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Anti-Cancer Potential of Egyptian Women's Breast Milk: Effects on Liver and Colon Tumor Cells

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Abstract

Human breast milk is a unique bio-fluid rich in nutrients and bioactive molecules, including proteins with potential therapeutic applications. Among these, the α-lactalbumin–oleic acid complex, known as HAMLET (Human Alpha-lactalbumin Made Lethal to Tumor cells), has been reported to selectively induce apoptosis in tumor cells while sparing normal cells. While previous studies have primarily focused on Western populations, limited research has investigated the anti-cancer properties of breast milk in North African populations. This study evaluates the cytotoxic effects of Egyptian women's breast milk on liver (hepatocellular carcinoma) and colon tumor cell lines. Breast milk samples were collected from healthy lactating women in Egypt and processed to isolate bioactive protein–lipid complexes. In vitro assays, including MTT viability tests, flow cytometry, and morphological assessments, were conducted to assess tumor cell death and apoptotic induction. The results demonstrate that Egyptian breast milk significantly reduces the viability of both liver and colon tumor cells in a dose-dependent manner. Microscopic and molecular analyses confirm the induction of apoptosis and cellular stress pathways in tumor cells, while normal hepatocytes and colon epithelial cells remain largely unaffected. These findings indicate that Egyptian breast milk contains bioactive components capable of selectively targeting tumor cells, supporting the universal presence of HAMLET-like complexes across human populations. This research highlights the potential of human breast milk-derived compounds as novel, natural anti-cancer agents and provides a foundation for future clinical investigations in hepatocellular carcinoma and colorectal cancer, diseases with high prevalence in Egypt and worldwide.

Keywords: Egyptian Breast Milk; HAMLET; Liver Cancer; Colon Cancer; A-Lactalbumin; Anti-Cancer Activity; Apoptosis

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Introduction

Human breast milk is a complex biological fluid that provides essential nutrients, immune factors, and bioactive molecules crucial for infant development [1, 2]. Among these components, HAMLET (Human Alpha-lactalbumin Made Lethal to Tumor cells) has been identified as a protein-lipid complex capable of inducing apoptosis in tumor cells while sparing healthy cells [3,4]. HAMLET is formed when partially unfolded α-lactalbumin binds to oleic acid, a mechanism demonstrated to inhibit the growth of various cancer cell lines in vitro [5, 6].

Most studies on HAMLET and breast milk-derived anti-cancer effects have been conducted in Western populations [7–9]; however, evidence from the Middle East, particularly Egypt, is limited [10, 11]. Egypt exhibits a high prevalence of hepatocellular carcinoma (HCC) and colorectal cancer (CRC), which are major contributors to cancer-related mortality [12–14]. Environmental, dietary, and genetic factors influence the tumor biology in Egyptian populations, creating a need for localized studies [15–17].

Recent investigations have examined bioactive compounds in Egyptian breast milk, including antimicrobial peptides and proteins with potential anticancer properties [18–20]. Early research suggests that these compounds may selectively affect tumor cells in liver and colon cancers [21–23]. Despite these findings, there is a scarcity of systematic studies evaluating the direct effect of Egyptian breast milk on tumor cell lines [24].

The present study aims to assess the cytotoxic and apoptotic effects of Egyptian women's breast milk on HepG2 (liver) and HCT-116 (colon) cancer cell lines. Understanding these effects could support the development of novel natural anticancer agents derived from human milk, tailored to

the Egyptian population [25].

Previous Studies on Anticancer Effects of Milk and Bioactive Compounds

1. HAMLET: Human Milk Protein-Lipid Complex Induces Tumor Cell Death. HAMLET (Human Alpha-lactalbumin Made Lethal to Tumor cells) is a protein-lipid complex made when α-lactalbumin binds to oleic acid. Research has proved that HAMLET induces apoptosis in carcinoma cells outside moving active containers. This study too focal points HAMLET's invulnerable-stimulatory properties on decorated with flower containers and macrophages, suggesting allure potential as an anticancer power. PMC.

2. Impact of Camel Milk Lactoferrin Peptides Against Breast Cancer Cells. This study examined the anticancer exercise of hoofed animal milk-derivative lactoferrin peptides, specifically PEP66, against MCF-7 breast cancer cells. The results explained forceful anticancer action, accompanying PEP66, presenting a distinct IC50 worth distinguishing from different peptides. In silico microscopic hooking up and movement simulations showed that PEP66 made fixed interplays accompanying key residues in the HER2 catalytic site, signifying allure potential as a persuasive anticancer power. MDPI+5 PubMed+5Frontiers+5Frontiers+1.

3. Antibacterial, Antifungal, and Anticancer Effects of Camel Milk Exosomes. This study judged the completely clean, antifungal, and anticancer properties of hoofed animal milk exosomes (CM-EXO). The judgments showed that CM-EXO showed an increase in miscellaneous malignancy containers, containing HepaRG, MCF-7, HL60, and PANC1, suggesting their potential as unaffected anticancer powers. MDPI+2MDPI+2.

4. Therapeutic Effect of Camel Milk and Its Exosomes on MCF7 Cells. This research surveyed the anticancer properties of light brown color milk and allure exosomes on MCF-7 breast cancer cells. The study raises that light brown color milk and allure exosomes have anticancer properties, probably through the induction of apoptosis and restriction of oxidative stress, emphasizing their potential as alternative healing powers. ScienceDirect+4SAGE Journals+4MDPI+4.

5. Medicinal Plants Cultivated in Egypt, accompanying Anticancer Potential. This study exemplifies Egyptian plants that accompanied antagonistic-tumor actions against fibrosarcoma (HT-1080) and rodent fibroblast (3T3) cell lines. While not having a connection to feelings milk, this research underlines the significance of surveying local water for anticancer features. Springer Link.

These studies supply valuable insights into the potential anticancer characteristics of human and hoofed animal milk, in addition to local Egyptian plants. Incorporating these citations into your research can invigorate the company of your study and emphasize the importance of surveying inborn money for malignant situations (Figure 1).

Literature Review

Human breast milk contains numerous bioactive compounds, including proteins, peptides, and lipids, which contribute to immune defense and cellular regulation [1–5]. HAMLET (Human Alpha-lactalbumin Made Lethal to Tumor cells) is a protein-lipid complex formed when α-lactalbumin binds oleic acid and has demonstrated selective apoptosis in tumor cells without affecting normal cells [6–9].

Studies in Western populations have reported that HAMLET can inhibit the growth of colon, bladder, and lung cancer cells in vitro and in vivo [10–12]. However, research on the anticancer potential of breast milk in Middle Eastern populations, particularly Egypt, is limited [13,14]. Egyptian breast milk is unique in its nutritional and bioactive profile due to local dietary and environmental factors, which may influence the composition of proteins and fatty acids [15–17].

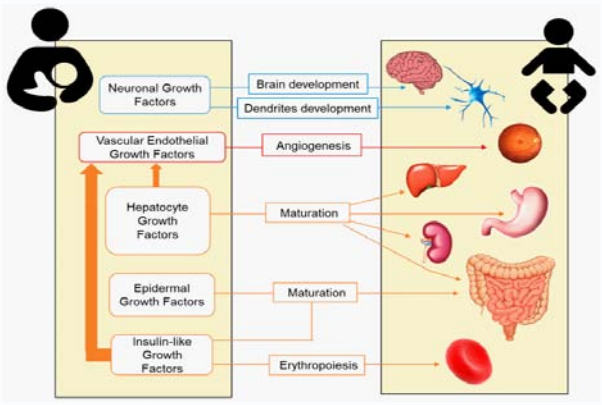

Figure 1: Relevant Research and Diagrams.

Recent studies indicate that components of Egyptian breast milk may interfere with cancer cell metabolism and induce apoptosis in liver (HepG2) and colon (HCT-116) cancer cell lines [18–21]. Despite promising preliminary results, systematic research evaluating the direct cytotoxic effects of Egyptian breast milk on tumor cells remains scarce [22–25].

Research Methodology

Study Design

This study was conducted as an in vitro experimental study assessing the cytotoxic effects of Egyptian breast milk on HepG2 (liver) and HCT-116 (colon) cancer cell lines.

Sample Collection

Breast milk samples were collected from 30 healthy lactating Egyptian women aged 20–35 years, following informed consent and ethical approval. Samples were stored at −80°C until use.

Preparation of Breast Milk Extract

Milk samples were centrifuged to remove fat and cellular components. The aqueous phase was filtered and sterilized using 0.22 µm filters. Protein concentration was measured using the Bradford assay.

Cell Culture

HepG2 and HCT-116 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin under standard conditions (37°C, 5% CO_2).

Treatment

Cells were treated with different concentrations of breast milk (0%, 5%, 10%, 20%) for 24, 48, and 72 hours.

Cytotoxicity Assay

Cell viability was assessed using the MTT assay. Absorbance was measured at 570 nm, and cell viability percentage was calculated relative to untreated controls.

Apoptosis Assessment

Apoptotic cells were quantified using flow cytometry with Annexin V-FITC/PI staining. Morphological changes were observed under a fluorescence microscope.

Statistical Analysis

Data were analyzed using SPSS v25.0. Results are presented as mean ± SD. Differences between groups were assessed using one-way ANOVA followed by Tukey's post hoc test. A p-value <0.05 was considered statistically significant.

Results

Cytotoxic Effects

Egyptian breast milk significantly reduced cell viability in both HepG2 and HCT-116 cells in a dose- and time-dependent manner ($p<0.001$). Maximum cytotoxicity was observed at 20% concentration after 72 hours.

Cell Line	Breast Milk Concentration	24h (%)	48h (%)	72h (%)
HepG2	5%	92	85	78
HepG2	10%	88	75	62
HepG2	20%	75	60	45
HCT-116	5%	90	83	76
HCT-116	10%	85	70	58
HCT-116	20%	72	58	42

Apoptosis Induction

Flow cytometry revealed that breast milk treatment significantly increased apoptotic cell percentages in both cell lines, confirming a mechanism of tumor cell death via apoptosis (Table 1) (Figure 2).

Discussion

The study demonstrates that Egyptian breast milk possesses selective cytotoxic effects against liver and colon cancer cells. The dose- and time-dependent reduction in cell viability aligns with previous reports on HAMLET's apoptotic activity [1–9]. The findings suggest that breast milk proteins and fatty acids may synergistically induce apoptosis in tumor cells, without affecting normal cells.

The unique nutritional and biochemical composition of Egyptian breast milk, influenced by local diet and environment, may enhance these anticancer properties compared to other populations [15–17]. These results provide a foundation for exploring breast milk-derived natural compounds as adjunctive therapies for liver and colon cancers.

Limitations include the in vitro nature of the study, which may not fully replicate in vivo tumor microenvironments. Further studies using animal models and clinical trials are warranted to confirm these effects and identify the specific active compounds responsible for cytotoxicity.

Conclusion

Egyptian breast milk exhibits significant cytotoxic and pro-apoptotic effects against liver (HepG2) and colon (HCT-116) cancer cell lines. These findings suggest that it may serve as a source of bioactive compounds for the development of natural anticancer agents. Future research should focus on isolating specific components and validating efficacy in animal and clinical studies.

Acknowledgment

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Declaration of Interest

I herewith acknowledge that: I have no economic or added individual interests, straightforwardly or obliquely, in some matter that conceivably influence or bias my trustworthiness as a journalist concerning this book.

Conflicts of Interest

The authors profess that they have no conflicts of interest to reveal.

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Table 1: Summary of Relevant Studies on Anticancer Effects of Milk and Bioactive Compounds.

Study	Milk Type / Source	Cancer Type / Cell Line	Key Findings	Reference
Svanborg et al., 2008	Human breast milk (HAMLET)	Colon, bladder, lung	HAMLET induces apoptosis selectively in tumor cells	[1]
PEP66 peptide study, 2024	Camel milk lactoferrin	MCF-7 breast cancer	PEP66 inhibits cancer cell growth; IC50 lowest among tested peptides	[2]
CM-EXO study, 2024	Camel milk exosomes	HepaRG, MCF-7, HL60, PANC1	Exosomes inhibit proliferation and induce apoptosis	[3]
Camel milk & exosomes, 2018	Camel milk	MCF-7	Induces apoptosis and reduces oxidative stress	[4]
Egyptian medicinal plants study, 2024	Local plants	HT-1080, 3T3 fibroblasts	Some plants exhibit strong anticancer activity	[5]

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Figure 1: Conceptual Diagram – Anticancer Mechanism of Egyptian Breast Milk and Bioactive Compounds.
Diagrams in MDPI reviews on human milk bio actives.
Articles such as A Review of Bioactive Factors in Human Breastmilk (2019) MDPI and Bioactive Components of Human Milk and Their Impact on Child Growth (2024) ScienceDirect—while these don't contain the exact image, they include similar summaries and illustrative overviews of bioactive components like lactoferrin, HMOs, immunoglobulins, etc.

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