purified protein against the following microorganisms (*Candida albicans*, *Aspergillus fumigatus*). one well for camel milk, the second well for camel milk purified protein, the third well for saline (as a negative control). There is no observed effect either by camel milk, or camel milk purified protein on either *Candida albicans* or *Aspergillus fumigatus* as shown by **(Figure.2)**.



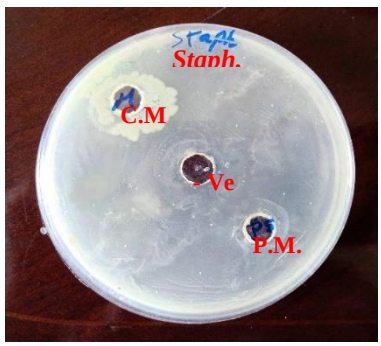


Figure.1. Well diffusion assay to evaluate the antibacterial effect of camel milk and its purified immunoglobulins against *Staph. aureus* and *E. coli.*

C.M: Camel milk; P.M.P: Purified milk protein
-ve: Negative control (saline)

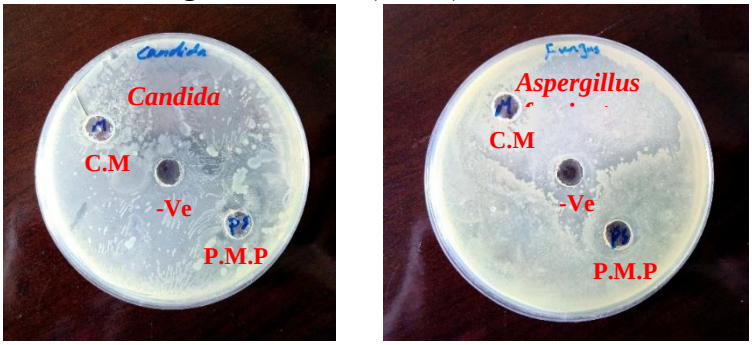


Figure.2. Well diffusion assay to evaluate the antifungal effect of camel milk and its purified immunoglobulins against *Candida albicans* and *Aspergillus fumigatus*.

C.M: Camel milk; P.M.P: Purified milk protein
-ve: Negative control (saline)

Discussion:

Camel milk was found to be the most similar in composition to human milk but differs from the milk of other ruminants. It contains low sugar content, and low cholesterol content but contains high levels of vitamin C, high mineral content (Na, K, Fe, Mg, Zn, and Cu) and it has high levels of protective proteins (lactoferrin, lysozyme, lactoperoxidase and immunoglobulins) (**Kumar *et al.*, 2015**). It has a potent therapeutic effect against several microbial and non-microbial diseases (**Asresie and Yusuf., 2014**, **Kumar *et***

Camel milk lactoferrin effect may be bacteriostatic or bactericidal or both (**Al-Majali *et al.*, 2007**), and it affects both Gram negative and Gram-positive bacteria *in-vitro* (**Jenssen and Hancock., 2009)**.

The antimicrobial activity of camel milk protective proteins (lactoferrin, lysozyme, lactoperoxidase and immunoglobulins) when assessed against some microorganisms (*Staph.aureaus, E. coli, S.typhimurium, Lactococcus* and rotavirus), this revealed that lactoperoxidase has bacteriostatic effect on Gram positive bacteria and has bactericidal effect on Gram negative bacteria, and also

high antibody titers was found against rotavirus (**Kula, 2016**).

Camel milk was found to have therapeutic effect to treat tuberculosis (**Mal *et al.*, 2006**). Camel milk protective proteins act as bactericidal and viricidal agents and it have immunologic, properties (**Yagil, 2013**).

In this study the antibacterial effect of camel milk, and its purified protein was tested against *Staph aureus* and *E. coli.* camel milk showed inhibitory effect against *E. coli* and *Staph. aureus* and this agrees with the previous studies that proved the antibacterial activity of camel milk as mentioned by **(Konuspayeva *et al.*, 2005**), **(Al-Majali *et al.*, (2007), (Jenssen and Hancock., 2009), (Cardoso *et al.*, 2013)**, **(Yagil, 2013), (Kula, 2016**) **and (Rasheed, 2017)** who reported that camel milk act as antibacterial agent against Gram +ve and Gram –ve bacteria because it contains lctoferrin and lactoperoxidase which have potent antibacterial effect also **(Konuspayeva *et al.*, 2005**), **(Kula, 2016**) **and (Rasheed, 2017)** who mentioned that camel milk has high levels of lysozyme and immunoglobulins which has bactericidal and bacteriostatic effect.

On the other side camel milk purified protein showed no inhibitory activity this may be due to precipitation of albumin, transferrin and Immunoglobulins by adding the organic solvent (ACN) to milk. This data in agreement with those mentioned by **(Chertov *et al.*, 2004)** and **(Merrell *et al.*, (2004)** and **(Tirumalai *et al.*, 2003**) who mentioned that ACN addition to serum at a percentage of 20% may disrupt proteins association with serum albumin and this reduces the possible biomarkers loss. This protein association disruption may lead to some protein denaturation. And as mentioned by **(Khabiri *et al., 2010)*** protein precipitation by organic solvents affect the protein surfaces physicochemical properties.

By testing the antifungal activity of camel milk, and camel milk purified protein against *Candida albicans* and *Aspergillus fumigatus*. They showed no inhibitory activity against both of them, which disagree with some studies that proved the anticandidal activity of camel milk mentioned by **(Mohamed and Emam, 2017)** who mentioned that there is good *in vitro* and *in vivo* anticandidal effect of camel lactoferrin (LF). This may be due to the difference in the feeding conditions as camels in the other study are grazing in the desert and may be eating some medicinal plants.

Conclusion:

The antibacterial effect of camel milk, and its purified immunoglobulins against *Staph aureus* and *E. coli* was assessed *in-vitro*, by well diffusion assay and this revealed that camel milk has antibacterial activity against both of them *in-vitro* while camel milk purified immunoglobulins showed no activity *in-vitro*.

Evaluation of the antifungal activity of camel milk, and camel milk purified immunoglobulins against *Candida albicans* and *Aspergillus fumigatus in-vitro* showed no effect.

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ملخص البحث

يعتبر لبن النوق فى هذه الأيام ذو أهمية عظيمة بسبب قيمته الغذائية وأيضا بسبب تأثيراته العلاجية وتم عمل الكثير من الدراسات عليه فى العقد الأخير.
فى هذه الدراسة تم تقييم التأثير المضاد المضاد للبكتيريا والفطريات للبن النوق والأجسام المناعية المنقاة منه على بعض الميكروبات. لذا تم تجميع عينات لبن من نوق سليمة وتم اختبارها على بعض الميكروبات باستخدام اختبار الانتشار. تم اختبار التأثير المضاد للبكتيريا للبن النوق والأجسام المناعية المنقاة منه ضد المكورات العنقودية الذهبية والإشريكية القولونية ووجد أن لبن النوق له تأثير مثبط ملحوظ ضد الميكروبين، فى حين أن الأجسام المناعية المنقاة من لبن النوق لم تظهر أى تأثير ضد الميكروبين. تم اختبار التأثير المضاد للفطريات للبن النوق والأجسام المناعية المنقاة منه ضد فطر المبيضات المبيضة وفطر الرشاشيات ولكن لم يظهر لبن النوق أو الأجسام المناعية المنقاة منه تأثير مثبط ضد أى منهما.
النتائج النهائية لهذه الدراسة أظهرت أن لبن النوق له تأثير مضاد للبكتيريا ولم يظهر أى تأثير مضاد للفطريات وأن الأجسام المناعية المنقاة من لبن النوق لم تظهر أى تأثير مضاد للبكتيريا أو الفطريات.