

Summary of the DVM **Thesis** No., X Faculty of Veterinary Medicine, Urmia University

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Author: Amirhossein Emamjomeh

Title: Evaluation of Buffalo Milk-derived Exosomes on HCT-116 Cell Line

Exosomes are naturally occurring extracellular nanoparticles that mediate intercellular communication by conveying bioactive molecules like proteins, microRNAs, mRNAs, DNA, and other compounds. Exosomes have been isolated from the milk of various animals, including cows, camels, donkeys, goats, horses, sheep, pigs, mice, and humans. The purpose of this study was to isolate and characterize exosomes from buffalo milk. Since no research has been conducted on the effects of buffalo milk exosomes on HCT-116 colon cancer cells, in the second phase of this study, the effects of buffalo milk exosomes on this cell line were investigated using the MTT assay. A centrifugation and solvent extraction method was employed to isolate exosomes from buffalo milk. To achieve this, milk was collected from a healthy buffalo at the onset of lactation stage from a buffalo breeding center in the northwestern part of the country and transported to the laboratory under strictly cold conditions. To remove fat globules, cellular debris, and large milk proteins such as casein, the samples were subjected to centrifugation at 3000 g for 10 min, 9000 g for 30 min, 14000 g for 30 min, and 21500 g in two stages for 30 and 60 min. Next, the resulting sample was combined with the Exocib kit containing polyethylene glycol in a 1:5 ratio and centrifuged again after 12 hours of incubation at 4 °C to precipitate. To sterilize the sample for cellular studies, it was passed through 0.45 and 0.22 µm filters. Transmission electron microscopy (TEM) and Zetasizer were used to verify and characterize exosomes, while protein content was analyzed using the Bradford protein assay kit. TEM images revealed exosomes with round and cup-shaped morphologies. The particle distribution spectrum in this study was measured between 90 and 105 nm using a Zetasizer. The protein content of the sample was also determined to be 2.17 mg/mL. The MTT assay was employed to assess the impact of exosomes on cell viability. The findings of the MTT assay indicated that exosomes exhibited an inhibitory effect on the viability and growth of HCT-116 cells in a concentration-dependent manner. In summary, the results of this study demonstrated the potential of buffalo milk exosomes as a therapeutic agent for colon cancer.

Keywords: Exosome, Colorectal cancer, Milk, Buffalo