Article

Cytotoxic potential of camel whey and milk on cervix cancer (HeLa) cell line

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Abstract: Camel milk is an important nutritional source that has historically been used in the treatment of cancer. Therefore, the main aim of the present study is to determine the in vitro anticancer effect of both camel milk and whey against cervix cancer (HeLa) cells. To perform that, skimmed milk as well as whey immunoglobulins concentrate samples were prepared at different concentrations (0, 1, 2.5, 5, 7.5 and 10 mg/ml). Then, the in vitro effect of the prepared concentrations on HeLa cells morphology and growth was investigated by tissue culture technique. Moreover, the anticancer activity of camel milk and whey against HeLa cells was estimated by the 3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. The obtained results displayed that both camel milk and whey reduced the viability of HeLa cells specially at 7.5 and 10 mg/ml. In addition, the viability of treated HeLa cells reduced after addition of both camel milk and whey to approximately 15% in a concentration dependent manner. In conclusion, this study showed the in vitro cytotoxic effect of camel milk and whey as they inhibited the growth of HeLa cells.

Keywords: camel; whey and milk; cytotoxic potential; cervix cancer (HeLa) cell line

1. Introduction

Cancer management in humans is a major challenge for modern medicine as there are no available medications that can selectively kill cancer cells without any effect on other normal living ones (Coufal et al., 2007; Kontou et al., 2011). The standard available therapies depend on surgery, chemotherapy, radiotherapy, hormone therapy, and immunotherapy (Khorshid et al., 2010). The primary method for cancer treatment among the previous methods is chemotherapy which has long lasting side effects (Rood et al., 2004). The drawbacks of some methods that are used for cancer treatment underline the necessity for the development of alternative ones with minimal side effects. One of these alternatives are natural products which known to be one of the important sources of therapeutically effective substances (Alebie et al., 2017). A novel chemoprevention that depend on dietary constituents is camel milk. It is a beneficial source of many useful organic substances that have a promising therapeutic values (Alghamdi and Khorshid, 2012).

Camel milk is an important nutritional source as it has many properties that make it very useful choice for the treatment of diverse diseases in some parts of the world (Attia et al., 2001). In addition to its high content of minerals and vitamins as well as high concentrations of insulin, camel milk has low content of cholesterol and sugars (Farah et al., 1992). Moreover, studies demonstrated that camel milk contains great concentrations of protective proteins, including lysozyme, lactoferrin, lactoperoxidase, peptidoglycan recognition protein (PGRP) enzyme, immunoglobulin G, and secretory immunoglobulin A (El Agamy et al., 1992). Furthermore, the protective proteins in camel milk may have a possible role for enhancing the immune defense mechanism (Yagil, 1982). Among these proteins, whey proteins that play an important role as an anti-tumor and anticarcinogenic agent (Zarogoulidis., 2015). Whey proteins protect the immune system by acting as an immunodulatory factors through which they activate different immune cell functions (Badr et al., 2017). The unique properties of camel IgG antibodies which lack light chains enable them to posses different biological
activities (Harmsen and De Haard, 2007). Traditionally, camel milk has cured and treated numerous cases of cancer. The anticancer potential of camel milk was referred to its cytotoxic effect, anti-apoptotic effect and anti-proliferative effect (Alebie et al., 2017). However, few studies have been published in literature regarding the medicinal properties of camel milk and whey against cancer (Almahdy et al., 2011; Korashy et al., 2012; Habib et al., 2013; El Miniawy et al., 2014; Vladimir et al., 2017). According to that, this study was investigated to determine the ability of camel milk and whey to inhibit the growth of HeLa cells in vitro.

2. Materials and Methods

2.1. Milk collection and whey immunoglobulins preparation

Veterinary specialist collected milk samples from one female camel (Jenin, West Bank). For whey preparation, the casein was precipitated from the pooled skimmed milk samples by milk renneting with commercially available rennin to obtain good crude contraction (Brussow et al. 1987). The coagulated milk was heated to 56°C for 10 min. Casein separation from lacto serum was carried out by filtration. For final clarification, the lacto serum was again centrifuged for 30 min at 10,000 rpm at 4°C. The supernatant was filtered using a millipore filter (0.45µm), then the filtered supernatant was lyophilized to get powder of whey immunoglobulins pool. Total protein content of camel whey sample was determined by Biuret method (Gornall et al., 1949). For milk preparation other skimmed milk sample was directly freeze-dried to produce milk powder.

2.2. Identification of camel whey proteins using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Components of whey immunoglobulin concentrate and milk samples were fractionated by SDS-PAGE. This was performed in the discontinuous buffer system using 12% acrylamide-bisacrylamide separating gel (pH 8.8) and 4% acrylamide-bisacrylamide stacking gel (pH 6.8). Samples were mixed in 3:1 ratio with sample buffer (pH 6.8). For band size determination, molecular weight protein standards were used. The gel was stained with coomassie brilliant blue R-250 and destained by 30% methanol until clear bands were seen.

2.3. Anti-cancer activity

2.3.1. In vitro morphological study

The human cervical cancer cell lines (HeLa cells) were obtained from the American Type Culture Collection [ATCC], Manassas, VA, USA. For screening experiment, the cells were seeded into 12-well plates in 900 μl of RPMI medium (Biological Industries, USA) containing 5% FBS, at plating density of (2*10^4 cells/well). Whey and milk samples were solubilized in 0.2 M phosphate buffer (pH, 7), then 100 μl of various concentrations (1, 2.5, 5, 7.5 and 10 mg/ml) was added in duplicates to the prepared 12-well plates and incubated at 37ºC, 5% CO2, 95% air and 100% relative humidity for 24 h. To observe the morphological changes of the cells an inverted microscope was used (Labomed, USA).

2.3.2. MTT assay

The anticancer effect of the camel milk and whey samples against HeLa cells was estimated by the 3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay using (cell growth determination kit MTT based, Sigma). Cells (2x10^3 cells/well) were incubated with various concentrations of the compounds (0, 1, 2.5, 5, 7.5 mg/ml) at 37°C 5% CO2, 95% air and 100% relative humidity for 24 h in a FBS-free medium. Aseptically MTT solution was added in an amount equal to 10% of the culture volume. Then cultures were returned to incubator and incubated for 4 hours. After the incubation period, the resulting MTT formazan crystals were dissolved by the addition of MTT solvent in an amount equal to the original culture volume. The addition of MTT solvent was performed after the removal and disposal of the culture fluid as HeLa cells were still attached to the culture surface. The absorbance at 570 nm was measured using micro plate reader (Labotech, UK). The relative cell viability was determined by the amount of MTT converted to the insoluble formazan salt. The data are expressed as the mean percentage of viable cells as compared to the respective control.

3. Results

3.1. Identification of camel whey and milk proteins

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done to determine the protein contents of camel whey and milk samples. The obtained results showed that whey sample lack casein, and contain high concentrations of IgG immunoglobulins in addition to moderate concentration of albumin and very low concentration of lactoferrin (Figure 1).
3.2. **In vitro** morphological study

Results showed that both camel milk and whey reduced the viability of HeLa cells specially at 7.5 and 10 mg/ml (Figures 2 and 3).

3.3. MTT Results

Viability of HeLa cells that treated with camel milk was reduced to 12.1-15.1% within in studied concentrations range (Figure 4). The same observation was noticed after whey treatment as the viability of HeLa cells reached 13-15.4% (Figure 5). Cell growth was decreased with the increasing concentration of both camel milk and whey.

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Figure 1. The SDS-PAGE pattern of camel milk (1) and whey (2), in addition to molecular weight standards (3).

**Figure 2.** *In vitro* effect of camel milk on HeLa cells at different concentrations (mg/ml).

**Figure 3.** *In vitro* effect of camel whey on HeLa cells at different concentrations (mg/ml).
4. Discussion

Chemoprevention by dietary constituents has a valuable role in the control of diverse diseases including cancer (Kontou et al., 2011). Camel milk is an example for an excellent source of these constituents that exhibit different biological activities (Yagil et al., 1982). In the present study camel milk and its whey proteins emerged as a powerful anticancer agents that reduced the in vitro growth of Hela cells. According to the obtained results, they showed a toxic effect on the studied cells that approximately reached 85%. Apparently, camel milk is one of the natural products that has cytotoxic potential against cancer cells like murine hepatoma hepa 1c1c7 cells (Korashy et al., 2012). In the running study, the cytoxicity of camel milk and whey could be referred to the presence of casein, lactoferrin and immunoglobulins (Konuspayeva et al., 2007). It was recorded that camel milk increased the expression of chemo-protective genes which in turn increased the levels of several antioxidant enzymes. These enzymes prevent the formation of highly reactive oxygen species and so protect DNA and cell damage (Habib et al., 2013). Other study showed that casein and its product formed during pepsin hydrolysis had the ability to protect mammalian cells against certain genotoxic compounds (Van Boekel et al., 1993). Moreover, lactoferrin, the other component of camel milk exerts antitumor activity due to the immune-inducing and immunomodulatory properties (Al-Majali et al., 2007).

On the other hand, other studies indicated the ability of camel milk to induce apoptosis in various cancer cell lines. According to these studies, the apoptotic effect was on account of the activation of both extrinsic and intrinsic pathways. In this aspect, the induction of apoptosis occurred by several ways like enhancement of reactive oxygen species (ROS) production, activation of caspase-3 expression and induction of receptor-mediated apoptosis (Sidgi Hasson et al., 2015). Almahdy and his colleagues found that camel α-lactalbumin initiated apoptosis in the examined HepG2 and HeLa cells. Additionally, a complex from camel milk albumin and oleic acid displayed a pronounced antitumor activity through the induction of apoptosis in a variety of tumor cells (El-Fakharany et al., 2018). However, the exact potency of camel milk in apoptotic activation is not well recognized (Almahdy et al., 2011).

Otherwise, camel milk caused a powerful anti-proliferative effect on various cancer cell lines. This anti-proliferative effect is mostly due to the apoptotic and oxidative stress-mediated mechanisms (Habib et al., 2013,
Korashy et al., 2012, Sidgi Hasson et al., 2015). In this regards, lactoferrin inhibited cancer cells proliferation through various mechanisms. One possible mechanism is the activation of cell signaling pathways that lead to cell apoptosis (Fujita et al., 2004). Other possibility is that lactoferrin can function as a transcription factor (Habib et al., 2013). Moreover, lactoferrin affect cell cycle by arresting the treated cells at the G1 phase and thus down-regulate their proliferation (Damiens et al., 1997).

Besides the previous studied milk proteins, camel antibodies are also have paid attention to scientists in cancer research. The hallmark of camel milk is that it contains a unique immunoglobulin type called the heavy chain antibodies along with nanobody which is also called the variable domain of the heavy chain antibody (VHH) (Evers et al., 2008). Those molecules are devoid of the light chains that normally present in conventional antibodies. The small size of heavy chain antibodies offer them the ability to penetrate different tissues that cannot be reached by other antibodies (Cortez et al., 2002). In spite of the high sequence homology between camel heavy chain antibodies and human ones, camel antibodies are less hydrophilic. In addition to that, camel VHH domains are non-immunogenic and specifically target solid and metastatic tumors (Muyldermans et al., 1994). Moreover, these antibodies have the ability to recognize epitopes which are less antigenic for conventional ones (Lauwereys et al., 1998). All the mentioned features allow camel antibodies to overcome the stickiness problems of conventional antibody domains as well as to exhibit a rapid pharmacokinetic clearance (Cortez-Retamozo et al., 2002). In overall, heavy chain antibodies that are naturally found in camel milk could be a very useful tools for cancer targeting, diagnosis and therapy.

5. Conclusions
In conclusion, this study showed the cytotoxic potential of both camel milk and whey as they inhibited the growth of HeLa cells in vitro. Nevertheless, the milk and whey components that are responsible for such anticancer activity are not determined in the running research. So there is a need for further studies to figure out the bioactive compounds in camel milk and whey and their exact mechanism of action against HeLa cells.

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Conflict of interest
None to declare.

References
Almahdy O, EM EL-Fakharyan, E EL-Dabaar, TB Ng and EM Redwan, 2011. Examination of the Activity of Camel Milk Casein against Hepatitis C Virus (Genotype-4a) and Its Apoptotic Potential in Hepatoma and HeLa Cell Lines. Hepat. Mon., 11: 724-730.


