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The Inhibitory Effects of Human, Camel and Cow's Milk against Some Pathogenic Fungi in Iraq

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Abstract

The present study investigates the use of the agar dilution method and the inhibitory effects of different concentrations (10%, 20% and 30%) of human, camel and cow's milk on thirteen different species of fungi from the genera *Aspergillus, Trichophyton, Microsporum, Penicillium* and *Fusarium*. The results show that all the tested concentrations of each of the three milks were capable of inhibiting the growth of the thirteen fungal species, but the greatest inhibitory effect was recorded with the concentration of 30%. Human milk gave the highest growth inhibition rate on all fungal species and the complete growth inhibition (100%) was recorded in respect to *Aspergillus fumigatus* with a chi-square x2 value of 9.462. For camel and cow milk, inhibition rates of 96% and 92%, with chi-square-x2 values of 8.684 and 9.140, respectively, were recorded. Overall, the inhibitory effects were observed to be concentration-dependent.

Keywords: Human, Camel and Cow's Milk, Fungi.

1. Introduction

Milk represents a major source of well-known antimicrobial substances, in addition to its recently discovered immunomodulatory effects. These are important in shaping the immune system of new-borns since the neonatal immune system is not fully developed, making it difficult for new-borns to protect themselves from infections (Garofola and Goldman, 1999; Mete *et al.*, 2006).WHO (2003) and Oftedal (2012) confirmed that feeding infants with maternal milk for the first six months of life, with continued breast feeding for the first one to two years of life (or longer), is the normative standard, due to the nutritional composition of human milk and the non-nutritive bioactive factors that promote survival and health development.

Some infants may not exclusively breast-feed during the first months of life, replacing human milk with cow milk modified to mimic the composition of human milk (Posati and Orr, 1976). Many nutritional problems have been reported as a result of the use of cow's milk for infant feeding, especially cow's milk allergy (El-Agamy, 2007; El-Agamy *et al.*, 2009); therefore, other types of milk have been proposed as a substitute for human milk including buffalo (Shamsia, 2005), goat (Park and Haenlein, 2006) and sheep (Haenlein and Wendorff, 2006).

Camel milk has the ability to inhibit the growth of pathogens not only because it contains more nutrients compared to cow milk, but because it also has therapeutic and antimicrobial agents (El-Ziney and Al-Turkiy, 2007). It has several beneficial characteristics, such as the absence of diabetes in populations that consume it and tolerance by patients who show intolerance to lactose. Even though camel milk does contain lactose, it is however in a lower concentration than the amount in human milk; it is a nutrient for individuals who are allergic to cow milk (Cardoso *et al*., 2010; Ehlayel *et al*., 2011).

Wernerg (2003) and Shamsia (2009) explained that camel milk contained high fat, protein (especially casein), ash, Ca, Mg, P, K, Na, Fe, Cu, whey protein, lactose and Zn, vitamins and niacin. Camel milk proteins contained a satisfactory balance of essential amino acids and many enzymes with antibacterial and antiviral properties, such as lactoferrin, which prevents microbial growth in the gut, lactoperoxidase, peptidoglycan recognition protein (PGRP),which has a broad antimicrobial activity, lysozyme, which inhibits the growth of bacteria and has a highly effective influence on the storage of camel milk, and immunoglobulin, all of which gives camel milk

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tremendous advantages over conventional antibodies. El-Agamy and Nawar (2000) found that camel milk contained 1.64 mg/ml of immunoglobulin G versus 0.67 for cow milk. A comparative study of lysozyme concentration in milk of different species (El-Agamy *et al*.,1997) showed that camel milk contained a significantly higher content of lysozyme than the cow, buffalo, sheep and goat milks, but a very low content as compared to lysozyme content of human, mare and donkey milks. The same study showed that the camel milk also contained a significantly higher level of lactoferrin (0.22 mg/ml) than cow, buffalo, sheep and goat milks, but very low level compared with the human milk.

Many studies have focused on the antimicrobial and antiviral effects of milk or milk constituents, but only few studies have been conducted to investigate its antifungal effects (Anderson *et al.*, 2000). The aim of the present study, therefore, is to evaluate the antifungal effects of human, camel and cow milk on clinical fungal isolates.

2. Materials and Methods

2.1. Fungal Isolates

Thirteen different species of fungi from the genera *Aspergillus, Trichophyton, Microsporum, Penicillium* and *Fusarium* were isolated from different sources and characterized, as shown in (Table.1):

Table1. Clinical fungal isolates and their sources

Fungus Species	Sources of Isolation
Aspergillus niger	Lower respiratory tract infection
Aspergillus fumigatus	Lower respiratory tract infection
Aspergillus flavus	Lower respiratory tract infection
Aspergillus terreus	Otomycosis
Trichophyton mentagrophytes	Dermal infection
Trichophyton rubrum	Dermal infection
Trichophyton gypsum	Lower respiratory tract infection
Trichophyton violaceum	Dermal infection
Trichophyton tonsurans	Dermal infection
Microsporum audouinii	Dermal infection
Microsporum canis	Dermal infection
Penicillium spp.	Otomycosis
Fusarium oxysporum	Lower respiratory tract infection

2.2. Milk Samples

Samples of human milk were obtained from lactating women two months after labour (lactation after colostrum, with a breast pump). Fresh camel's and raw cow's milk samples were collected from apparently healthy animals also after two months after labourbred in the living stock station at the College of Veterinary Medicine, Baghdad University, Baghdad.

The milk samples were placed in sterile containers and transported to the laboratory in a cool box. Human, camel and cow milk samples were passed separately through a Millipore filter (0.22mm) (Bio-Rad) before determining their anti-fungal activity.

2.3. Evaluation of Anti-Fungal Activity of Milk

The following technique was used to determine the anti-fungal activity of the studied milk types according to Wang *et al*. (2005):

100 ml of each type of milk was prepared and each of these volumes was mixed separately with sterilized SDA (Sabouroud dextrose agar) in order to prepare the required concentrations (10%, 20% and 30%). These concentrations were shacked well, poured into Petri dishes and left to solidify in sterile conditions. A 5 mm piece of mycelia growth from mould cultured for seven days was deposited in the centre of each plate. The inoculated plates were incubated at 28ºC for 7-10 days. Replicates were prepared for each treatment. The diameters of the fungal colonies were measured and then the anti-fungal activity of each concentration of the studied milk was calculated by measuring the growth inhibition rate using the following formula (1):

Growth inhibition rate (%) = ({Growth in control – Growth in treatment/ Growth in control} x100).

2.4. Statistical Analysis

The statistical evaluation of the results was performed using Analysis System-Microsoft SAS (2012). The chi-square test was used to compare significance between the growth inhibition percentages.

3. Results and Discussion

The inhibitory effects of human, camel and cow milk at concentrations of 10, 20 and 30% for each of the different fungal species were determined as described previously. The results, shown in (Tables 2, 3 and 4), indicate that for all the fungal species the growth inhibition rate (%) was related to the milk concentration, so that a 30% concentration gave the highest growth inhibition rate, followed by the 20% and 10% concentrations.

For the genus *Aspergillus*, a concentration of 30% exhibited the highest growth inhibition rate on *Aspergillus fumigates* (100%, 96% and 92%, respectively, for the three types of milk) with chi-square-x2 values of 9.462, 8.684 and 9.140, respectively. For the genus *Trichophyton*, the greatest inhibition was recorded for *Trichophyton rubrum* (92%, 87% and 88%, respectively) with chi-square-x2 values of 11.053, 9.417 and 10.427. While for the genus *Microsporum, Microsporum canis* was the most inhibited species with the growth inhibition rates of 75%, 79% and 65% and chi-square-x2 values of 10.628, 8.938 and 9.326.Finally, the genus *Penicillium* and *Fusarium* exhibited growth inhibition rates of 73%, 59% and 51%, and 62%, 55% and 51%, respectively, with chi-square-x2 values of 10.819, 9.155 and 8.629 and 10.062, 8.951 and 8.627, respectively. Chi-square-x2values of 10.622, 8.264 and 8.619 were recorded for each type of milk, respectively.

Table2. The inhibition growth rate (%) of human milk on different clinical fungal isolates

Clinical isolates	Inhibition growth rate (%) with different Concentrations of milk (%)			Chi-square–χ^2 value
	10 %	20 %	30 %	
Aspergillus niger	19	38	62	10.316 **
Aspergillus fumigatus	52	80	100	9.462 **
Aspergillus flavus	30	73	92	10.702 **
Aspergillus terreus	12	23	35	7.934 **
Trichophyton mentagrophytes	40	62	88	10.538 **
Trichophyton rubrum	40	73	92	11.053 **
Trichophyton gypseum	18	30	65	10.812 **
Trichophyton violaceum	19	42	73	10.931 **
Trichophyton tonsurans	19	37	72	10.944 **
Microsporum audouinii	15	32	53	8.592 **
Microsporum canis	22	40	75	10.628 **
Penicillium spp.	22	47	73	10.819 **
Fusarium oxysporum	17	30	62	10.062 **
Chi-square–χ^2 value	9.327 **	11.289 **	11.752 **	-----

** (P<0.01).

Table3. The inhibition growth rate (%) of camel milk on different clinical fungal isolates

Clinical isolates	Growth inhibition rate with different milk concentrations (%)			
	10 %	20 %	30 %	Chi-square–χ^2 value
Aspergillus niger	37	51	82	9.745 **
Aspergillus fumigatus	52	75	96	8.684 **
Aspergillus flavus	29	44	65	8.927 **
Aspergillus terreus	14	39	62	8.634 **
Trichophyton mentagrophytes	25	59	77	9.108 **
Trichophyton rubrum	30	52	87	9.417 **
Trichophyton gypseum	19	32	55	8.405 **
Trichophyton violaceum	`15	41	66	10.271 **
Trichophyton tonsurans	22	41	70	9.528 **
Microsporum audouinii	22	40	63	8.623 **
Microsporum canis	37	52	79	8.938 **
Penicillium spp.	19	33	59	9.155 **
Fusarium oxysporum	20	39	55	8.951 **
Chi-square–χ^2 value	8.219 **	9.855 **	9.891 **	-----

** (P<0.01).

Table4. The inhibition growth rate (%) of cow milk on different clinical fungal isolates

Clinical isolates	Growth inhibition rate with different milk concentrations (%)			
	10 %	20 %	30 %	Chi-square–χ^2 value
Aspergillus niger	11	20	37	6.946 **
Aspergillus fumigatus	40	65	92	9.140 **
Aspergillus flavus	22	37	52	7.351 **
Aspergillus terreus	9	22	40	7.150 **
Trichophyton mentagrophytes	20	47	72	8.255 **
Trichophyton rubrum	23	52	88	10.427 **
Trichophyton gypseum	15	29	44	6.922 **
Trichophyton violaceum	`13	32	56	8.437 **
Trichophyton tonsurans	19	37	59	8.922 **
Microsporum audouinii	12	32	45	7.832 **
Microsporum canis	25	44	65	9.326 **
Penicillium spp.	12	29	51	8.629 **
Fusarium oxysporum	12	30	51	8.627 **
Chi-square–χ^2 value	7.849 **	9.535 **	9.702 **	** (P<0.01).

** (P<0.01).

The results of this study show that the human milk represents the most effective type of milk against fungal growth, compared to camel and cow milk which were ranked second and third, respectively, according to their overall inhibitory effect on the studied species of fungi. Our results agree with those of Mete *et al.* (2009) who demonstrated that human, cow and infant's formula milks have an antifungal activity *against Rhizopus*, *Penicillium*, *Alternaria* and *Aspergillus* and determined that the human milk had a more pronounced antifungal effect than that found in cow milk, after comparing the fungal growth in human and cow milk-rubbed agar.

The results obtained by the present study may be indicative of the effect of some of the constituents of the different types of milk as inhibitors of fungal growth. Lönnerdal (2003) referred to some of these constituents, especially of human milk, which contains wide varieties of proteins that contribute to its unique qualities. The positive health effects of milk proteins can be presented as antioxidative, anti-microbial, antihypertensive, immuno-modulatory and anti-thrombotic (FitzGerald and Meisel, 2000).

There are several types of milk proteins with antimicrobial activity, such as immunoglobulin, casein, lysozyme, lactoferrin, haptocorrin, α-lactoalbumin and lactoperoxidase. These proteins are relatively resistant to proteolysis in the gastrointestinal tract and contribute to the defence of breast fed infants against numerous types of microbes (bacteria, fungi and viruses). These enzymes are present in the milk of cows, ewes, goats, buffalos, pigs, camels and humans (Wernerg, 2003; Seifu *et al*., 2005 and Siseciaglu *et al*., 2010), but their concentration fluctuates depending on several factors such as species, health status of the animal and stage of lactation. Thus, the level of immunoglobulin-G in camel milk is 1.64 mg\mL-1 compared to 0.70, 0.67, 0.55, 0.63 and 0.86 mg\mL-1 for goat, cow, sheep, buffalo and human milk, respectively (El-Agamy and Nawar, 2000). While, the content of the glycoprotein lactoferrin, sometimes known as lactotransferrin, in camel milk (0.22 mg\mL-1) is significantly higher than that in goat, sheep, buffalo and cow milks and very low compared with that of human milk (El-Agamy *et al.,* 1997). At the same time, lactoperoxidase, which is purified from different milk sources, exerts bactericidal activity generally on Gram negative bacteria and antifungal activity especially on *Aspergillus niger, Pencillium schrysogeum, Aspergillus flavus, Alternaria* sp., *Trichoderma* sp., *Corynespora cassiicola, Phytopthora meadii, Claviceps* sp. and *Corticiums almonicolor*, and thus, contributes to non-immune host defence systems (Ueda *et al*., 1997; Ozdemir *et al*.,2002; Uguz and Ozdemir, 2005). Camel milk also has a unique property in that it includes the presence of lactic acid bacteria (LAB), especially *Lactobacillus* sp. strains, as shown by Laref and Guessas (2013) who found that these bacteria have the ability to inhibit the germination of candida and completely inhibit the mycelium growth of *Aspergillus* sp., *Trichoderm* sp., *Pencillium* sp. *Fusarium roseum* and *Stemphylium* sp. on a solid medium by using the overlay method and confrontation assay.

Wakabayashi *et al*.(2006), Kruzel *et al*.(2007) and Legraut *et al*. (2008), show that lactoferrin is an essential element of non-specific innate immunity in humans and other mammals (the concentration of lactoferrin in cow's milk is lower than it is in human's milk). At the same time, lactoferrin protects the intestinal epithelium cells and inhibits the growth of *E. coli* and other pathogenic intestinal bacteria, mainly *Enterobacteriaceae*, while stimulating the growth of useful intestinal micro flora like *Bifidobacterium.*

Shamsia (2009) determined the antimicrobial factors of both camel and human milk and concluded that camel milk is richer in Immunoglobulin (1.54 mg/ml) than human milk (1.14mg/ml). However, its contents of lactoferrin and lysozyme were very low, (0.24mg/ml) and (0.06mg/ml), respectively, as compared with human milk, which contains (1.95mg/ml) lactoferrin and (0.65mg/ml) lysozyme. Shamsia (2009) also reported that camel milk contained more fat, protein, especially casein, and ash contents but lower whey protein and lactose contents than the human milk. The lower casein and higher whey protein contents in human milk make it very nutritious for the new born due to the resultant soft coagulum after milk ingestion and the higher digestibility and absorption of soluble proteins (Fox and Mc Sweeney, 1998)

In conclusion, the present study confirms that there is a positive relationship between the concentrations of the milk proteins mentioned above and the inhibitory growth rate of milk against fungi and that human milk has a stronger inhibitory effect than camel or cow milk.

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