ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
June 2024; 23(3):321-329.
DOI: 10.18502/ijaai.v23i3.15641

Assessment of the Anti-cancer Effects of Camel Milk Exosomes (CMEXOs) on Murine Colorectal Cancer Cell Line (CT-26)

Samira Karbasi[1], Nafiseh Erfanian[2], Hamideh Dehghan[3], Asghar Zarban[1,4], Mohammad Hasan Namaei[5], Mohammad Yahya Hanafi-Bojd[6], and Saeed Nasseri[6]

[1] Cardiovascular Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran
[2] Student Research Committee, Department of Molecular Medicine, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran
[3] Student Research Committee, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran
[4] Department of Clinical Biochemistry, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran
[5] Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran
[6] Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran

Received: 16 July 2023; Received in revised form: 10 January 2024; Accepted: 22 January 2024

ABSTRACT

Today, camel milk consumption in the Middle East is trendy because it is believed that it reduces the risk of cancer. Recently, studies have discovered that most of milk's beneficial effects are because of its nanoparticles, especially exosomes. The objective of the present research was to investigate the anti-cancer effects of camel milk exosomes (CMEXOs) in the murine colorectal cancer cell line (CT-26).

After isolation and characterization of CMEXOs, we investigated their effects on the proliferation and migration of CT-26 cells using MTT and scratch assay. Additionally, we employed real-time quantitative PCR (RT-qPCR) to analyze the expression levels of IL-6 and TNF-α genes in CT-26 cells.

Our findings verified the existence of exosomes measuring approximately 114.1±3.4 nm in diameter. Through MTT and migration assays, we established that CMEXOs exhibit dose-dependent anti-proliferative and anti-migration effects on the CT-26 cell line. Furthermore, our study showed that treatment with CMEXOs led to a reduction in *TNF-a* and *IL-6* gene expression in CT-26 cells.

While additional in vivo studies are required, our data demonstrate that CMEXOs have anti-proliferative and anti-migration effects on CT-26, possibly by influencing crucial genes within the inflammation pathway.

Keywords: Camel milk; Colorectal cancer; Exosomes; *IL-6; TNF-a*

Corresponding Author: Saeed Nasseri, PhD;
Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran. Tel: (+98 53) 3238 1569, Fax: (+98 56) 3243 8007, E-mail: saeed.nasseri922@gmail.com

The first and second authors contributed equally to this study

INTRODUCTION

Colorectal cancer (CRC) is the world's third most common cancer, caused by the growth of abnormal cells in the colon or rectum.[1] According to global statistics

from the International Agency for Research on Cancer, there were 940,000 CRC deaths and around 1.9 million new cases globally in 2020; however, more men than women are involved.[2] The development of CRC is influenced by various factors, aging, genetics, lifestyle, and environmental factors, such as obesity, insufficient exercise, smoking, and alcohol consumption.[2,3] Currently, surgery and chemotherapy are the two main treatment options for CRC; still, there are some limitations, including drug resistance and severe side effects which may lead to failure in the treatment process.[4] As a result, researchers were encouraged to search for natural bioactive molecules from different sources to develop new anti-tumor drugs to overcome these limitations. Recent pharmacological study on milk has identified several therapeutic properties, including the anti-cancer effect that could be exploited in modern medicine.

Camel milk is a valuable food source for people in hot and dry regions. It is used in different parts of the world due to its perceived ability to improve immune capacity and decrease the jeopardy of many diseases.[5] Camel milk is one of the rich sources of lactoperoxidase, lysozyme, alpha-lactalbumin, lactoferrin, immunoglobulins, and exosomes. Camel milk's anti-oxidant, anti-bacterial, anti-diabetic, anti-viral, anti-cancer, and anti-inflammatory properties might be attributed to the high contents of those biological components.[6-8] Among all types of exosomes in different milk sources, camel milk exosomes (CMEXOs) have received much awareness in the last few years owing to their diagnostic and therapeutic uses.[9,10] CMEXOs have excellent biocompatibility, low adverse immune effects, no systemic toxicity, and inflammatory responses, making them promising candidates for developing new therapeutic approaches to treating various diseases, including types of cancer.[11,12]

The anti-cancer property of CMEXOs has been described via augmentation of tumor cell apoptosis, suppression of metastasis, oxidative stress, and inflammation.[13] In this current research, we evaluate the impact of CMEXOs at various dosages on the proliferation and migration/invasion properties of murine colorectal cancer cell line (CT-26) cells and investigate the underlying cytotoxic mechanisms.

MATERIALS AND METHODS

Isolation of CMEXOs

Twenty camel milk samples were collected from healthy lactating female camels from the middle of the lactation period and stored at -80°C until use. Following the manufacturer's instructions, exosomes were isolated from camel milk using a commercial exosome isolation kit (Zantox, Birjand, Iran). Milk samples were centrifuged at 300g for 30 min at 4°C to remove fat. The supernatants were centrifuged at 2000 g for 1 h at 4°C, 12000g for 1 h, and then at 14000 g for 2 h at 4°C to pellet the vesicles; this was done to remove the remaining cell debris. The supernatant was filtered using 0.22 µm Whatman filters (Millipore, Cork, Ireland). Then 1 mL of the filtered supernatant was mixed with 200 µL of the Zantox kit regent and incubated overnight at 4°C. After centrifugation at 2000g for 45 min at 4°C, the sediment obtained was dissolved in 200 µL of phosphate-buffered saline (PBS), (Sigma-Aldrich, USA) and stored at -80ºC until the experiments were performed.

Assessment of the Characteristics of CMEXOs

Dynamic Light Scattering (DLS)

The size distribution of CMEXOs was determined using a NanoBrook 90Plus DLS instrument. The exosomes diluted in PBS were examined using a monochromatic laser beam with a detection angle of 173 degrees. The size of exosomes was calculated based on the scattering intensity distribution.[14]

Transmission Electron Microscope (TEM)

Samples were imaged with the help of a TEM to investigate the morphological and structural characteristics of CMEXOs. The samples were first fixed with 1% glutaraldehyde (Sigma-Aldrich, USA). A sample drop was placed on a carbon-coated grid to dry at room temperature. The grids were then stained with uranyl acetate for 10 min after washing twice in sterile PBS for 5 min. Finally, the images were prepared by TEM Philips EM 208S transmission electron microscope at K780 voltage.

Scanning Electron Microscope (SEM)

Fixed exosome samples were diluted with 2% paraformaldehyde in distilled water. One to five µl of each sample were placed on silicon chips, and after washing with water, it was dried with dehydrating acetone for 5 min. A SEM with Kv36 voltage was used to record the images.

Protein Quantification

The total protein content of the exosomes was determined using the bicinchoninic acid (BCA) assay.

This analysis was performed with a commercially available kit (Parstoos, Iran) following the recommended protocol provided by the manufacturer.

Cell Culture and Maintenance

The CT-26 cell line (ATCC CRL-2638) used in this study was provided by the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). The cells were cultured and maintained in a controlled environment at 37°C in a 5% CO2 atmosphere using the recommended medium from ATCC. The RPMI (Bioidea, Iran) 1640 growth medium was supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), (GIBCO Invitrogen Corporation, CA, USA) and 100 IU/mL penicillin-streptomycin (Bioidea, Iran) to support cell growth and viability.

MTT Assay

Cancer cells were plated onto 96-well microplates (1×10^4 cells per well, 100 µL per well) and incubated at 37 °C in a 5% CO2 incubator for cytotoxicity assay. After 24 h, CMEXOs were administered in RPMI 1640 medium with concentrations of 0.625-1.25-2.5-5-10 mg/ml based on the previous report.[15] After 24 h incubation at 37°C, the cells were treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), (Sigma-Aldrich, USA) (5 mg/mL) reagent for four hours. Dimethyl sulfoxide (DMSO), (Merck, Germany), (10%) was added to dissolve the blue formazan-formed crystals. Formazan dye absorbance was quantified at 570 nm with a reference wavelength of 630 nm. The experiment was repeated three times with triplicate samples. The following equation was used to calculate cell viability percentage: (sample-blank)/(control-blank) × 100%, where sample represents the absorbance of cells treated with CMEXOs; blank represents the absorbance of CMEXOs and control represents the absorbance of untreated cells. The half-maximal (50%) inhibitory concentration (IC50) was elevated using graph pad prism software after recording the absorbance at 570 -630 nm.

Scratch Assay

A scratch assay was used to evaluate CMEXO migration potential on the CT-26 cell line. Cells were planted in 12 well plates at a density of $8x10^5$ cells per well. After 24 h of treatment, a straight scratch was formed with a 200 µL tip. Microscopy was used to observe cell migration 24 h post treatment with different concentrations of CMEXOs (1.2, 1.4, and 1.8 IC_{50}). The lesion's boundary areas were assessed and photographed. Cellular migration was evaluated by measuring the ratio between the reduced open space after 24 h and the open space at 0 h with Digimizer 5.4.9 software.

Real-time Quantitative PCR (RT-qPCR)

Total RNA was isolated using a total RNA extraction kit (Parstous, Tehran, Iran). The isolated RNA was immediately used in RT-qPCR to generate first-strand complementary DNA (cDNA) (cDNA Synthesis Kit, Thermo Scientific, USA). The RT-qPCR for *TNF-α* and *IL-6* was carried out using specific primers (Supplementary Table 1). This analysis was carried out with the Quanti Tect SYBR Green PCR master mix (Amplicon, Denmark) and gene amplification was performed in the ABI Step One™ Real-Time PCR System (Applied Biosystems, Foster City, CA). β-actin gene was used to normalize the relative expression of interested genes calculated by ΔΔCT method.

Statistical Analysis

Statistical analyses were conducted utilizing SPSS software (version 18.0, SPSS Inc., USA). The applied method involved ANOVA, accompanied by Tukey's post hoc analysis for pairwise comparisons, with results presented as mean±standard deviation (SD). Minitab Statistical Software was employed to assess outcomes with significance denoted by p-values<0.05.

RESULTS

CMEXOs Characterization

The particle size analysis of the CMEXOs suspension was conducted using a NanoBrook 90Plus DLS device, as illustrated in Figure 1. The data revealed that the particles exhibited an average size of 114.1±3.4 nm (Mean±SD). To further investigate CMEXO structural integrity, we employed both TEM and SEM methodologies. The findings, presented in Figure 2A, demonstrate that CMEXOs maintained their membrane integrity and retained their characteristic spherical structure during the isolation process. Notably, the exosome diameter was approximately 114 nm, consistent with their spherical morphology, which was also corroborated through SEM imaging (Figure 2B).

CMEXOs Inhibited CT-26 Cell Line Viability

The MTT assay assessed CMEXO inhibitory effects on CT-26. Data showed that exosomes had a significant cytotoxic effect on CT-26 cells with an IC_{50} value of 125 µg/mL compared to the untreated group (Figure 3).

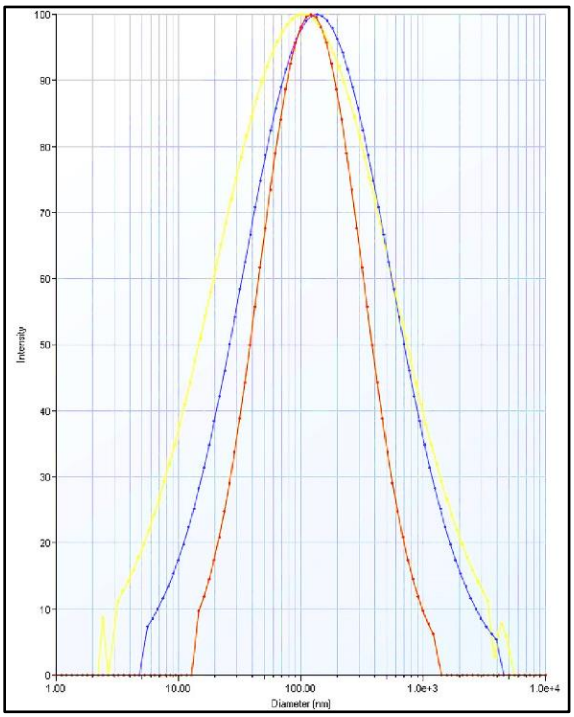

Figure 1. The DLS using a NanoBrook 90Plus DLS device measured the particle size distribution (by intensity) of camel milk exosomes (CMEXOs). Each colored peak represents one reading by the Dynamic light scattering (DLS) device.

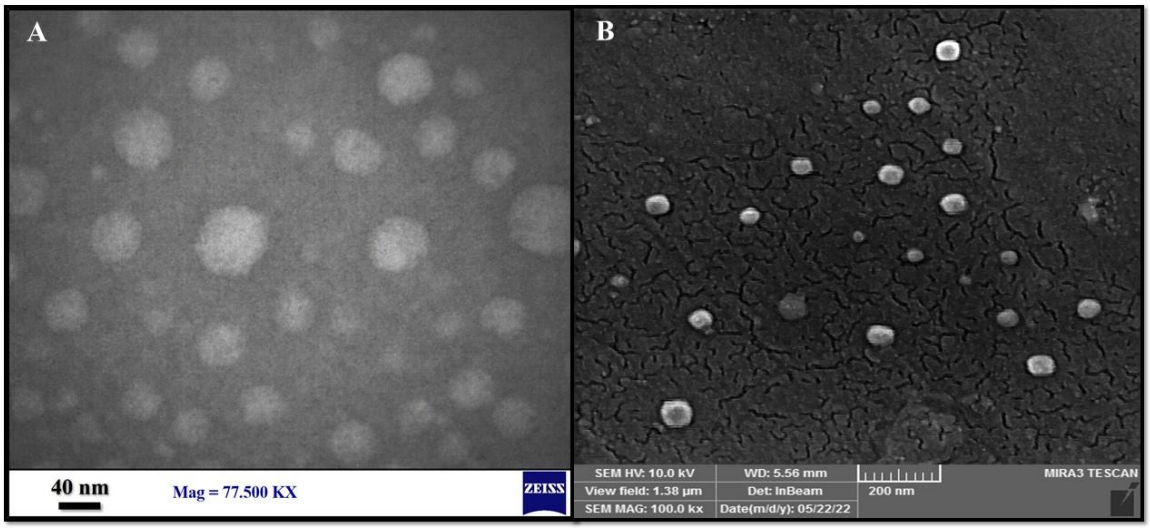

Figure 2. The microscopic images obtained via the transmission electron microscope (TEM) method are shown in A. B presents images captured using the scanning electron microscope (SEM) method. These images vividly illustrate the preservation of the membrane integrity and spherical nature of camel milk exosomes (CMEXOs).

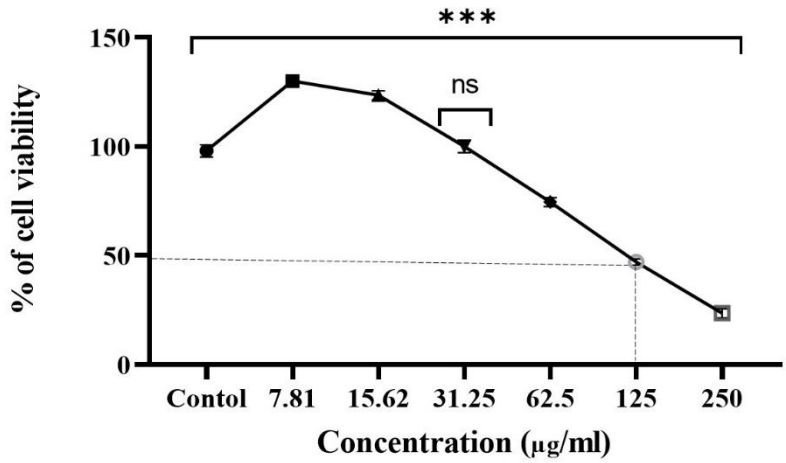

Figure 3. The MTT assay results demonstrate the pronounced impact of camel milk exosomes (CMEXOs) on reducing the viability of CT-26 cells. Notably, the MTT analysis revealed the half-maximal inhibitory concentration (IC50) value of 125 µg/mL for the exosome-treated group. (*: p<0.001).**

CMEXOs Inhibited the Migration of CT-26 Cell Line

The scratch assay was used to measure the cell migration in CT-26 cell culture plate with/without treatment with CMEXOs. Data indicated that at 1.2 IC_{50} dose, CMEXOs considerably reduced the migration of CT-26 cells after 24 h compared to the untreated control group (p<0.05) (Figure 4).

CMEXOs Reduced IL-6 and TNF-α Expression in CT-26 Cell Line

RT-qPCR data demonstrated that treatment of the CT-26 cell line with CMEXOs' effectively inhibited the elevated levels of *IL-6* and *TNF-α* genes in CT-26 cells compared to untreated cells (Figure 5).

.

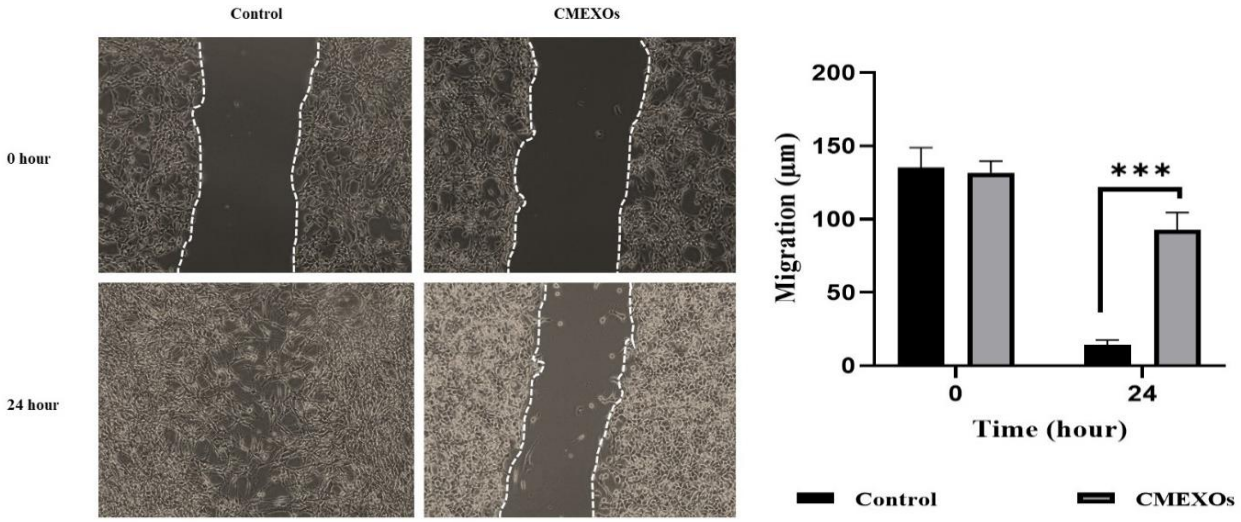

Figure 4. A scratch test was conducted to assess the impact of CMEXOs on CT-26 cell migration inhibition. Cells in the wells were treated with varying concentrations of camel milk exosomes (CMEXOs) (1.2, 1.4, and 1.8 times the half-maximal inhibitory concentration (IC50) value). Images were captured at both 0 and 24 hours, and the extent of migration at these time points was quantified. The results indicated significant reductions in cell migration at 24 hours compared to baseline (*: p<0.001).**

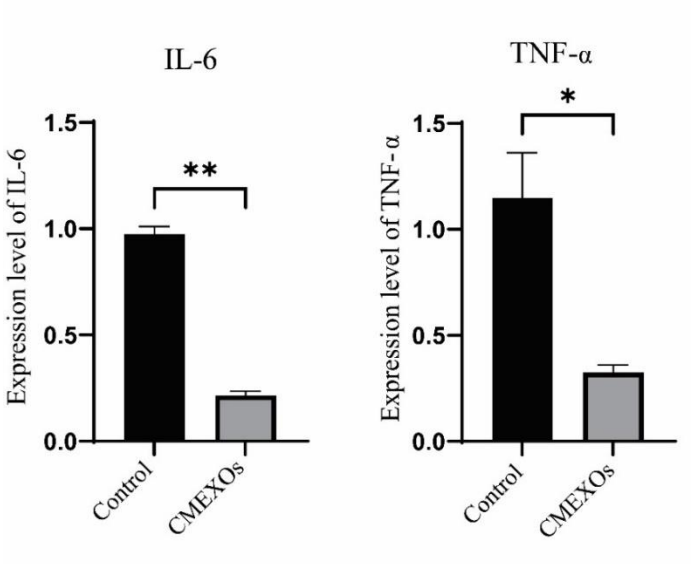

Figure 5. Gene expression levels of *IL-6* and *TNF-α* in CT-26 cell line in response to camel milk exosomes (CMEXOs) treatment. Y-axis represents the n-fold expression levels of the target gene in comparison with untreated cells (*: $p<0.05$, **: $p<0.01$).

DISCUSSION

The present study aimed to assess the anti-proliferative, anti-migratory and anti-inflammatory effects of CMEXOs on CT-26 cells. Following the successful isolation and characterization of CMEXOs, we explored their potential anti-tumor and anti-inflammatory impacts on CT-26 cell line. Our findings demonstrated that CMEXOs effectively suppressed the proliferation of CT-26 cells in a dose-dependent manner and significantly attenuated their migration after 24 h, as compared to the untreated group.

Exosomes are naturally occurring small extracellular nanovesicles, with diameters ranging from 30 to 150 nm, released into the extracellular environment through exocytosis. They originate from endosome in-budding, forming multi-vesicular bodies (MVBs) containing intra-luminal vesicles, which serve as messengers for intercellular communication. Exosomes possess the intrinsic ability to transfer various cargoes, including circulating DNA, micro-RNA, long non-coding RNA, and proteins, into their environments.[16] Exosomes have been demonstrated in various physiological fluids such as saliva, urine, and milk.

Milk contains abundant exosomes from multiple cellular sources, and studies have shown that milk exosomes can withstand strongly acidic conditions in the stomach and degradative conditions in the gut. Moreover, they can traverse biological barriers to reach the targeted tissues.[17-19] Research has highlighted the anti-tumor activities of exosomes derived from human, camel, or cow milk, stimulating a potent immune response against tumors.[20,21] CMEXOs, in particular, have been reported to inhibit inflammation, oxidative stress, metastasis, and enhance tumor cell apoptosis, making exosome-based cancer therapies, a promising solution to mitigate the side effects of current cancer treatments.[11,22] Exosomes exert their anti-cancer effects through multiple mechanisms, including the transfer of tumor-suppressive miRNAs.[23,24] delivery of tumor-suppressive proteins,[25] immune modulation,[26] induction of apoptosis,[27,28] and microenvironment modification,[29,30]

In line with our results, several in vitro studies have extracted exosomes from different natural sources and investigated their anti-cancer effects on CT-26 cells. For instance, exosomes derived from tea leaves exhibited inhibitory effects on the proliferation of various tumor cell lines, including CT-26 cells,[31] Furthermore, in vivo investigations have explored the anti-cancer properties of tumor-derived exosomes (TEXs) enriched with different miRNAs (TEXomiR) in CT-26 cell lines or CT-26 tumor-bearing mice. These studies demonstrated that TEXomiR treatment inhibited migration and tumor progression of colorectal cancer cells in vitro, and elicited a robust anti-tumor immune response in vivo in

a mouse model of CRC.[32-34] CMEXOs have been investigated for their anti-cancer and anti-migration effects on various cell lines, including breast cancer cell line (MCF-7),[11] hepatic progenitor cell line (HepaRG),[35] and pancreatic cancer cells (PANC1).[36]

Our gene expression findings indicated that CMEXOs could reduce the expression levels of IL-6 and TNF-α genes, both central pro-inflammatory cytokines in colorectal cancer. IL-6, produced by various cells, including tumor and immune cells, can stimulate the growth, invasion, and metastasis of CRC cells, contributing to the development of a pro-tumorigenic microenvironment. Elevated IL-6 levels have been observed in CRC patients' sera, particularly in metastatic cases. TNF-α, another pro-inflammatory cytokine, regulates cellular communication within the tumor microenvironment and promotes metastatic transformation. High TNF-α expression is strongly associated with tumor recurrence and lymph node metastases in CRC patients. The accumulation of inflammatory mediators like IL-6 and TNF-α leads to local and systemic immunosuppression associated with cancer progression. Notably, inflammatory factors such as IL-6 and TNF-α can down-regulate DNA repair pathways and cell cycle checkpoints, resulting in the accumulation of random genetic alterations and destabilization of the cancer cell genome. Hence, CMEXOs, by reducing IL-6 and TNF-α cytokines, have the potential to contribute to colorectal cancer treatment.[34,37-39] Previous studies have demonstrated that camel milk exosomes can mitigate the effects of cyclophosphamide on the expression levels of IL-6, TNF-α, and IFN-γ in rats.[40]

It is important to acknowledge the limitations of our study, including variations in isolation methods, the type of milk (fresh or frozen), and species differences that can affect the size and number of isolated exosomes. Future research should investigate exosomes from other milk sources. Additional in vitro and in vivo studies are necessary to assess the anti-tumor efficacy of CMEXOs on different types of CRC cells.

Our current research represents a pioneering effort to assess CMEXO inhibitory effects on the CT-26 cell line. Our findings provide compelling evidence that CMEXOs effectively suppress the proliferation, migration, and inflammatory attributes of CT-26 cells. To solidify these observations and pave the way for potential clinical applications, further in vivo and in vitro studies are imperative to corroborate the anti-cancer properties of CMEXOs against the CT-26 cell line.

STATEMENT OF ETHICS

This research study was approved by the ethics committee of the Birjand University of Medical Science (IR.BUMS.REC.1401.163).

FUNDING

This work was supported by Birjand University of Medical Science (BUMS), Iran (5893).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

We thank all the participants who collaborated in line with the research goals of this project.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J. Clin. 2019;69(1):7-34.
2. Wang H, Tian T, Zhang J. Tumor-associated macrophages (TAMs) in colorectal cancer (CRC): from mechanism to therapy and prognosis. Int J Mol Sci. 2021;22(16):8470.
3. Siskova A, Cervena K, Kral J, Hucl T, Vodicka P, Vymetalkova V. Colorectal adenomas—genetics and searching for new molecular screening biomarkers. Int J Mol Sci. 2020;21(9):3260.
4. Cacciola NA, Salzano A, D'Onofrio N, Venneri T, Cicco PD, Vinale F, et al. Buffalo milk whey activates necroptosis and apoptosis in a xenograft model of colorectal cancer. I Int J Mol Sci. 2022;23(15):8464.
5. Althobaiti NA, Raza SHA, BinMowyna MN, Aldawsari RD, Abdelnour SA, Abdel-Hamid M, et al. The potential therapeutic role of camel milk exosomes. Ann Anim Sci. 2022.
6. Khan MZ, Xiao J, Ma Y, Ma J, Liu S, Khan A, 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):788.
7. Swelum AA, El-Saadony MT, Abdo M, Ombarak RA, Hussein EO, Suliman G, et al. Nutritional, antimicrobial

and medicinal properties of Camel's milk: A review. Saudi J Biol Sci. 2021;28(5):3126-36.
8. Mostafa GA, Bjørklund G, Ayadhi L. Therapeutic effect of camel milk in children with autism: its impact on serum levels of vasoactive intestinal peptide. IJMSCI. 2021;8(10):5698-707.
9. Iigo M, Alexander DB, Long N, Xu J, Fukamachi K, Futakuchi M, et al. Anticarcinogenesis pathways activated by bovine lactoferrin in the murine small intestine. Biochimie. 2009;91(1):86-101.
10. Shariatikia M, Behbahani M, Mohabatkar H. Anticancer activity of cow, sheep, goat, mare, donkey and camel milks and their caseins and whey proteins and in silico comparison of the caseins. Mol Biol Res Commun. 2017;6(2):57.
11. Badawy AA, El-Magd MA, AlSadrah SA. Therapeutic effect of camel milk and its exosomes on MCF7 cells in vitro and in vivo. Integr. Cancer Ther. 2018;17(4):1235-46.
12. Bunggulawa EJ, Wang W, Yin T, Wang N, Durkan C, Wang Y, et al. Recent advancements in the use of exosomes as drug delivery systems. J Nanobiotechnology. 2018;16(1):1-13.
13. Abels ER, Breakefield XO. Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. Cell Mol Neurobiol. 2016;36:301-12.
14. Reif S, Elbaum Shiff Y, Golan-Gerstl R. Milk-derived exosomes (MDEs) have a different biological effect on normal fetal colon epithelial cells compared to colon tumor cells in a miRNA-dependent manner. J Transl Med. 2019;17:1-10.
15. Park H-W, Cho SH. Management of Elderly Asthma: Key Questions and Tentative Answers. Allergy Asthma Immunol Res. 2023;15(1):8.
16. Sarko DK, McKinney CE. Exosomes: origins and therapeutic potential for neurodegenerative disease. Front Neurosci. 2017;11:82.
17. Javeed N, Mukhopadhyay D. Exosomes and their role in the micro-/macro-environment: a comprehensive review. J Biomed Res. 2017;31(5):386.
18. Zempleni J, Aguilar-Lozano A, Sadri M, Sukreet S, Manca S, Wu D, et al. Biological activities of extracellular vesicles and their cargos from bovine and human milk in humans and implications for infants. J Nutr. 2017;147(1):3-10.
19. Zhong J, Xia B, Shan S, Zheng A, Zhang S, Chen J, et al. High-quality milk exosomes as oral drug delivery system. Biomater Res. 2021;277:121126.
20. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. Cancer Lett. 2016;371(1):48-61.
21. Pieters BC, Arntz OJ, Bennink MB, Broeren MG, van Caam AP, Koenders MI, et al. Commercial cow milk contains physically stable extracellular vesicles expressing immunoregulatory TGF-β. PLoS One. 2015;10(3):e0121123.
22. Nam GH, Choi Y, Kim GB, Kim S, Kim SA, Kim IS. Emerging prospects of exosomes for cancer treatment: from conventional therapy to immunotherapy. J Adv Mater. 2020;32(51):2002440.
23. Kosaka N, Iguchi H, Yoshioka Y, Hagiwara K, Takeshita F, Ochiya T. Competitive interactions of cancer cells and normal cells via secretory microRNAs. J Biol Chem. 2012;287(2):1397-405.
24. Wadhonkar K, Singh N, Heralde III FM, Parihar SP, Hirani N, Baig MS. Exosome-derived miRNAs regulate macrophage-colorectal cancer cell cross-talk during aggressive tumor development. Colorectal Cancer. 2023;12(1):CRC40.
25. Han J-H, Park D, Jung B, Yoo H-G, Cho K-O, Kim E. Exosomal delivery of Fas-associated factor 1 (FAF1), a multifunctional tumor suppressor and genuine exosome cargo, effectively antagonizes tumor growth. Cancer Res. 2023;83(7_Supplement):1728-.
26. Elsayed R, Elashiry M, Tran C, Yang T, Carroll A, Liu Y, et al. Engineered Human Dendritic Cell Exosomes as Effective Delivery System for Immune Modulation. Int J Mol Sci. 2023;24(14):11306.
27. Kheradjoo H, Nouralishahi A, Hoseinzade Firozabdi MS, Mohammadzadehsaliani S. Mesenchymal stem/stromal (MSCs)-derived exosome inhibits retinoblastoma Y-79 cell line proliferation and induces their apoptosis. Nanomed Res J. 2022;7(3):264-9.
28. Wang L, Xu P, Xie X, Hu F, Jiang L, Hu R, et al. Down regulation of SIRT2 reduced ASS induced NSCLC apoptosis through the release of autophagy components via exosomes. Front cell dev biol. 2020;8:601953.
29. Bobrie A, Krumeich S, Reyal F, Recchi C, Moita LF, Seabra MC, et al. Rab27a supports exosome-dependent and-independent mechanisms that modify the tumor microenvironment and can promote tumor progression. Cancer Res. 2012;72(19):4920-30.
30. Wang Z, Chen J-Q, Liu J-L, Tian L. Exosomes in tumor microenvironment: novel transporters and biomarkers. J Transl Med. 2016;14(1):1-9.
31. Chen Q, Zu M, Gong H, Ma Y, Sun J, Ran S, et al. Tea leaf-derived exosome-like nanotherapeutics retard breast tumor growth by pro-apoptosis and microbiota modulation. J Nanobiotechnology. 2023;21(1):1-19.

32. Rezaei R, Baghaei K, Hashemi SM, Zali MR, Ghanbarian H, Amani D. Tumor-derived exosomes enriched by miRNA-124 promote anti-tumor immune response in CT-26 tumor-bearing mice. Frontiers in med. 2021;8:619939.
33. Asadirad A, Baghaei K, Hashemi SM, Dehnavi S, Ghanbarian H, Mortaz E, et al. Dendritic cell immunotherapy with miR-155 enriched tumor-derived exosome suppressed cancer growth and induced antitumor immune responses in murine model of colorectal cancer induced by CT26 cell line. Int Immunopharmacol. 2022;104:108493.
34. Belluco C, Nitti D, Frantz M, Toppan P, Basso D, Plebani M, et al. Interleukin-6 blood level is associated with circulating carcinoembryonic antigen and prognosis in patients with colorectal cancer. Ann Surg Oncol. 2000;7:133-8.
35. El-Kattawy AM, Algezawy O, Alfaifi MY, Noseer EA, Hawsawi YM, Alzahrani OR, et al. Therapeutic potential of camel milk exosomes against HepaRG cells with potent apoptotic, anti-inflammatory, and anti-angiogenesis effects for colostrum exosomes. Biomed Pharmacother. 2021;143:112220.
36. Ali M, Elsayed GR, Salama MF, El-Magd MA. Camel milk exosomes had a selective anticancer effect on PANC1 cells and a proliferative effect on H6c7 cells. KVMJ. 2022;20(1):1-5.
37. Coskun Ö, Oztopuz O, Ozkan O. Determination of IL-6, TNF-alpha and VEGF levels in the serums of patients with colorectal cancer. CMB J. 2017;63(5).
38. Ueda T, Shimada E, Urakawa T. Serum levels of cytokines in patients with colorectal cancer: possible involvement of interleukin-6 and interleukin-8 in hematogenous metastasis. J Gastroenterol. 1994;29:423-9.
39. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. J Carcinog. 2009;30(7):1073-81.
40. Ibrahim HM, Mohammed-Geba K, Tawfic AA, El-Magd MA. Camel milk exosomes modulate cyclophosphamide-induced oxidative stress and immuno-toxicity in rats. Food Funct. 2019;10(11):7523-32.