

EVALUATION OF THE ANTI-CANCER EFFECT OF CAMEL MILK ON TONGUE SQUAMOUS CELL CARCINOMA CELL LINE USING FLOW CYTOMETRIC ANALYSIS, NUCLEAR MORPHOMETRIC ASSAY, AND WESTERN BLOTTING TECHNIQUE: AN IN-VITRO STUDY

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ABSTRACT

Background: Cancer is the leading cause of death in developing nations after cardiac diseases. Fighting cancer is a highly challenging task. Natural compounds are becoming a major source for many anti-cancer drugs. Camel milk is well known for its benefits to human health and is believed to be able to treat several ailments, including cancer.

Aim: The current study was designed to explore the possible effects of camel milk on tongue squamous cell carcinoma cell line (HNO-97 cells).

Methods: The anticancer effectiveness of camel milk was assessed using cell viability assay, cytological evaluation, flow cytometric analysis, nuclear morphometric assay, western blotting technique, and statistical analysis.

Results: The findings demonstrated that camel milk had a dose-dependent cytotoxic impact on HNO-97 cells. Camel milk mainly triggered apoptosis on HNO-97 cells. Nuclear morphometric report revealed that, the mean nuclear area factor values of the HNO-97 treated groups were less than those of the control group. Regarding western blotting technique, caspase-3 and P53 expression levels were significantly increased in the treated groups.

Conclusion: Camel milk considerably increased the cytotoxic effect on HNO-97 cells.

KEYWORDS: Camel milk, tongue squamous cell carcinoma cell line, apoptosis, flow cytometric analysis, nuclear morphometric assay, and western blotting technique.

Ethical Regulation: All investigations included in the current study were conducted in compliance with the ethical guidelines of the Research Ethics Committee of the Faculty of Dentistry, Minia University, Egypt, with approval number 667 on Tuesday, 27/12/2022.

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INTRODUCTION

Oral cancer is the sixth most common cancer globally, accounting for 300,000 cases (2.1% of all cancer cases) and 145,000 deaths worldwide ^[1]

Squamous cell carcinoma (SCC), which originates from the mucosal surface of the oral cavity, accounts for 90% of all documented cases of cancer ^[2].

In 2023, there were 354,864 oral cancer patients and 177,384 fatalities, according to latest global estimates ^[3]. SCC mostly affects the lateral border of the tongue ^[4].

The most prominent risk factors for tongue squamous cell carcinoma (TSCC) are drinking alcohol and smoking cigarettes. These factors have the potential to trigger a variety of genetic and epigenetic pathways that contribute to genomic instability, cancer development, and progression ^[5].

Surgery, chemotherapy, radiation, or combinations of these treatments are available for the treatment of TSCC. Chemotherapy remains the most often used treatment for metastatic cancer or when organ preservation required. However, drug toxicity and tumor cell resistance are considered obstacles of this treatment option ^[6].

Cell death is an important biological process. This organism-controlled process kills old or damaged cells and replaces them with new ones ^[7]. Cell death is critical in prevention and treatment of cancer. The most common types of cell death are apoptosis and necrosis ^[8].

Apoptosis is an intracellular program-mediated cell death process. It is a complicated process that governs cell homeostasis in eukaryotes. It can take place in both normal and pathologically altered tissues ^[9].

The most common apoptotic routes are intrinsic (mitochondrion-initiated), and extrinsic (death receptor-mediated) processes ^[10].

Membrane blebbing, chromatin condensation, nuclear fragmentation with organelle preservation are the morphologic characteristics that characterise apoptosis. Following the procedure, apoptotic bodies are phagocytized by phagocytic cells without causing inflammation ^[11].

Necrosis can occur throughout several conditions, including cancer, neurological, and autoimmune disorders. Necrosis is triggered by factors such as toxic stress or damage to the body ^[12].

The distinctive features of necrosis include the swelling of cells and organelles as well as the rupture of plasma membranes, which allows the release of cellular components that trigger an inflammatory response ^[13].

Various natural substances are currently used in the treatment of TSCC. Several studies have explored the positive impact of natural compounds on cancer with very low toxicity on normal cells. Researchers have recently concentrated on these compounds' potential applications as anti-inflammatory, anti-oxidant, antibacterial, and anti-carcinogenic agents ^[14].

Camel milk is an opaque, white, salted, highly acidic liquid ^[15]. Camel milk is a special type of nourishment that is regarded as a superfood with therapeutic properties ^[16].

Camel milk is a highly nutritious diet that is rich in minerals, rich in vitamins (C, B2, A, and E), low in sugar, low in cholesterol, and high in lactoferrin, lysozyme, and lactoperoxidase ^[17].

Camel milk has antioxidant properties and regulates genes that prevent the growth of cancer cells. Camel milk contains lactoferrin and immunoglobulins. Lactoferrin is a specific kind of glycoprotein that has anticancer properties ^[18].

The extracellular effect of lactoferrin involves binding to several membrane receptors, whereas the intracellular effect involves apoptosis and cell cycle arrest ^[19].

Camel milk appears to have a thrombolytic impact by limiting the expansion and multiplication of metastatic cancer cells because it inhibits coagulation and fibrin production [20].

Cent research indicates that camel milk can help treat a variety of diseases, such as colon and breast cancer. For this reason, it will be crucial to the management of oral cavity cancers [21].

Flow cytometry (FCM) is a sophisticated technology that is able to carry out quantitative multiparametric analysis of cell populations at the single-cell level [22].

Western blotting technique is the gold standard method for identifying and quantifying a specific protein in a complicated mixture isolated from cells or tissue [23,24].

MATERIAL AND METHODS

MATERIAL

Cell Culture Protocol:

Human tongue squamous cell carcinoma cell line (HNO-97) was obtained from Cell Culture Department VACSERA-EGYPT. The cells were imported in the form of a frozen vial bearing the reference number "CRL-1628" from the American Type Culture Collection.

In accordance with standard laboratory protocol, the cells were cultured on Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum in a humidified CO₂ incubator at 37 °C.

The Tested Reagent:

- Camel milk was purchased as liquid from the camel farm.

Preparation of Camel Milk

- Camel milk was driven by milking via a vacuum pump from camel farm. The milk was harvested under cooling conditions.

- Camel milk was distributed in 50 ml total volume falcon tube.
- The tube was centrifuged at 10000 rpm in cooling centrifuge.
- The supernatant was harvested in sterile conditions.
- The milk was tested for sterility on bacterial culture media and sterile milk was kept at -80 till use.

METHODS

Cell Viability Assay

The vitality of the malignant cells following treatment with varying dosages of camel milk was evaluated using the Methyl Thiazole Tetrazolium (MTT) assay. MTT comprises yellow tetrazolium salt, a water-soluble salt that is converted by lactate dehydrogenase (LDH) in the mitochondria to an insoluble purple MTT-Formosan complex. The resultant purple Formosan intracellular crystals were measured using spectrophotometry.

To carry out the MTT experiment, $1.2-1.8 \times 10^3$ HNO-97 cells were pre-cultured in 96 micro-titer plates (5×10^4 cells/mL) and exposed to varying amounts of camel milk for a whole day. Following the addition of 10 μ L of MTT (0.5 mg/ml stock) solution to each well, the wells were incubated for 24 hours at 37°C.

After that, each well was filled with 100 μ L of dimethyl sulfoxide to dissolve the purple Formosan crystals, and the medium was taken out of the wells. Using the spectrophotometer, the absorbance at 570 nm was determined.

The data obtained were analyzed to determine the pre IC₅₀, IC₅₀ (the half-maximal inhibitory concentration), and post IC₅₀ doses of camel milk.

Photomicrography and Cytological Evaluation

Using a digital video camera mounted on a light microscope, microscopic slides were

photomicrographed at an oil immersion power of x1000.

Nuclear Morphometric Analysis

Image analysis software (Image J, 1.27z, NIH, USA) was used to analyze the photomicrographed fields. The 8-bit grayscale format was applied to the images. Automatic phase color coding was applied to the designated region.

The formula to calculate the nuclear area factor (NAF) was $NAF = \text{Nuclear circularity} \times \text{Object area}$.

Flow Cytometric Analysis

One of the most widely used methods for examining different phases of the cell cycle and apoptosis is flow cytometric analysis (FCM). Using a fluorescence activated cell sorting (facscan) flow cytometer, FCM was carried out.

Propidium Iodide/Annexin-V Staining Assay

Propidium iodide/annexin-V identifying was carried out based on the changes that occur at the cell surface at different phases of apoptosis and the translocation of phosphatidylserine (PS) from the inner portion of the plasma membrane to the outer layer exposed to the external surface of the cell.

Western Blotting Technique

Western blotting technique is a laboratory technique used to find a specific protein in a tissue sample. The antibody is combined with radioactive or fluorescent labels or enzymes to produce a reaction that, when added to a reagent, results in the coloring or emission of light.

The present study employed this methodology to assess the expression levels of caspase-3 and P53.

Statistical Analysis

To ascertain the significance of differences between groups, experimental data were statistically

evaluated using One-way Analysis of Variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) version 16.0 window software. For the ANOVA test, the data were presented as mean \pm standard deviation. *P*-value ≤ 0.05 was considered statistically significant.

RESULTS

1- Cell Viability Assay

In the current investigation, the cytotoxic impact of camel milk on HNO-97 cells was evaluated for 24 hours. The acquired data indicated a dose-dependent pattern of cytotoxicity.

Table 1 illustrates how the mean viability percentage of the treated HNO-97 cells dropped in comparison to control cells as the camel milk concentration rose from 1 μ M/ml to 4 μ M/ml.

Table (1): The mean viability percentage (%) of camel milk-treated HNO-97 cells with low concentration (1 μ g/ml), medium concentration (2 μ g/ml), and high concentration (4 μ g/ml) for 24 hours' incubation.

Sample	HNO-97 cells treated with camel milk for 24h incubation		
Concentration μ g/ml	Pre IC ₅₀ (1)	IC ₅₀ (2)	Post IC ₅₀ (4)
Viability%	92.66%	50%	27.88%

2. Photomicrography and Cytological Evaluation

As shown in photomicrographs, the control cells were round and displayed characteristics indicative of malignancy, including hyperchromatism, increased mitosis, nuclear pleomorphism, and an increased nuclear-cytoplasmic ratio.

On the contrary, HNO-97 cells treated with camel milk displayed the morphologic characteristics

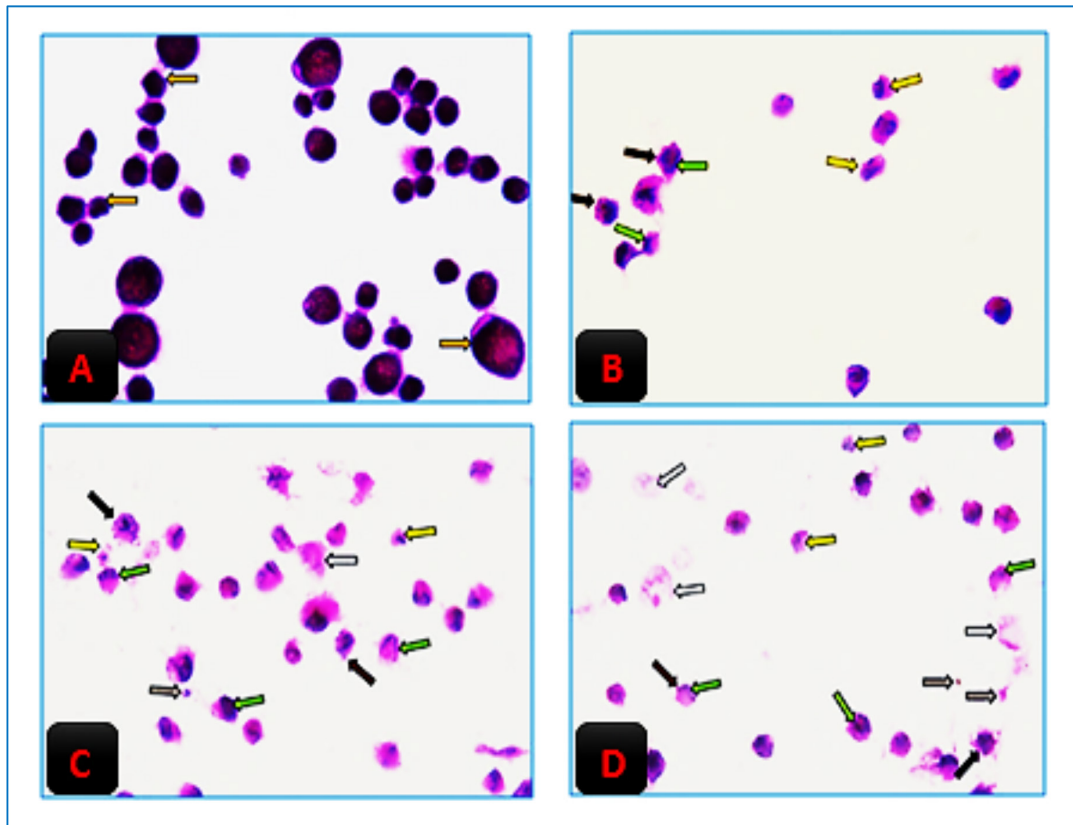


Fig. (1): Photomicrographs of HNO-97 cells showing: (A) control cells, (B) Pre IC₅₀-treated cells, (C) IC₅₀-treated cells, and (D) Post IC₅₀-treated cells, (H & E ×1000 oil immersion).

of apoptosis. Peripheral chromatin condensation, abnormalities in the nuclear and cellular membranes, nuclear shrinkage, membrane blebbing, and nuclear fragmentation were all these criteria.

Necrosis-related morphological features, including nuclear and cellular enlargement, elevated cytoplasmic eosinophilia, and cell membrane rupture, were seen in some treated HNO-97 cells. Comparing the postIC₅₀ treated group to the other groups, these characteristics were more apparent, as shown in figure 1.

3. Nuclear Morphometric Analysis

Comparing the mean nuclear circularity of HNO-97 treated cells to control HNO-97 cells, the recorded results showed a decrease in these values.

For the control group, pre IC₅₀, IC₅₀, and post IC₅₀ treatment groups, the mean values of nuclear circularity were, respectively, 0.88, 0.87, 0.77, and 0.65.

The findings showed that, in comparison to control HNO-97 cells, the mean values of object area in the HNO-97 treated cells reduced. The object area means for the control group, pre IC₅₀, IC₅₀, and post IC₅₀ treatment groups were 96.62, 86.80, 68.20, and 22.30, respectively.

The acquired results demonstrated that, in comparison to control HNO-97 cells, the mean values of NAF in HNO-97 treated cells dropped. The NAF mean values were, for the control group, pre IC₅₀, IC₅₀, and post IC₅₀ groups, 78.98, 74.25, 50.82, and 15.59, respectively.

4. Flow Cytometric Analysis

Cell cycle analysis using FCM revealed that the number of cells entering the cell cycle reduced progressively and, in a dose-dependent manner, when comparing camel milk-treated groups to the control group in the G2/M phase. The findings show that, as Figure 2 shown, camel milk caused cell cycle arrest in the G2/M phase.

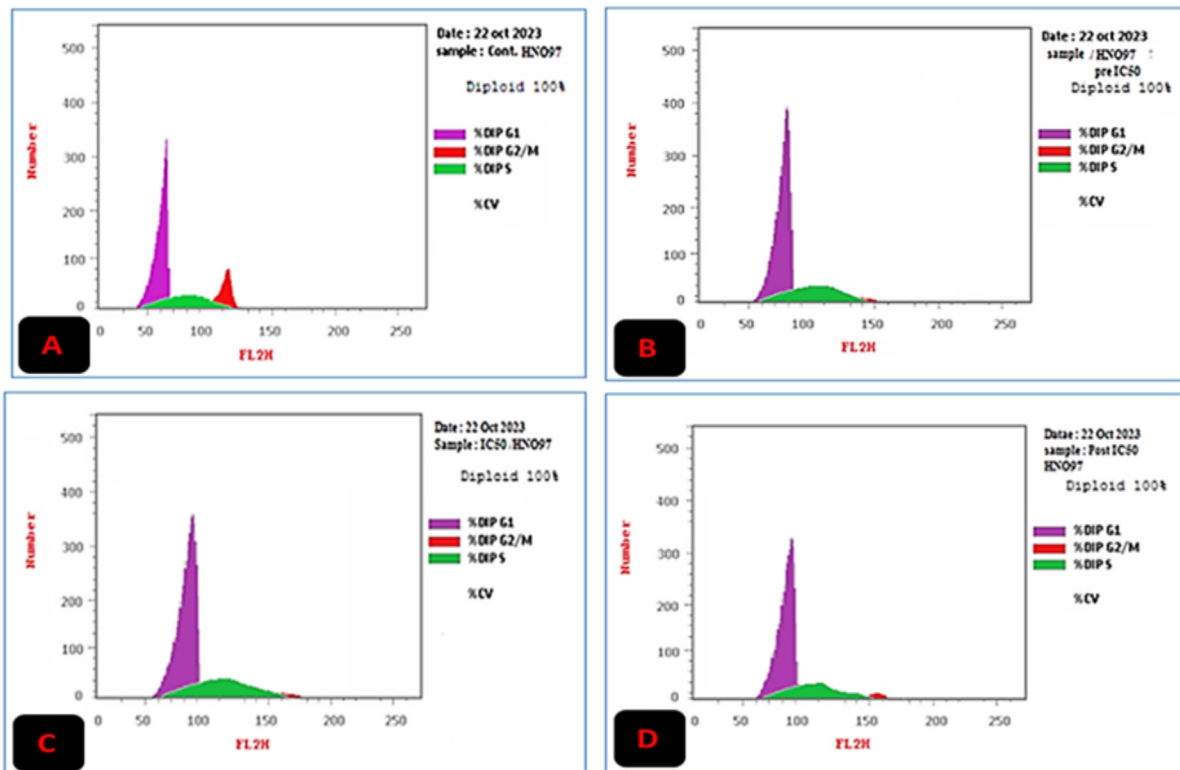


Fig. (2): Cell cycle distribution histograms of HNO-97 cells showing (A) control cells, (B) Pre IC50-treated cells, (C) IC50-treated cells, and (D) Post IC50-treated cells.

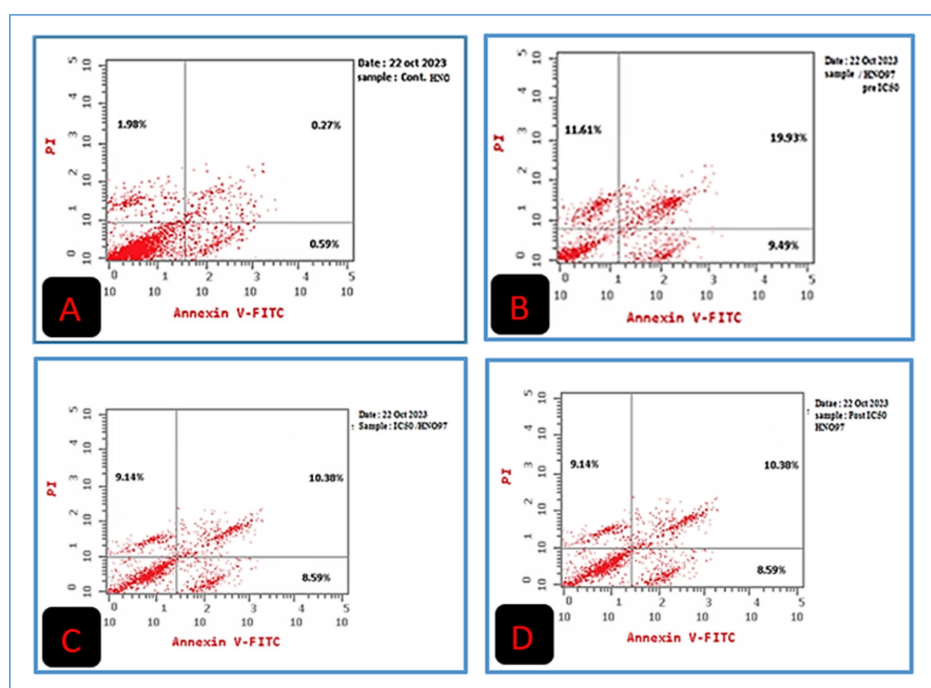
5. Propidium Iodide/Annexin-V Staining Assay

The results of the propidium iodide/annexin-v staining test demonstrated necrotic cells (in the upper left quadrant), early and late apoptotic cells (in the lower right quadrant and upper right quadrant), and control cells (in the lower left quadrant).

This experiment showed, as indicated in table 2 and figure 3, that when camel milk dosage was raised from 1 $\mu\text{g/ml}$ to 4 $\mu\text{g/ml}$, the number of live cells decreased, and the number of apoptotic and necrotic cells increased.

TABLE (2) Propidium Iodide/Annexin-V staining assay showing various phases of apoptosis and necrosis.

Ser	Code	Apoptosis			Necrosis
		Total	Early	Late	
1	Cont. HNO-97 cells	2.84	0.59	0.27	1.98
2	Pre IC ₅₀ treated HNO-97 cells	22.07	6.61	3.45	6.01
3	IC ₅₀ treated HNO-97 cells	28.11	8.59	10.38	9.14
4	Post IC ₅₀ treated HNO-97 cells	41.03	9.49	19.93	21.61

Fig. 3: Propidium Iodide/Annexin-V staining assay showing various phases of apoptosis and necrosis: (A) control cells, (B) Pre IC₅₀-treated cells, (C) IC₅₀-treated cells, and (D) Post IC₅₀-treated cells.

6. Western Blotting Technique

The findings of this method showed elevated Caspase-3 and P53 levels, as shown in table 3.

TABLE (3) Expression levels of both Caspase-3 and P53 in HNO-97 cells using Western blotting technique.

Ser	Compound Code	Western Blotting		β-actin
		OD		
		Casp-3	P53	
1	Cont. HNO-97 cells	0.127	0.144	✓
2	Pre IC ₅₀ treated HNO-97 cells	0.416	0.326	✓
3	IC ₅₀ treated HNO-97 cells	0.661	0.487	✓
4	Post IC ₅₀ treated HNO-97 cells	0.834	0.752	✓

7. Statistical Analysis

One-way The ANOVA test was employed to compare several groups. Tables 4 and 5 illustrate that the P-value < 0.05 was chosen as the significance level.

TABLE (4) Descriptive statistics of one-way ANOVA showing the mean values of NAF \pm stranded deviation of different groups.

Group	N	NAF		95% CI		P-value
		Mean \pm SD	Standard error	Lower limit	upper limit	
Control	50	78.98 \pm 66.91	9.463	59.9	98.00	
Pre IC ₅₀	50	74.25 \pm 43.13	6.099	61.99	86.51	
IC ₅₀	50	50.82 \pm 42.85	6.060	38.64	63.00	< 0.0001
Post IC ₅₀	50	15.59 \pm 26.59	3.761	8.03	23.15	

TABLE (5) Descriptive statistics of one-way ANOVA test for comparison between different groups.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	125808.673	3	41936.224	18.887	
Within Groups	435202.887	196	2220.423		<0.0001
Total	561011.560	199			

DISCUSSION

Oral cancer (OC) is ranked among the top 10 cancers worldwide. Biomedical science has a continuing struggle as survival rates have not grown significantly in recent years, despite breakthroughs in cancer therapy [25].

It has been shown that alcohol and tobacco use are the primary risk factors for OC [26].

Chemotherapy, radiation therapy, and surgery are among the OC approaches to therapy. There is a strong correlation between major morbidity and traditional therapeutic techniques. Thus, it became necessary to look for natural alternative medicines that are effective against cancerous cells while having little to no influence on healthy ones [27].

Camel milk is a natural white liquid that has nearly the same amounts of lactose, fat, and protein as bovine milk [28]. Both in vitro and in vivo studies have demonstrated the anti-cancer properties of camel milk [29].

Camel milk has been shown to inhibit human breast cancer BT-474 and laryngeal HE-p2 cells [30].

Additionally, camel milk has been shown to function as an antioxidant and a DNA damage repair agent, which inhibits the development of human colorectal cancer HCT 116 cells [31].

This study aimed to clarify the underlying processes and investigate any potential effects of camel milk on HNO-97 cells. This was done using cell viability assay, cytological evaluation,

flow cytometric analysis, nuclear area factor assay, western blotting technique, and statistical analysis.

There were four groups in the current study: group I was the control group; group II treated HNO-97 cells with a camel milk pre-IC₅₀ concentration; group III treated HNO-97 cells with an IC₅₀ concentration; and group IV treated HNO-97 cells with a camel milk post-IC₅₀ concentration.

First, a cell viability assay was performed to investigate the influence of the camel milk on HNO-97 cells after 24 hours' incubation, and to calculate the pre IC₅₀, IC₅₀, and Post IC₅₀ values of camel milk.

The pre IC₅₀, IC₅₀, and post IC₅₀ values were found to be 1 μg/ml, 2 μg/ml, and 4 μg/ml, respectively. The MTT experiment showed that when the concentration of camel milk rose, the viability percentages dropped. For the pre IC₅₀, IC₅₀, and post IC₅₀, the corresponding viability percentages were 92.66%, 50%, and 27.88%.

The dose at which 50% of a cell population is destroyed at a certain time is known as the IC₅₀ value. Finding the IC₅₀ is necessary to understand the pharmacological and biological characteristics of chemotherapy medicines [32].

The MTT experiment demonstrated that camel milk significantly and in-vitro cytotoxicity affected HNO-97 cells during an incubation period of 24 hours. It also successfully suppressed the proliferation of HNO-97 cells.

Those findings were compatible with those of (Krishnankutty, Iskandarani et al. 2018), who discovered that camel milk inhibited the growth of human colorectal HCT 116 and breast MCF-7 cancer cells with IC₅₀ of 31 and 51 μL/mL, correspondingly [30].

The cytological investigation, which showed that morphological apoptotic criteria increased with drug concentration, was supported by the findings of the MTT experiment [33].

Second, HNO-97 cells demonstrated a significant rise in apoptotic cells both at the microscopic and cellular levels during a 24-hour treatment period.

The HNO-97 cells lost their distinctive look at pre IC₅₀ concentration, and they showed signs of apoptosis, including chromatin condensation, cytoplasmic blebbing, and irregularities in the nuclear and cellular membranes.

The apoptotic morphological changes, including nuclear shrinkage, nuclear fragmentation, blebbing of the cell membrane, and apoptotic bodies, were more visible after incubation of HNO-97 cells with IC₅₀ concentration.

Both the necrotic and apoptotic characteristics enhanced with post IC₅₀ incubation.

The apoptotic process appears to be the method by which the different anticancer medications suppress the development of cancer cells [34].

Third, to determine NAF, the morphometric parameters such as nuclear circularity and nuclear surface area were evaluated.

NAF is regarded as a reliable indication of apoptosis as surface area and circularity are early markers of the process. Additionally, because it is predicated on two crucial morphological markers of apoptosis, it is a sensitive predictor of the effectiveness of anticancer therapy [35].

The results showed that the HNO-97 treated groups' mean NAF levels were reduced than those of the control group. In comparison to control cells (78.98), the mean NAF values for camel milk treated groups were 74.25, 50.82, and 15.59 for the treated groups respectively.

Fourth, a decline in the proportions of cells in G2/M was detected by flow cytometric analysis. Cell cycle arrest at the G2/M phase was therefore caused by camel milk. This was in agreement with (Murali, Mudgil et al. 2021), who established the link between human colorectal cancer and camel milk-induced G2/M cell cycle arrest [36].

Fifth, utilizing propidium iodide/annexin-v staining, we conducted another confirmatory experiment to validate whether camel milk induced necrosis or apoptosis. As compared to the control group, the treated groups had a dose-dependent increase in apoptotic cells.

Sixth, the results of the western blotting approach demonstrated a considerable up-regulation of the P53 and caspase-3 genes in treated HNO-97 cells as compared to the control group.

Caspase-3 is a crucial mediator of apoptosis. Caspases-3 in apoptotic cells are triggered by both internal and extrinsic causes. During apoptosis, Caspase-3 controls DNA fragmentation and chromatin condensation [37].

One crucial gene for preventing cancer is P53. It contains the genes for proteins that bind to DNA, regulate gene expression, and stop genome alterations. It can cause apoptosis by triggering the outer mitochondrial membrane's apoptotic effector proteins, BAK and BAX, which impair the membrane's integrity and release cytochrome c [38].

At last, statistical analysis demonstrated that there existed a statistically noticeable difference among the various groups. P-value is less than 0.0001. This validated the study findings that showed a positive correlation between camel milk concentrations and the decreased tendency of HNO-97 cells to survive. *P*-value < 0.0001.

CONCLUSION

On HNO-97 cells, camel milk significantly inhibits the growth of cancer cells.

REFERENCES

1. Rahman, M.A., The Role of Lysophosphatidic Acid in the Pathogenesis of Oral Squamous Cell Carcinoma. 2019, University of Malaya (Malaysia).
2. Johnson, D.E., et al., Head and neck squamous cell carcinoma. Nature reviews Disease primers, 2020. 6(1): p. 92.
3. Doghish, A.S., et al., Decoding the role of miRNAs in oral cancer pathogenesis: A focus on signaling pathways. Pathology-Research and Practice, 2023: p. 154949.
4. Faisal, M., et al., Prognostic outcomes of treatment naïve oral tongue squamous cell carcinoma (OTSCC): A comprehensive analysis of 14 years. European Archives of Oto-Rhino-Laryngology, 2021. 278: p. 3045-3053.
5. Parashar, P., et al., Unraveling molecular mechanisms of head and neck cancer. Critical reviews in oncology/hematology, 2022: p. 103778.
6. Zhang, H. and W. Han, Protein post-translational modifications in head and neck cancer. Frontiers in Oncology, 2020. 10: p. 571944.
7. Filep, J.G. and A. Ariel, Neutrophil heterogeneity and fate in inflamed tissues: implications for the resolution of inflammation. American Journal of Physiology-Cell Physiology, 2020. 319(3): p. C510-C532.
8. Koren, E. and Y. Fuchs, Modes of regulated cell death in cancer. Cancer discovery, 2021. 11(2): p. 245-265.
9. Thakur, P.C., et al., Inhibition of endoplasmic-reticulum-stress-mediated autophagy enhances the effectiveness of chemotherapeutics on pancreatic cancer. Journal of translational medicine, 2018. 16: p. 1-19.
10. Paladino, S., et al., Nrf2 pathway in age-related neurological disorders: insights into MicroRNAs. Cellular Physiology and Biochemistry, 2018. 47(5): p. 1951-1976.
11. Jaeschke, H., O.B. Adelusi, and A. Ramachandran, Ferroptosis and acetaminophen hepatotoxicity: are we going down another rabbit hole? Gene Expression, 2021. 20(3): p. 169.
12. Baust, J.G., et al., Mechanisms of tissue injury in cryosurgery. The Application of Heat in Oncology: Principles and Practice, 2023: p. 45-71.
13. Nirmala, J.G. and M. Lopus, Cell death mechanisms in eukaryotes. Cell biology and toxicology, 2020. 36 :p. 145-164.
14. Lin, S.R., et al., Natural compounds as potential adjuvants to cancer therapy: Preclinical evidence. British journal of pharmacology, 2020. 177(6): p. 1409-1423.
15. El-Aziz, A., et al., Physicochemical properties and health benefits of camel milk and its applications in dairy products: A review. Egyptian Journal of Chemistry, 2022. 65(5): p. 101-118.

16. Swelum, A.A., et al., Nutritional, antimicrobial and medicinal properties of Camel's milk: A review. *Saudi Journal of Biological Sciences*, 2021. 28(5): p. 3126-3136.
17. Awais, M.A., et al., A Review on Camel Milk and Its Medicinal Properties. *Riphah Journal of Allied Health Sciences*, 2022. 1(1).
18. Alkhulaifi, M.M., et al., Assessment of Broad-Spectrum Antimicrobial, Antibiofilm, and Anticancer Potential of Lactoferrin Extracted from Camel Milk. *Applied Biochemistry and Biotechnology*, 2023: p. 1-17.
19. Cutone, A., et al., Lactoferrin's anti-cancer properties: Safety, selectivity, and wide range of action. *Biomolecules*, 2020. 10(3): p.456 .
20. Shaban, A.M., et al., Antibacterial, Antifungal, and Anticancer Effects of Camel Milk Exosomes: An In Vitro Study. *Veterinary Sciences*, 2023. 10(2): p. 124.
21. Yang, M., et al., Development and validation of a predictive model for immune-related genes in patients with tongue squamous cell carcinoma. *Open Life Sciences*, 2022. 17(1): p. 1657-1668.
22. Manohar, S.M., P. Shah, and A. Nair, Flow cytometry: Principles, applications and recent advances. *Bioanalysis*, 2021. 13(3): p. 181-198.
23. Kumar ,M., et al., MS Western, a method of multiplexed absolute protein quantification is a practical alternative to western blotting. *Molecular & Cellular Proteomics*, 2018. 17(2): p. 384-396.
24. Büyükköroğlu, G., et al., Techniques for protein analysis, in *Omics technologies and bio-engineering*. 2018, Elsevier. p. 317-351.
25. D'souza, S. and V. Addepalli, Preventive measures in oral cancer: An overview. *Biomedicine & Pharmacotherapy*, 2018. 107: p. 72-80.
26. Rodríguez-Álvarez, M.I., et al., Prevalence and risk factors of human papillomavirus in male patients: a systematic review and meta-analysis. *International journal of environmental research and public health*, 2018. 15(10): p. 2210.
27. Nandini, D., et al., Novel therapies in the management of oral cancer: An update. *Disease-a-Month*, 2020. 66(12): p. 101036.
28. Alhadrami, G. and B. Faye, Camel. 2022.
29. Badawy, A.A., M.A. El-Magd, and S.A. AlSadrah, Therapeutic effect of camel milk and its exosomes on MCF7 cells in vitro and in vivo. *Integrative cancer therapies*, 2018. 17(4): p. 1235-1246.
30. Krishnankutty, R., et al., Anticancer activity of camel milk via induction of autophagic death in human colorectal and breast cancer cells. *Asian Pacific journal of cancer prevention: APJCP*, 2018. 19(12): p. 3501.
31. Khan, M.Z., et al., Research development on anti-microbial and antioxidant properties of camel milk and its role as an anti-cancer and anti-hepatitis agent. *Antioxidants*, 2021. 10(5): p. 788.
32. Perego, P., et al., Cellular pharmacology studies of anti-cancer agents: recommendations from the EORTC-PAMM group. *Cancer Chemotherapy and Pharmacology*, 2018. 81: p. 427-441.
33. Woo, J.S., et al., Apigenin induces apoptosis by regulating Akt and MAPK pathways in human melanoma cell A375SM. *Molecular Medicine Reports*, 2020. 22(6): p. 4877-4889.
34. Fan, T., et al., Inhibiting MT 2-TFE 3-dependent autophagy enhances melatonin-induced apoptosis in tongue squamous cell carcinoma. *Journal of pineal research*, 2018. 64(2): p. e12457.
35. Gao, J., et al., Selenium exerts protective effects against fluoride-induced apoptosis and oxidative stress and altered the expression of Bcl-2/caspase family. *Biological Trace Element Research*, 2021. 199: p. 682-692.
36. Murali, C., et al., Camel whey protein hydrolysates induced G2/M cellcycle arrest in human colorectal carcinoma. *Scientific reports*, 2021. 11(1): p. 7062.
37. Lossi, L., C. Castagna, and A. Merighi, Caspase-3 mediated cell death in the normal development of the mammalian cerebellum. *International journal of molecular sciences*, 2018. 19(12): p. 3999.
38. Rizzotto, D., L. Englmaier, and A. Villunger, At a crossroads to cancer: how p53-induced cell fate decisions secure genome integrity. *International Journal of Molecular Sciences*, 2021. 22(19): p. 10883.