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Effects of Heating and Storage on the Antifungal Activity of Camel Urine

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Abstract

Camel urine, considered a 'miraculous' drug used in Prophetic Medicine, since the pre-Islamic era camel milk and urine were used as drinking medicine for different health problems. In addition, camel urine has proven to be effective as an antimicrobial agent, and may not have side effects for humans. Furthermore, camel urine may be resistant to factors such as high temperatures and an extensive waiting period in laboratory conditions, which can reduce the effectiveness of antibiotics. The aim of our study was to examine the effectiveness of camel urine as an antifungal agent following exposure to high temperatures and long time periods in laboratory conditions. After maintaining camel urine in natural laboratory conditions for 6 weeks at temperatures of up to 100°C, we tested camel urine on the fungi *Aspergillus niger* and *Fusarium oxysporum*, and on the yeast *Candida albicans*. We then measured the dry weight of each microorganism, and determined their minimum inhibitory and fungicidal concentrations. Our results showed that after maintained for 6 weeks, camel urine did not lose its antifungal activity; dry weights following treatment were decreased 100% of the dry weight prior to treatment for *Aspergillus niger* and *Candida albicans*, and 53.33% for *Fusarium oxysporum*. Our study demonstrates that camel urine is a highly effective and resilient antifungal agent for treating human and plant fungal diseases.

Keywords: Camel urine; Antifungal; *Candida albicans*; *Aspergillus niger*; *Fusarium oxysporum*

Introduction

The camel is mentioned in the Holy Qur'an as a particularly important animal[1], and is referred to by other names such as al-ibil, al-nagah, al-jamal, al-ishar and al-him [1]. Ccamel urine is considered a 'miraculous' drug used in Prophetic Medicine since the pre-Islamic era[2] [2], which has been used as traditional and folk medicine for women's hair; gums and teeth; skin injuries; snake bites; stomach pain; tumors; the common cold; diarrhea and nausea; diabetes; jaundice; scabies; and eye, skin, liver and nail infections [1-5]. Camel urine is also commonly used against cancer and respiratory tract infections in alternative medicine [6].

Camel urine has been proven to be effective as an antimicrobial agent, and may not have any side effects for humans [7]. Muhammad (1998) reported that patients who were given camel urine to treat digestion problems recovered after two months of treatment [8]. Al-Yousef et al. (2012) found that camel urine has no cytotoxic effect against mononuclear cells, and has strong immune activity by inducing IFN-γ and inhibiting Th2 cytokines IL-4, IL-6 and IL-10. Kidney, liver and stomach tissues infected with *Escherichia coli* in mice recovered with no histopathological effects after treatment with camel urine of concentrations up to 100% [9-12]. Studies have tested the antimicrobial activity of camel urine against pathogenic microorganisms including the fungi *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Aschocayta sp.*, *Pythium aphanidermatum*, *Sclerotinia sclerotiorum*, *Candida albicans*; and the bacteria *Staphylococcus aureus*, *Streptococci*, *E. coli*, *Pseudomonas aeroginosa* and *Klebsiella pneomoniae*. The results of these studies showed high antimicrobial activity against the tested microorganisms, even when accompanied by changes in anions and cations [4,13-18].

Antimicrobial activity of camel urine is due to factors such as high salt concentrations, alkalinity, natural bioactive compounds from the plants camels eat, resident bacteria, and excreted antimicrobial agents. Compared with other cattle, camel urine is alkaline due to high concentrations of potassium, magnesium and albuminous proteins, and low concentrations of uric acid, sodium and creatine [19-20]. The different composition of camel urine compared to other cattle and goats is due to the type of plants they consume and their feeding habits; camels prefer browse with high concentrations of minerals that decline more slowly when they dry instead of other types of forage such as grasses [21-23]. Further, camels eat a variety of types of vegetation including thorny bushes, halophytes, salty and sour plants, shrubs and aromatic species that are avoided by cattle and goat (e.g., *Haloxylon aphyllum*, *H. persieum*, *Salsola gemmaseens*, *S. orientabs*, *Astragalus*, *Aristida karelinii* and *A. pinnate*) [17,18,20,24].

The aim of our study was to investigate the resistance of camel urine to heating at high temperatures and storage for extensive waiting periods in laboratory conditions, which can reduce the effectiveness of antibiotics.

[1] 'Do they not look at the camel, how it was created?' (Surah Number 88: Al-Ghâshiyah).

[2] Several Hadith in Sunnah talked about using camel urine and milk as medicine (the Saying of the prophet Muhammad, Volume 8, Book 82, Number 794: Narrated Anas): 'Some people from the tribe of 'Ukl came to the Prophet and embraced Islam. The climate of Medina did not suit them, so the Prophet ordered them to go to the (herd of milk) camels of charity, and to drink their milk and urine (as a medicine).'

Materials and Methods

Study materials

The molds *Aspergillus niger* and *Fusarum oxysporium* were isolated and identified at the Cairo MIRCEN, Ain Shams University, Cairo, Egypt. Tested fungi were incubated at 28 ± 2°C. *Candida albicans* ATCC CA 10231 was incubated at 30 ± 2°C. Camel urine was collected from north Jeddah from live camel in the desert in sterilized dark bottles that were taken directly to the laboratory.

To investigate the effect of storage time and heating on camel urine antifungal activity, collected camel urine was divided into two major groups. The first group was further subdivided into three portions that were heated at 60, 80 and 100°C for 60 min. The second group was further subdivided into three portions that were stored for 3, 6 and 9 months before laboratory analyses. The positive control was fresh camel urine at 4°C.

Laboratory analyses

The antimicrobial activity of camel urine was determined in vitro in response to *A. niger*, *F. oxysporum* and *C. albicans*. Activity levels were measured using disc diffusion and broth dilution, methods previously described by the Clinical and Laboratory Standards Institute (CLSI; formerly known as the National Committee for Clinical Laboratory Standards) [25,26]. For disc diffusion we used filter paper discs (1 mm diameter impregnated with 100 µL), which were placed on the pre-inoculated agar surface. Negative controls were prepared with sterilized discs. Plates were then incubated at 28°C for *A. niger* and *F. oxysporum* for 7 days, and at 30°C for *C. albicans* for 48 h. The inhibitory zones of each disc were measured. All tests were performed in triplicate.

The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of camel urine that inhibited the growth of fungi were investigated using a broth-microdilution method. *C. albicans*, *A. niger* and *F. oxysporum* were cultured and resuspended in 1 mL mueller-hinton broth (OXOID) to obtain a final concentration of 100 cfu mL-1. Camel urine was serially diluted with Mueller-Hinton broth using methods approved by the National Committee for Clinical Laboratory Standards (M27- A) [27]. After incubation, the MIC was determined as the lowest concentration of extract for which there was no visible growth compared with the control [28,29]. The MFC was determined by inoculating 0.1 mL of negative growth at the MIC onto sterile Sabouraud Dextrose Agar SDA for *C. albicans* and Potato Dextrose Agar PDA for A. niger and *F. oxysporum* (OXOID) plates(Table 1). The plates were incubated at 30°C for 48 h for *C. albicans*, and at 28°C for 7 days for *A. niger* and *F. oxysporum*.

The lowest concentration of camel urine that did not demonstrate growth of the tested fungi was considered the MFC; the negative control was a plate grown with media only [30,31].

The dry weight of the tested fungi was measured to determine the effects of recommended doses in Arab folk-medicine. 1 mL samples of *A. niger* and *F. oxysporum* spores, and *C. albicans* suspension (108 cfu mL-1) were inoculated into 5, 10 and 15 mL samples of treated camel urine with SDB/PDB in 250 mL Erlenmeyer flasks. Flasks were incubated with shaking (180 rpm) at 30°C for 7 days for *A. niger* and *F. oxysporum*, and for 48 hours for *C. albicans*. Afterwards, samples were collected and centrifuged at 10,000 rpm for 10 min. Fungal mycelia and yeast cells were collected. Growth was estimated as dry weight by washing with triple-distilled water and drying at 80°C on Whatman no. 1 filter paper until constant weight [32].

The lowest MICs of 1 µL mL-1 were obtained with untreated camel urine; with urine treated at 60°C and 80°C, and stored for 2 months for *C. albicans*; and with all treatments except for urine stored for 6 months for *A. niger* (Table 2). The most resistant fungus was *F. oxysporum* with MIC values ranging from 2 to 8 µL mL-1. MFC values ranged from 4 to 32 µL mL-1, and were lowest for *A. niger* and greatest for *F. oxysporum* (Table 3).

Statistical Analysis

The results were analyzed by paired-samples t-test using the IBM SPSS 20 statistical software to compare the mean values of each treatment. The results are expressed as means ± SE. Probability levels of less than 0.01 were considered highly significant.

Results

We observed high inhibitory growth of *C. albicans*, *A. niger* and *F. oxysporum* after treatment with fresh camel urine, which provided evidence for camel urine as an active antifungal agent (Table 1). The most sensitive tested fungi were *C. albicans* and *A. niger*, while the inhibition of *F. oxysporum* only decreased by 22% when camel urine was stored for 6 months.

		Incubation temperature			Storage time (months)		
	Fresh	60°C	80°C	100°C	2	4	6
C. albicans	45 ± 0.891**	42 ± 0.895**	39 ± 0.891**	36 ± 0.895**	44 ± 0.589**	40 ± 0.895**	37 ± 0.566**
A. niger	43 ± 1.166**	38 ± 0.895**	35 ± 1.166**	30 ± 0.566**	41 ± 0.589**	37 ± 0.873**	35 ± 0.589**
F. oxysporum	39 ± 0.895**	36 ± 0.895**	34 ± 0.589**	29 ± 0.589**	37 ± 0.891**	35 ± 0.566**	32 ± 0.566**

Table 1: Inhibition of *C. albicans*, *A. niger* and *F. oxysporium* growth after incubation with 100 µL of camel urine.

		Incubation temperature				Storage time (months)	
	Fresh	60°C	80°C	100°C	2	4	6

C. albicans	1	1	1	2	1	2	2
A. niger	1	1	1	1	1	1	2
F. oxysporum	2	2	4	4	2	4	8

Table 2: MIC (µL/ml) of *C. albicans*, *A. niger* and *F. oxysporium* growth after treatment with serial concentrations of camel urine.

		Incubation temperature				Storage time (months)	
	Fresh	**60°C**	**80°C**	**100°C**	**2**	**4**	**6**
C. albicans	8	8	16	16	8	8	16
A. niger	4	4	8	8	4	4	4
F. oxysporum	8	16	16	32	16	32	32

Table 3: MFC (µL/ml) of *C. albicans*, *A. niger* and *F. oxysporium* growth after treatment with serial concentrations of camel urine.

Heating camel urine at different temperatures did not affect fungal dry weight (Table 4). Fungal growth was completely inhibited by 15% concentration of camel urine for all treatments and all tested fungi, and by 5 and 10% concentrations for most treatments. The activity of camel urine after heating at different temperatures increased compared with untreated camel urine; there was still 100% growth inhibition after treatment at 100°C for all tested fungi and all concentrations of camel urine. However, storage time increased the effect of inhibition for *C. albicans* and *F. oxysporum* at camel urine concentration of 5 and 10% (Table 5).

		Urine concentration (%)			
Temperature		**0**	**5**	**10**	**15**
Untreated	*C. albicans*	20	5 ± 2.207**	0	0
	A. niger	230	130 ± 0.333**	0	0
	F. oxysporum	300	180 ± 2.848**	93 ± 2.309**	0
60°C	*C. albicans*	20	10 ± 1.528*	10 ± 1.528**	0
	A. niger	230	0	0	0
	F. oxysporum	300	0	0	0
80°C	*C. albicans*	20	10 ± 1.000**	10 ± 1.732*	0
	A. niger	230	0	0	0
	F. oxysporum	300	0	0	0
100°C	*C. albicans*	20	0	0	0
	A. niger	230	0	0	0
	F. oxysporum	300	0	0	0

Table 4: Dry weight (mg) of *C. albicans*, *A. niger* and *F. oxysporium* after incubation with different concentrations of camel urine at different temperatures.

Storage time			**Urine concentration (%)**		
		0	**5**	**10**	**15**
Fresh	*C. albicans*	20	5 ± 2.207**	0	0
	A. niger	230	130 ± 0.333**	0	0

	F. oxysporum	300	180 ± 2.848**	93 ± 2.309**	0
2 months	*C. albicans*	20	10 ± 0.333**	10 ± 2.028*	10 ± 0.333**
	A. niger	230	110 ± 0.667**	0	0
	F. oxysporum	300	290 ± 2.028**	210 ± 0.333**	0
4 months	*C. albicans*	20	10 ± 0.333**	10 ± 0.333**	0
	A. niger	230	0	0	0
	F. oxysporum	300	240 ± 0.333**	140 ± 2.309**	0
6 months	*C. albicans*	20	10 ± 2.646*	0	0
	A. niger	230	0	0	0
	F. oxysporum	300	200 ± 0.333**	100 ± 0.333**	0

Table 5: Dry weight (mg) of *C. albicans*, *A. niger* and *F. oxysporium* after incubation with different concentrations of camel urine for different periods of time.

Discussion

Camel urine is an efficient antimicrobial compound, particularly against *Aspergillus* sp., as demonstrated by our study and others [13,15-17]. Our results on the effects of heating and storage time on the antimicrobial activity of camel urine were consistent with the results of several other studies [33,34]. High inhibitory growth of the tested fungi, which were grown in an acidic environment, was due to the high alkalinity of camel urine as a result of high concentrations of K, Mg, Ca and proteins, and low concentrations of carbohydrate and cellulose [13,19-21].

Active compounds from plants that camels eat are excreted into the urine and increase its antimicrobial activity; these desert plants include *Haloxylon aphyllum*, *H. persieum*, *Salsola gemmaseens*, *S. orientabs*, *Astragalus*, *Aristida karelinii*, *A. pennate*, *Citrullus colocynlhis schrad*, *Acacia eherenbergiana hayne*, *Dipterygium glaucum*, *Convolvulus hystrix vahl*, *Rhyzya stricta, Decne* and *Anabasis setifera* Mog [5,21,35]. Camels spend more than 80% of their total feeding time on dicotyledons [21,36], which have more extracellular compounds compared to plants eaten by cattle, goat and sheep. Camels also graze on a variety of plants including thorny shrubs, halophytes and aromatic species that are avoided by cattle, goat and sheep [24], which ensures that active compounds such as flavonoids, alkaloids, terpenes, volatile and essential oils, anthraquinones, and phenolics are excreted in the urine [37-41].

Inhibited growth of *C. albicans*, *A. niger* and *F. oxysporum* reveals that the antimicrobial activity of camel urine was not affected by heating or storage time, perhaps because it was a high dose 100 µl; these results are reflected in the MIC and MFC. There was more of an effect of heating and storage time on the recommended dose of camel urine in Arab folk-medicine, which may be due to changes in the camel urine structure and composition as a result of treatment. Al-Awade and Al-Judaibi (1999) explain that camel urine is very effective against microorganisms because of several components including bacteria that can survive under extreme conditions. These bacteria have special characteristics that enable them to live in conditions with high osmotic concentrations and alkalinity, and without nutrition. Further, these bacteria stay highly motile even after incubation at low temperatures. Our results show that the antimicrobial activity of camel urine increases after storage and heating up to 100°C, which completely inhibited the growth of *C. albicans*, *A. niger* and *F. oxysporum*. Heating may increase the concentration of active compounds in urine by lysis of the bacterial cells, which in turn secrete enzymes and antibiotics. Storage time had no effect on the 15% concentration of camel urine. At high concentrations, more antibiotics are secreted by the bacteria, alkaline concentrations are higher and there are more active compounds from the plants.

The increased inhibitory effects on *C. albicans* and *F. oxysporum* at concentrations of 5 and 10% may be due to low concentrations of active compounds in the urine, which may allow the fungal cells to become more permeable to antibiotics and active compounds [14,42,43].

The high antifungal activity of camel's urine reflected on the inhibition of the tested fungi and the results agreed with Al-Judaibi's results of camel's urine on *A. niger* and *C.albicans* compared with the antifungal agents Mycostatin, Pevaryl and Nizoral [44]. Several studies determined the effect of camel's urine on the cells and the results showed the efficient as repaired to the damaged cells, including the tumor cells and can be used as anticancer and antiplatelet activity against ADP-induced agent [8-11,45-47].

Conclusion

In conclusion, camel urine is a highly effective and resilient antifungal agent for treating human and plant fungal diseases. Our results confirm the traditional uses of camel urine as an antimicrobial agent, and may not have side effects for humans. In addition, heating and storage of camel urine did not alter the main fungicidal effects.

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