

## THE UPCOMING THERAPY FROM CAMEL'S URINE

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### ABSTRACT

Cancer presents a serious problem in Egypt with high incidence. Intensive research work. was done to control the disease. The ideal treatment that selectively kill the malignant cell and keep other normal ones safe from hazards of cancer therapy has not yet been achieved. Camel was mentioned in the book of Muslims as special creature. The profit Mohamed advised people to use camel's urine and milk for treatment of some ailments. in the current study camel urine samples were collected from camels reared in wadi Elnotron, El Alameen road in north west desert of Egypt. Urine samples were fractionated using HPLC. Freshly collected urine samples were diluted in different proportions 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048 with sterile saline solution (0.85% NaCl). Cytotoxic effect of camel urine to cancer cell lines were done. Colon (HCT), lung (A549), breast (MCF-7) and liver (HepG2) cancers were used in the study. the antiviral effect of camel urine was examined using sterile adenovirus inoculated into pre-cultured Vero cells tissue culture flask. results revealed that camel urine has anticancer effect and antiviral effect up to 8-fold of dilution. the main component defined in fractionated urine was dimethylamine and formamide. The study showed that camel urine had cytotoxic effect for different cancer cell lines and antiviral effect up to 8 folds of dilution. The dimethylamine was a major component in camel urine was known as a component of anticancer chemotherapy.

Key words: Camel's urine- cancer cell line- adenovirus

### INTRODUCTION

Although it may sound unpleasant, camel urine has been consumed extensively for years in the Middle East .Since ancient time the profit Mohamed advised people to use camel's urine and milk for treatment of some ailments [1]( Alhaidar et al 2011).Bedouins used to drink about 100 ml of camel urine every day either alone or mixed with camel milk [2] (Al Yousef et al 2012).Recently scientific research has been carried out to explore the potency of urotherapy for treatment of disease as complementary medical practice [3]( Al-abdalall, 2010). incidence rates of cancer in Egypt was not reported till 2014. Since then, the seriousness of the cancer problem in Egypt on a national and regional level has been demonstrated. The common protocol for treatment of cancer is by complete removal by surgery, kill the cancer cell, using chemotherapy, radiotherapy, immunotherapy and hormone therapy. Also, **cryoablation** kills cancer cells with cold. However, recurrence or relapse of disease after complete eradication may take place. The camel urine attracted the greatest interest for treatment of different types of cancers like colon ( HCT), lung (A549), breast ( MCF-7 ) and liver (HepG2) cancers. Also, camel urine is used in treatment of ulcers, skin problems, chronic hepatitis, hepatitis C, stomach infections, weakened immune system and some cardiovascular diseases [4-6] (kula and Tegegne,2016, Gader and Alhaidar,2016,Yadav et al.,2015).The use of urine in the treatment of disease is more common than one would think, and electronic databases have numerous of literature on the different uses of urine [1] (Alhaidar et al., 2011). This work



was to verify the claims about the effectiveness of urotherapy of camel in Egypt. First, by evaluating different dilution of urine cytotoxicity for colon (HCT), lung (A549), breast (MCF-7) and liver (HepG2) cancers cell lines. Second, by evaluation of the antiviral ability of urine using adenovirus as a model.

## MATERIALS & METHODS

### *Sample collection*

Camel urine samples were kindly provided by Alteb alnabawy organization (the *head of Scientific Foundation for Experimental Studies and Research, Ismailia, Egypt, 41511. Website: www. Alteb alnabawy.org email: rafeekeltebelnabwy1@gmail.com cell phone 00201223851993*) from Tal sheriff region, 60 Km apart from wadi Elnotron, El Alameen Road in north west desert of Egypt. Approximately 250–300 ml of urine samples was collected in the early morning before sunrise and after sunset from ten male and female healthy camels aged 3-8 years old. Camels were clinically free of disease and had free access to water. Animals were fed on natural flora present in the locality such as Atriplex, Salicornia, Artemisia and Rosmarinus. The natural urination samples were directly kept in sterile culture tubes, transferred to the laboratory (<4 hours) and were stored at -20° C until used.

### *HPLC analysis of camel urine*

camel urine was analyzed using a Thermo LCQ Advantage Max ion trap mass spectrometer. For liquid chromatography separation, a Thermo Surveyor HPLC system and Waters Symmetry RP-18 column (5 µm, 2.1 mm × 150 mm; Waters, Milford, MA) were used. Glycosphingolipid, lipopolysaccharide, and oligosaccharide peaks were detected using a 95-min long linear gradient from 5 to 25% of mobile phase B [CH<sub>3</sub>CN acidified with 0.03% HCOOH (vol/vol)] in mobile phase A (HPLC-grade H<sub>2</sub>O acidified with 0.03% HCOOH). The flow rate was 0.4 mL/min, and column temperature was set at 50°C. After moving through the chromatography system, the flow was introduced in the ESI ion source in a negative ion mode ([M-H]<sup>-</sup>), which was adjusted at the following parameters: spray sheath gas (N<sub>2</sub>) flow rate 70 (arbitrary units), capillary voltage -47 V, tube lens offset -50 V, voltage 3.9 kV, auxiliary gas (N<sub>2</sub>) flow rate 10 (arbitrary units), injection volume 10 µL, capillary temperature 260°C. The scan range was m/z 150 to 2,000, and 150 ms was the maximum injection time with 2 microscans. The LCQ mass spectrometer was run sequentially. Full-scan MS was performed to acquire analytical data on anions within the scan range, followed by an MS/MS trial at a normalized collision energy of 35% to detect the most notable [M-H]<sup>-</sup> ion that was obtained in the first step [7,8] (Hamed et al., 2017; Hsu, 2016).

### *Sample preparation*

In order to evaluate the effect of increased inflow and dilution of camel urine, a simplified laboratory model has been developed. Freshly collected urine samples were diluted in different proportions -1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048 with sterile saline solution (0.85% NaCl). All samples were used for cytotoxicity.

### *Maintenance of cell lines:*

Cytotoxic effect of camel urine to cancer cell lines namely colon (HCT), lung (A549), breast (MCF-7) and liver (HepG2) cancers also Vero cells was included as a normal cell control.

Both African green monkey cells (Vero) and the tested cancer cell line (ATCC-HB-8065) were cultured in 75-cm<sup>2</sup> tissue culture flasks using EMEM and medium 199E (GIBCO-USA) supplemented with 10% (FBS) fetal bovine serum (GIBCO-USA). Cell lines were maintained according to the method described by Thomson (1998) where cells were grown until they reached confluent monolayer. Cells were incubated with trace trypsin in the incubator at 37°C until the cells detached from the surface. Cells were resuspended in growth medium to the desired concentration according to cell count. Cell suspension was incubated in new culture growth media in new flasks and incubated at 37°C until confluency. The cell number in the suspension was calculated by using trypan blue exclusion assay [9] according to Swain et al., (2010).



### ***Evaluation of urine cytotoxicity using MTT assay:***

The MTT (3- [4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide) assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity. Since for most cell populations the total mitochondrial activity is related to the number of viable cells [10] (Van Meerloo et al, 2011). Cellular cytotoxicity was determined using MTT assay [11] as described by Takahashi et al., (1989).

Cell lines were counted as 105 cells/ml and cultured in 96-well tissue culture plates. On confluence, growth media were discarded and 100 µl of each dilution of urine was inoculated into 8 wells of the plates. The plates were incubated at 37°C for 24 hrs. Media of different dilutions were decanted and plates were washed with PBS 3 times. Plates were stained using.

MTT (0.05 ml/well) for 4 hrs at 37°C. Developed MT Formazan complex was dissolved using 0.05 ml of DMSO.

Plates were incubated for 30 minutes at 37°C for cell lysis. Optical density (OD) was measured using ELISA reader at

wavelength of 550-570 nm. The percentage of viability was determined according to the method described by [12] Mossman et al., (1983) as follows:

Number of residual living cells = (OD of treated cells/ OD of untreated cells) X Number of negative control cells (104

cells/0.1ml). Percentage viability = (Number of residual living cells /Number of negative control cells) X 100.

### ***Adenovirus propagation on cell culture***

Sterile adenovirus was inoculated into pre-cultured Vero cells tissue culture flask. Infected flasks were incubated at 37°C.

(Jouan-France) for an adsorption period of 1-1.5 hr with gentle mixing at timed intervals of 15 min. Maintenance medium was added to infected flasks [13] (Joseph 1994). Non-infected cell culture control was included. Cultures were incubated at 37°C and examined daily under the inverted microscope (Hund-Germany) until the initial detection of cytopathic effect.

Flasks that showed 90% CPE were frozen and thawed three successive times for virus extraction [14] according to (Noor et al, 2018)

### ***Virus infectivity titer assessment***

The virus infectivity titer was assessed [15] according to Reed and Muench (1938) to determine the highest dilution of the virus that produced CPE in 50% of the infected cells (TCID<sub>50</sub>). The virus was 10-fold diluted in culture medium. Vero cells precultured on 96 well plates (104cells/well) were infected using the serially diluted virus on the reciprocal wells.

On confluency, the medium was decanted 100 µl/well of each dilution of the virus suspension into 8 wells (using separate

tips for each dilution). Two successive columns of non-inoculated wells were maintained as negative control. Plates were incubated at 37°C, seven days post incubation, the number of wells per each dilution showed CPE were recorded.

The 50% endpoint was determined as follows:

50% endpoint = (percentage of CPE of >50% – 50) / (percentage of CPE of >50 % – percentage with CPE of < 50%) X log dilution.

### ***Evaluation of anti-viral activity of camel urine against adenovirus***

The antiviral activity of camel urine was determined [16] according to the method described by Shinji (2005) where different dilutions of urine was prepared in EMEM. Vero cells were counted as 105 cells/ml



and cultured in 96-well tissue culture plates. On confluence, growth media were discarded. Pre cultured plates 100 µl/well (under test for antiviral activity) were.

dispensed to the reciprocal wells. One urine non treated plate was maintained for viral control titration. All plates were.

incubated at 37°C for 24 hrs. Growth/ treatment media were decanted; each virus dilution was inoculated at 100µl.

/Well. Plates were incubated at 37°C and examined daily under the inverted microscope. Three days post incubation, the virus titer was calculated in each plate. The antiviral activities were calculated by determining the virus mean titer in treated and untreated cells. The difference between both titers refers to the antiviral activity.

### Virucidal activity

Virucidal activity was monitored based on the evaluation of direct contact of adenovirus to urine followed by collecting samples from 37°C incubated urine–virus mix. Collected samples were titrated the treatment test was repeated in triplicates and the mean depletion rate was calculated [15] according to (Reed and Muench 1938). Statistical significance between treated and untreated cells was determined using one way ANOVA.

## RESULTS

Results (Table 1) of the HPLC of camel's urine showed that the major compounds of camel urine are die methylamine (68.59%) and formamide (20.96%) and furan (5.05%).

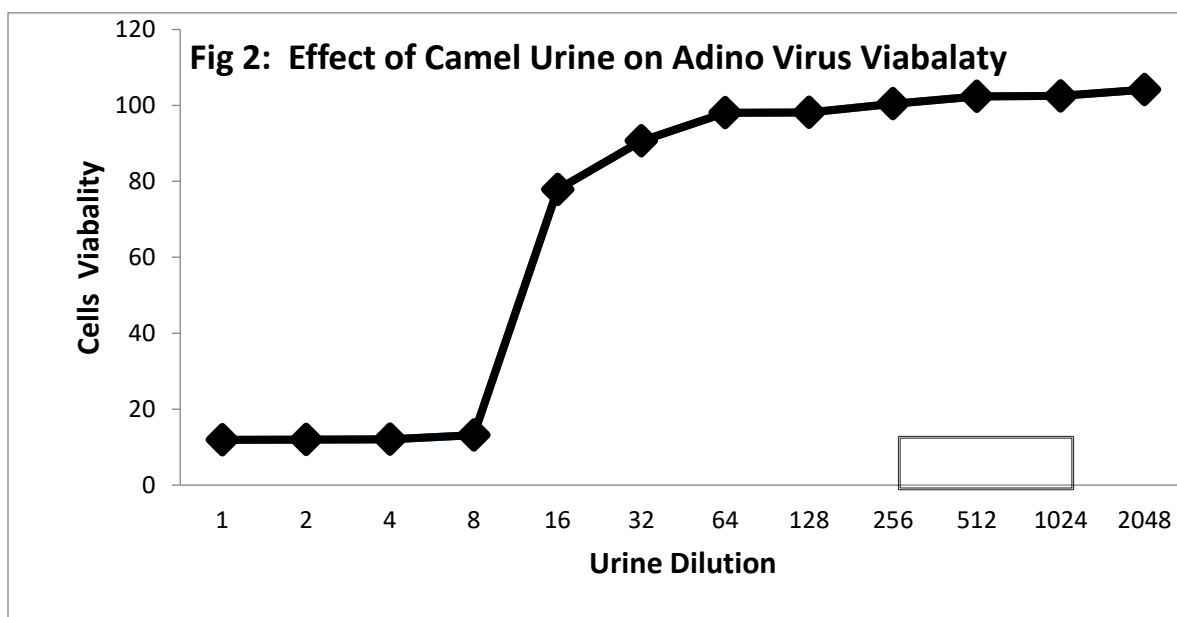
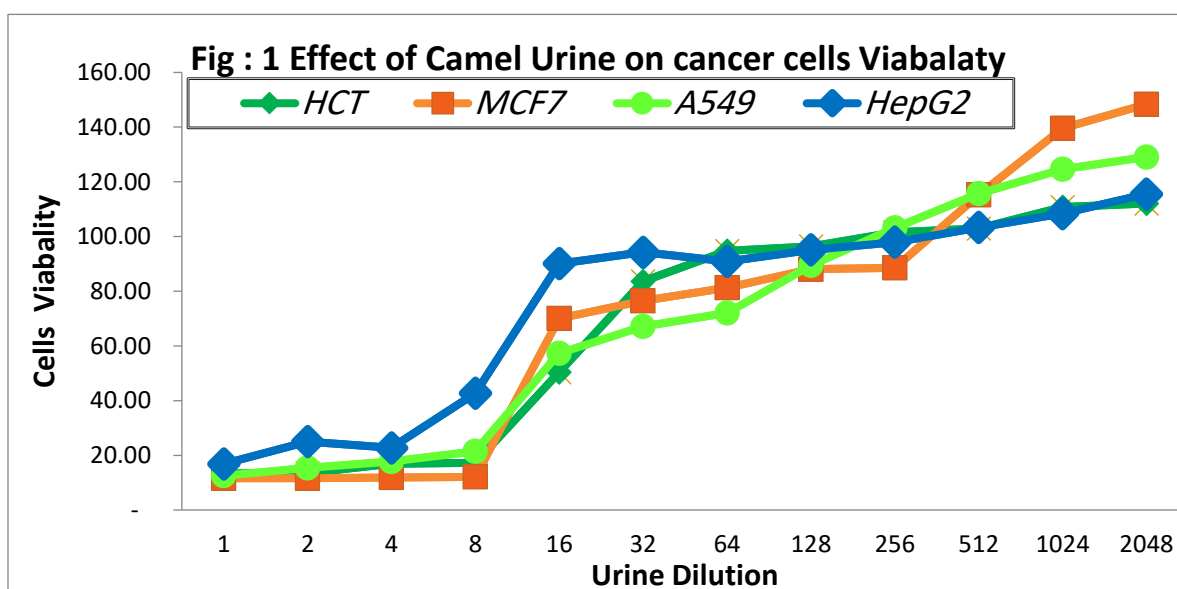
**Table (1) high performance liquid chromatography of camel's urine:**

Area Sum %	formula	Name of compound
0.41	C9H17N	1-(5-Bicyclo [2.2.1] heptyl) ethylamine
5.05	C4H6O	Furan, 2,3-dihydro-
20.96	CH3NO	Formamide
68.59	C2H7N	Dimethylamine
0.1	C4H8O	Cyclobutanol
0.14	C7H16O2	Hydroperoxide, 1-methylhexyl
0.06	C6H13ClO	2-Pentanol, 3-chloro-4-methyl-, (R*, R*)- (. +/-)-
0.07	C5H8O	4-Pentenal
0.14	C6H12O	Cyclobutanol, 2-ethyl-
0.06	C6H14O2	Hydroperoxide, hexyl
0.38	C5H10O	Pentanal
0.11	C10H14O5	2H-Oxireno [3,4] cyclopenta [1,2-c] furan-2-one, 1a,1b,4,4a,5,5a-hexahydro-4-(dimethoxy methyl)-, (1bR,1a-cis,4-trans,4a-cis,5a-cis)-
0.12	C9H14O2	3-Nonynoic acid
0.84	C6H12O	Hexanal
0.05	C8H10O2	3,8-Dioxatricyclo [5.1.0.0(2,4)] octane, 4-ethenyl-
0.05	C19H30O2	3,6-Octadecadiynoic acid, methyl ester
0.26	C7H14O	Heptanal
0.09	C14H24	5-Tetradecen-3-yne, (E)-
0.09	C10H16N2O2	ACETHYDRAZIDE, N2-[1-(2,3-DIHYDRO-6-METHYLPYRAN-2-YL) ETHYLIDENO]-
0.07	C10H18O	3-Decyn-2-ol
0.1	C15H28O2	3-(Prop-2-enoyloxy) dodecane
0.18	C8H16O	Octanal
0.04	C10H16O	carveol 1
0.17	C7H12O	2-Heptenal, (Z)-
0.04	C24H45N2O3	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion
0.29	C9H18O	Nonanal



0.2	C14H25F3O2	2-Trifluoroacetyldodecane
0.19	C8H16O3	Methoxyacetic acid, pentyl ester
0.1	C8H16O	cis-2-Ethyl-2-hexen-1-ol
0.12	C14H22N2O3	Acetamide, N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]-
0.12	C22H40	E, E-1,9,17-Docosatriene
0.44	C5H10O5	DL-Arabinose
0.04	C19H32	Z, Z, Z-1,4,6,9-Nonadecatetraene
0.07	C10H7N3O3S	1,2,4-Triazine-3,5(2H,4H)-dione, 6-benzoylthio-
0.05	C16H30O2	9-Tetradecen-1-ol, acetate, (E)-
0.09	C16H34	Hexadecane
0.05	C10H20O	Ether, 6-methylheptyl vinyl
0.08	C11H18O4	Oxalic acid, allyl hexyl ester

Results (Fig 1) showed that diluted camel urine showed anticancer effect up to 8 folds dilution the more dilution, the higher cancer cell viability. the same trend was observed in Fig (2) that showed antiviral effect of camel urine up to 8 folds of dilution.







## DISCUSSION

In the last century there was growing interest for using traditional and alternative medicine all over the world. Scientists have developed many therapeutic anticancer therapies and improved their properties by strong research work. The ideal treatment that selectively kill the malignant cell and keep other normal ones safe from hazards of such cytotoxic chemicals has not yet been achieved. There was a guidance from prophet Mohamed to use camel milk and urine in treatment of some ailments. Currently, intensive research work was done to support urine-therapy. [17] (Alyahya et al., 2016). In the current study the HPLC analysis of the collected urine from camels reared in desert and fed on the natural flora showed dimethylamine (68.59%) and formamide (20.96%) and furans (5%) as major compounds in camel urine (table 1). Surfing literature showed that dimethylamine is a component in many biologically active anticancer drugs. It was used as small molecules against cancer containing methyl (Z)-2-(2-chlorobenzoyl)-3-(dimethylamino) acrylate [18] (Zhang *et al.*, 2019). Diethylamine was used to produce new compound, trans-[PtCl<sub>2</sub>(OH)<sub>2</sub> (dimethylamine) (isopropylamine)], that exhibits greater cytotoxicity and reduce the side effects of cisplatin or cis-diamminedichloroplatinum, the well-known anticancer compound used in chemotherapy but unfortunately, was recorded to have nephrotoxicity and neurotoxicity [19,20]. (Cohen and Lippard, 2001 and Perez et al., 2002).

The current study showed cytotoxic effect of camel urine (Fig 1) against the colorectal (HCT 116), breast (MCF-7), lung (A549) and hepatic (HepG2) cancers. In the same time it did not harm normal human cells. Previous studies showed the same results of the lyophilized camel urine on cancer of the same cell types (Khorshid, 2009; Khorshid et al., 2009; Khorshid et al., 2011 and Khorshid, 2011) [21 - 24]. Camel urine contains antibodies estimated in size as one tenth that of human, the thing enables it to pass the blood brain barrier. It was also named as nanoantibodies and can be used in diagnosis and treatment of cancer as well as development of biosensors [25] (Muyldermans et al., 2009). Scientific research concentrates on the unique features of the camel's immune system and the possibility to develop immune therapy for cancer and other autoimmune diseases [26] (Gader and Alhaider, 2016). Recently, camel urine was fractionated and identified. PMF is one of these fractions that contain nanoparticles of cesium and rubidium as potential anticancer compounds (Khorshid et al., 2011) [27]. Moreover, other fractions of camel urine such as PM701 and its bioactive fraction PMF and sub-fraction PMFK were shown to have cytotoxic properties against different cancer cell lines (Raouf et al., 2009) [28]. Recent research work showed that camel urine down regulated the gene Cyp1a1, responsible for cancer cell replication in Hepa 1c1c7 cell line [1] (Alhaider et al., 2011).

Camel urine may acquire these therapeutic effects because camel consumes desert herbs rich in phytochemicals having pharmacotherapeutic effect [29] (Alkhamees and Alsanad, 2017). In Egypt, camel shows preference for some natural growing local desert plants like *Kochia*, *Acacia*, *Pasternak*, *Atriplex*, *Cassi* and *Salicornia*. These desert plants contain phytochemicals such as radicicol, steroids, triterpene and saponins which help in the storage and conservation of water to survive the desert weather conditions and at the same time showed anticancer effect [30] (Wang et al., 2008). The desert plants are subjected to very stressful environments (Heat and radiation) so that it contains many antioxidants that have antitumor activity [31] [Wang et al., 2007]. Previous studies showed that *Artemisia annua* extract inhibited the viability of breast (MDA-MB-231 and MCF-7), pancreas (MIA PaCa-2), prostate (PC-3), non-small cell lung cancer (A549) cells [32] (Sophia et al., 2019). *Salicornia* showed growth inhibitory effect against human cancer cell lines [33] (Jung et al., 2008). *Salicornia* contains Luteolin that showed antioxidant, antitumor, anti-inflammatory, antiapoptotic, and cardioprotective activities [34] (Kim et al., 2021).



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