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Growth Inhibition and Anti-Cancer Activity of the Human Hepatocarcinoma HepG2 Cell Line *in Vitro* Study Using Human, Camel and Cow Milk

Nada H. Al-Mudallal[a]*, Ali A. Taha[b], Madlen Qassm abbas[c], Farooq Ibrahem Mohammed[d]

[a]*Department of Microbiology, College of Medicine, University of Al-Iraqia, Baghdad, Iraq*

[b]*Applied Science Department, University of Technology, Baghdad, Iraq*

[c]*College of dentistry, Tikreet University*

[d]*Biotechnology research center, Al nahrain University*

Abstract

"Certain food has resilient anticancer amalgams which can be rummage-sale for the cure and stoppage of cancer. The study scrutinized the cytotoxicity of human, camel, cow milk on animal carcinomatous cell line using tissue culture teqnique. In the existing study the inhibitory special effects of human, camel and cow milk at concentrations of 1.25%, 2.5%, 5%, 10%, 20%, 30%, 40% and 50% alongside human hepatocarcinoma HepG2 cell line *in vitro* were strongminded. The results signpost that anti-cancer activity of each type of milk used in the study and the growth restraint rate (%) was related to the milk concentration, so that a (50%) concentration of gave the highest growth inhibition rate, followed by the 40%, 30% and 20, 10% concentrations. Theirs significant difference between the concentrations when compare between the three types of milk, also statistical analysis association between the effects of different type of milk displayed significant difference ($P < 0.05$). The results visibly displayed that *human and camel milk* have potent cytotoxic and cytostatic effects on studied the cell line. On the basis of these results we can conclude that human and camel milk has strong cytotoxic effect on human carcinomatous cells".

Keywords: Camel and Cow milk; anti-cancer activity; human hepatocarcinoma HepG2 cell line.

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* Corresponding author.

1. Introduction

"Chemoprevention by dietary ingredient in the form of purposeful food has a well-established useful role in healthiness promotion and arisen as a new methodology to switch cancers [1,2]. Milk exemplify a foremost source of well-known antimicrobial constituents, in adding to lately revealed immunomodulatory effects [2,3]. These are main in shaping the immune system of new-borns since the neonatal immune system is not completely developed, creation it difficult for new-borns to guard themselves from infections [3,4,5,6]long-established that nourishment infants with maternal milk for the first 6-months of life, with non-stop breast feeding for the first one to two years of life (or longer), is the normative typical, due to the nutritive conformation of human milk and the non-nutritive bioactive factors that stimulate existence and healthy development. Camel milk is a main nutritious source that consumed novel or sour and historically been consumed in the cure of various diseases and for the upkeep of kindly health. The key components of the camel milk have been once upon a time determined [7,8,9], in that camel milk is diverse from other ruminant milk; having minimum cholesterol and sugar, rise minerals and vitamins, and rise concentrations of insulin [2,10,11]. A comparative study of lysozyme concentration in milk of diverse types El-Agamy and his colleagues [12] displayed that camel milk included significantly higher up content of lysozyme than goat, buffalo, sheep and cow but very minimum content as compared to lysozyme contented of human, donkey and mare milks. The same study displayed that camel milk included also meaningfully higher up level of lactoferrin (0.22 mg/ml) than cow, buffalo, sheep and goat but very minimum match with that of human milk. Many studies have attentive on the antimicrobial and antiviral effect of milk or milk ingredients, but only a limited study have been conducted to check its antifungal effects [13]. But the anti-cancer activity has not ever been showing to scientific checkup therefor. The aim of this study is to estimate the anticancer effects of human, camel and cow milk on Human hepatocellular carcinoma (HepG2) and used the type of milk subjected in the study as anticancer agent".

2. Method

2.1 Milk Sample preparation

"Samples of human milk were gained from lactating women two months after labour (lactation after colostrum, with a breast pump). Fresh *She*-camel's and raw cow's milk samples were collected from apparently healthy animals also after two months after labour bred in the living stock station in college of veterinary medicine, Baghdad University, Baghdad. The milk samples were positioned in sterilized vessels and imparted to the laboratory in a cool box. Human, cow and camel milk samples were delivered independently through a Millipore filter (0.22mm) (Bio-Rad) before determination of their anti-cancer activity".

2.2 Evaluation of anti-cancer activity of milk

"Human, Camel, Caw milk stock solution was made by mixing 400µl of milk with 10 µl of DMSO and complete the volume up to one ml using serum free medium [14, 15]. Eight concentrations starting with 50% till 1.25% in a twofold dilution manner were added in a triplicate to the microtiter plate containing 200 µl/well

containing $1x10^5$ cells/well".

2.3 Culture of cell lines

"The cytotoxicity assay for HepG2 was done at Animal Cell Culture lab/ Biotechnology Research Centre/ AL-Nahrain University. Cells were cultured in DMEM media enhanced with 10% fetal bovine serum, Non-essential amino acids, L-glutamine, HEPES, streptomycin, Sodium Bicarbonate and penicillin. Cells were grown as a monolayer in humidified 5% CO2 incubator at 37°C. The experiments were performed when cells were healthy, active and at log phase of growth" [16].

2.4 Cytotoxicity assay

Culture of this cell line was incubated with different concentrations of each extracts, eight concentrations were used as follows 1.25%,2.5%, 5%, 10%, 20%, 30%, 40% and 50% in triplicate to detect the growth inhibition of HepG2 cell line, and to investigate its cytotoxic and anti-proliferative effects respectively. Complete medium was used as negative control.

2.5 Neutral red assay

The wells were washed with PBS and freshly prepared neutral red (100µl/well) was added and incubated for 2 hrs, after incubating the cells with extracts for 48hr. lastly; wells were washed again with PBS to remove excessive dye and eluent buffer (100µl/well) was added and measured the absorbance using ELISA reader at 492nm wave length. Percentage of IR was counted according to formula:

(%IR) = bsorption at 490nm for control- absorption at 490 nm for extracts/absorption at 490nm for control] x 100 [17].

2.6 Statistical Analysis

The obtained results (The values of the examined parameters were assumed in terms of mean ± standard error) were statistically analyzed using Duncan's multiple range tests in SAS software (version 17; SAS Inc., Chicago, IL, USA). The grade of significance was $P > 0.05$ [18].

3. Results and Discussion

Cell culture can be a more sensible and reproducible method for preliminary screening the inhibition rate of active ingredients against cancer cell lines [19, 20] as the active ingredients can be tested on animal cells in very controlled way. Cytotoxicity assays in overall are capable to detect many agents that potentially inhibit the biochemical activity of numerous animal or human cell lines [21]. Several methods have been developed to assess the growth inhibition of cancer cell lines, like neutral red assay [22].

"In the present study the inhibitory effects of human, camel and cow milk at concentrations of 1.25%,2.5%, 5%, 10%, 20%, 30%, 40% and 50% against human hepatocarcinoma HepG2 cell line *in vitro* were determined as described previously. The results shown in (**Table.1**) indicate that anti-cancer activity of each type of milk used in the study and the growth inhibition rate (%) was related to the milk concentration, so that a (50%) concentration of gave the highest growth inhibition rate, followed by the 40%, 30% and 20, 10% concentrations. **Theirs significant difference between the concentration When compare between the three type of milk,** also Statistical analysis comparison between the effect of different type of milk showed significant difference ($P < 0.05$)".

"The results shows that human milk give strong anticancer activity against **human hepatocarcinoma HepG2 cell line *in vitro*** the greatest inhibition was recorded for human milk followed by camel milk then cow milk, the anticancer activity increase with the increase of milk concentrations for all the type of milk subjected in current stydy. For the **HepG2 cell line**, a concentration of 50% exhibited the highest growth inhibition rate (87.45%, 85.22% , and 44.5%), respectively for the three types of milk) with *p* value 0.05".

Table 1: The cytotoxic effect expressed as the inhibition rate percentage (%IR)for different concentrations of three type of milk after 48 hours exposure on HepG2 cell lines.

Concentrations %	**Percentage of Growth inhibition (G.I %) in hep g2 cell line**		
	Human	camel	caw
1.25%	1 ±0.00 f	00.00±0.00 f	00.00±0.00 e
2.5%	3± 20 f	5 .66±0.00 g	00.00±0.00 e
5%	9.12±9.12 g	7.91± 0.19 g	00.00±0.00 e
10%	18 ± 9.12 h	14.1±2.13 h	8.22± 0.12 a
20%	21.00±4.39 c	17.89 ±1.82 c	13.9± 0.75 a
30%	39.10±1.96 b	34.92± 1.30 b	23± 7.12 b
40%	61.99±2.736 a	58.34±2.34 c	36.4± 8.1 c
50%	87.45 ±0.34 d	85.22±0.33d	44.5± 0.19 d

***(P<0.05), different letters= significant differences between mean.**

"α-lactalbumin in human milk is the main proteins. A structural derivative of α- lactalbumin harbors tumor-selective capabilities, named human α-lactalbumin made lethal to tumor cells is a molecular complex of α -lactalbumin and oleic acid [23,24,25] speedily invades the cancer cell. The mechanism is not fully understood, but invasion requires both the unfolding of a-lactalbumin and the presence of the fatty acid. The native protein in human milk does not invade cells expeditiously, no kills them; neither does stably unfolded a-lactalbumin mutants [26,27]. The interaction between phospholipid membranes and a-lactalbumin binds to negatively

charged lipid vesicles in a pH-dependent manner, as observed with fluorescent methods. Early studies discovered apoptotic features in cancer cells that die after treatment with milk. Cytochrome C release and mitochondrial damage were detected in both intact tumor cells and isolated mitochondria, and there was a weak caspase response, including activation of effector caspases-3 and -9, and of the DNA damage-related, nuclear caspase-2. The apoptotic response was not cause death for cell, however, as caspase inhibitors did not rescue the cells from dying [28,29]. The mitochondria are only one of several targets for milk in tumor cells [30]. The effect of milk on proteasomes and the participation of proteasomes in death of cell, Like non-malignant cells, tumor cells can undergo numerous types of death for cell, including necrosis, apoptosis, autophagic cell death, and mitotic catastrophe"[31].

"The current study provides, to our knowledge, the informations about the importance of human, camel, and cow milk significantly proliferation and inhibition growth of human liver HepG2 that's also may be related to molecular mechanism by which milk work as anti-cancer may be through different mechanisms: (a) the activation of caspase-3 at the mRNA and activity levels, (b)Activation of DR4, (c) the accumulation of intracellular ROS, and (d) the role of MAPK signaling pathway" [32].

"Shamsia, (33) determined the antimicrobial factors of both camel and human milk and concluded that camel milk is wealthier in Ig (1.54 mg/ml) than human milk (1.14mg/ml). It contents of lysozyme (0.06mg/ml) and lactoferrin (0.24mg/ml) were very low, as linked with human milk which contains (1.95mg/ml) lactoferrin and (0.65mg/ml) lysozyme. He also reported that camel milk contained higher fat, protein especially ash and casein contents but lower lactose and whey protein contents than human milk. The lower casein and higher whey protein contents in human milk make it very nutritious for new born due to the resulting soft-coagulum after milk ingestion and higher digestibility and absorption of soluble proteins [34]. Many biologically active peptides have been identified from dairy products and milk proteins by proteolysis with enzymes or microbial enzymes or by fermentation [35]".

4. Conclusion

In conclusion, the human and camel milk had a strong cytostatic, cytotoxic, inhibitory and antiproliferative effects against cancer cell line *in vitro*, but yet we do not know the active protein compound in these types of milk which showed these effects. There is need of more investigational studies to figure out the molecular mechanism of the bioactive protien compound in the human and camel milk so it could be used as therapeutic agent for cancer treatment.

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