

## BOVINE LACTOFERRIN (B-Lf) AS CHEMO PREVENTIVE AGENT SCREENED FROM NATURAL SOURCE MILK, WHEY AND COLOSTRUMS

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### ABSTRACT

Nutrition uptake and health care are very important for mankind. In the present scenario, quality of nutrition with healthy diet is major need. By the evolutionary progress, many diseases had provoke due to many reasons. To overcome, diet management is one of the major source. In these present days, one of the major effected problems is multi development diseases cancer. Cancer is the most common cause of cancer death in women worldwide. The survival condition of cancer person is more in risk. So, in our research, our main objective is to sought and to investigate cancer control agent from natural resources in our diet. After investigating many research articles, we came to know many proteins from natural source has property to manage the cancer survival rate. In our present research, we screened bovine lactoferrin (B-Lf), a natural product which enhances chemotherapy, could improve the chemotherapeutic effects. It has been proven, administration of Lf orally is very healthy to mankind and animals. So, we focused to evaluate to screen Lf from natural source like milk, whey and colostrum of buffalo, cow and goat. These animals are having capable to produce B-Lf in adequate level as required. The results reported, conformed to prove that b-Lf is in proper adequate quantity from milk, whey and colostrums. The potent isolated natural protein B-Lf is adjuvant capable of augmenting the chemotherapeutic activity. Among milk, whey and colostrums for producing protein, colostrums is strong to produce B-Lf in high level.

**KEY WORDS:** Bovine lactoferrin, cancer therapeutic agent, milk, whey, colostrum.

### INTRODUCTION

Cancer is a genetic complex disease that is caused primarily due to environmental factors. The causing agents for cancer (carcinogens) can be present in water and food and in air, along with chemicals and sunlight to which that people are exposed. As epithelial cells cover the skin, line the alimentary tracts and respiratory that metabolize the ingested carcinogens, over 90% of cancers occur in epithelia. Management of patients with cancers is costly, but there is a daunting prospect that 70% of patients are living in countries which have only 5% global resources. Steps in the improvement of the prognosis of patients diagnosed with cancer are mostly immediately achievable due to present-day technology, financial resources and essentially related to early detection. Cancer cannot be developed overnight, instead often evolves over many years which can be detected premalignant lesions through the development of full-blown malignancy (Lingia. R Rodrigues *et al.*, 2009).

### Cancer Therapeutics

1. Chemotherapy uses medications to treat cancer. Chemotherapy medications can be given to one in

different ways. The four most common ways to give chemotherapy are by, vein (intravenously), mouth (orally), Injection into muscle (intramuscular), Injection into the central nervous system (intrathecal)

2. Use of radiation beams to kill cancer cells. Radiation is able to treat certain areas that sometimes cannot be reached with chemotherapy. Radiation can be given externally or internally and at different intensities, depending on your diagnosis.
3. Immune system fights disease, including cancer. This can be done in different ways: for example, stimulating your own immune system to work harder or smarter, or giving your immune system components, such as man-made immune system proteins (Chessum N *et al.*, 2005).

### Novel advances in cancer therapeutics

Significant advances have been made in cancer therapy during the last decade as our understanding of molecular biology and carcinogenesis has evolved. Most traditional cancer drugs directly interfere with mitosis DNA synthesis, and repair systems. A new class of agents

induces tumor growth retardation (cytostasis) and apoptosis by exploiting aberrant tumor stroma, tumor vasculature, and cellular signaling mechanisms (Ashim K. Mitra *et al.*, 2015).

Many natural or synthetic cationic peptides have been reported to show anticancer activity with characteristics including the ability to kill target cells rapidly the broad spectrum of activity, and the specificity for cancer cells. Compared with the traditional cancer treatments (Nimjee SM *et al.*, 2005).

### Natural Peptides

#### Bovine lactoferricin B6

Several mucosal pathogenic bacteria are capable of entering into nonprofessional phagocytes, such as epithelial cells. Inside the host cells, they are immediately localized within the endosome where some bacteria are killed others are capable of surviving, and some others escape from the vesicles and multiply in the cytoplasm (Elizabeth Di Russo Case and James E. Samuel. 2016). Generally, invasive bacteria adhere to the host cells by specific molecules called adhesins that, in some cases, can also mediate the entry into host cells. Lactoferrin (Lf) is an iron-binding glycoprotein on the surface of professional phagocytes. Bacterial invasion of nonprofessional phagocytes is an active event requiring viable bacteria and cells, and this phenomenon ensures the bacteria a protective niche to replicate, persist, and avoid the host defenses. Normally bovine milk contains low concentrations of Lf approximately 0.1 mg/ml or less, but in dry udder secretion Lf concentration is marked high and can reach a level of 20 mg/ml or high. Bovine lactoferricin (bLf) has been associated with immune boosting and anti-carcinogenic properties. Increase in interferon (IFN- $\gamma$ ), caspase -1 and interleukin-18 (IL-18) as well as increase in the level of immune cells in various mice models has been shown by purified bLf- $\gamma$  (T Kutila *et al.*, 2003). bLf is approved by the Therapeutic Goods Administration (Australia), Food and Drug Administration (USA) and the European Food Safety Authority for use in food, sports medicine and nutritional products. Squamous and mammary gland cells and also reported to induce apoptosis in lung, stomach. Native bLf (15-20% iron saturated) has been shown to inhibit growth of colon, lung, bladder, colon and breast cancers. Specifically, apoptosis was reported in T47D and HS578D breast cancer cell line with native bLf (Jessica A *et al.*, 2005). Studies have also shown that native bLf significantly decreased the levels of intrinsic anti-apoptotic protein Bcl-2 in stomach cancer cells. Studies have shown that Fe-bLf can restore red and white blood cells following chemotherapy and increasing the sensitivity of the tumor to drugs used in chemotherapy. Fe-bLf when encapsulated in ceramic Nano carriers has also been proved to be very effective. Complete inhibition of tumor regrowth in xenograft of colon cancer in mice with both prevention and treatment models with tumor rejection and regression is observed when mice fed orally (Perthor Straten and Mads Hald Andersen, 2010). In addition, pre-clinical animal and human

clinical trials reported the high safety profile and anti-oxidant effect in colon cancer cell BLF shown by iron-free (Apo-bLf) and selenium saturated bLf (Se-bLf). Apo-bLf is thought to have enhanced anti-cancer properties through its capacity to bind to free Fe<sup>3+</sup>, which acts as an iron chelating agent. This could be of particular importance in breast cancer in relation to iron metabolism. In breast cancer cells abnormalities in iron metabolism is associated with chemo resistance. Iron is essential for a rapid growth rate and for many cellular processes. For cell growth, proliferation and angiogenesis high iron level are essential in tumor micro environment (Jessica A *et al.*, 2005).

### METHODOLOGY

#### Anticancer Peptides from natural sources

Extraction & Purification of Bovine lactoferricin is done following the references. (Ramisa Sharbafi and Alireza Rafiei., 2014, Zainab H *et al.*, 2015) Isolation & Purification of lactoferrin from various sources [bovine colostrums, whey, bovine milk].

#### Lactoferrin Extraction Protocol

Colostrum of cows is procured from local dairy farm. Cream is separated by centrifugation (10000  $\times$ g, 20 min at 4 $^{\circ}$  C). Casein removed from skim milk in acidic condition using 2N HCl incubated at 40 $^{\circ}$  C for 30 min. The acid whey is neutralized to pH 6.8 with 2N NaOH. Some extra proteins precipitated using ammonium sulfate in two steps and after centrifugation in 10000 $\times$ g, 30 min at 4 $^{\circ}$ C. The precipitate is dissolved in 20 mM phosphate buffer then dia filtration finally, the protein powder obtained by freeze drying. Lactoferrin has a cationic nature according to its amino acids composition thus it can be purified by cation exchange chromatography.

Lactoferrin was purified by carboxymethyl Sephadex-C50 chromatography using 0.2 M phosphate buffer (pH7.7) and linear gradient NaCl from 0.0 to 0.5 M.

During chromatography, protein in the eluents was monitored by ultraviolet absorption at 280 nm. Protein Analysis using Biuret Method. Extraction of lactoferricin from lactoferrin.

#### Pepsin Digestion of lactoferrin

Lactoferrin is dissolved in distilled water at a concentration of 5% (w/v). pH was adjusted to 2.5 (aspartic proteinase) or 7.0 (neutral proteinase). Hydrolysis was performed using pepsin concentration of 3% (w/w of substrate) at 37C for 4h. The reaction is terminated by heating at 80C for 15min. Hydrolysates were adjusted at pH 7.0, Precipitate formed was removed by centrifugation or filtration, and soluble material was freeze-dried.

**RESULTS**

Lactoferrin is a natural peptide isolated from different natural source buffalo, cow, and goat. Different protein

concentrations has been collected from colostrums, milk and whey of each and every nature sources like buffalo, cow and goat were tabulated below with different source.

**Table 1: Table represents the amount of protein after lactoferrin extractions.**

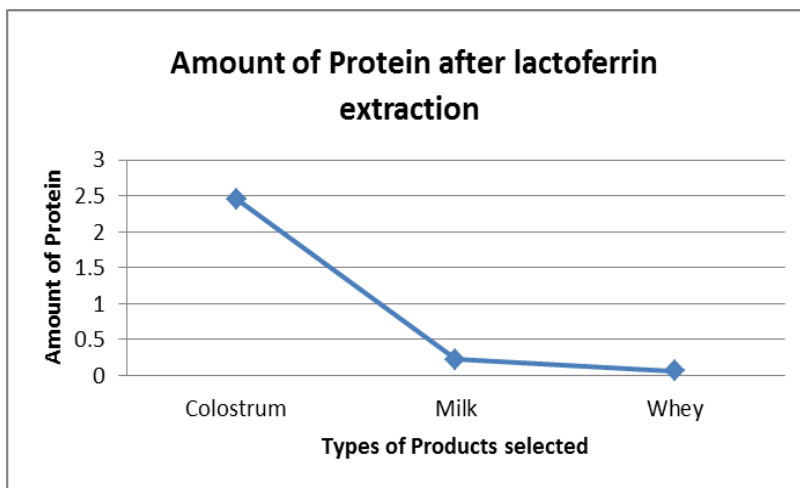
AOP = Amount of Protein after lactoferrin extraction			
Animal Type	Source	Batch No	AOP mg/ml
Buffalo	Colostrum	BCL1	2.58
		BCL2	2.39
		BCL3	2.41
		Av	2.46
	Milk	BML1	0.29
		BML2	0.21
		BML3	0.18
		Av	0.22666667
	Whey	BWL1	0.08
		BWL2	0.05
		BWL3	0.07
		Av	0.06666667
Cow	Colostrum	CCL1	2.34
		CCL2	2.18
		CCL3	1.78
		Av	2.1
	Milk	CML1	0.32
		CML2	0.35
		CML3	0.29
		Av	0.32
	Whey	CWL1	0.06
		CWL2	0.05
		CWL3	0.06
		Av	0.05666667
Goat	Colostrum	GCL1	2.14
		GCL2	2.28
		GCL3	2.79
		Av	2.40333333
	Milk	GML1	0.62
		GML2	0.53
		GML3	0.64
		Av	0.59666667
	Whey	GWL1	0.04
		GWL2	0.03
		GWL3	0.06
		Av	0.04333333

Different concentrations of AOP had estimated after protein after lactoferrin extractions. AOP has estimated in buffalo, cow and goat using milk, whey and colostrums. It has reported, average amount of AOP in

buffalo is 2.46 (colostrums), 0.22 (milk) and 0.66 (whey), cow had 2.1 (colostrums), 0.32 (milk) and 0.05 (whey) and in goat AOP 2.40 (colostrums), 0.59 (milk) and 0.43 (whey).

**Table 2: Table represents the concentration of protein from buffalo.**

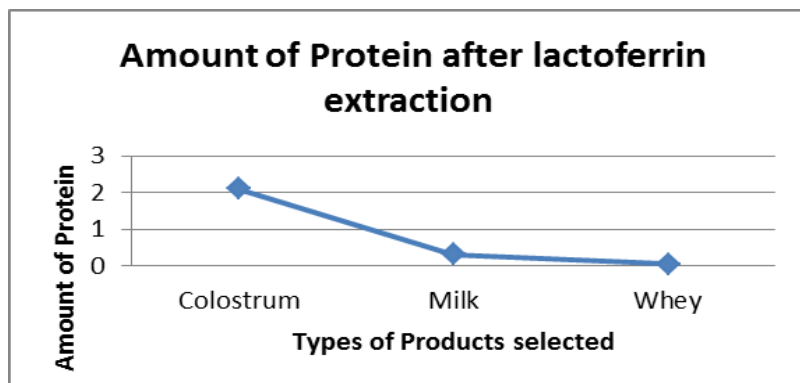
Buffalo	Type of Product	Colostrum	Milk	Whey
	Amount of Protein	2.46	0.226	0.066



Graph 1: Graph represents the lactoferrin exaction from buffalo.

Table 3: Table represents the concentration of protein from cow.

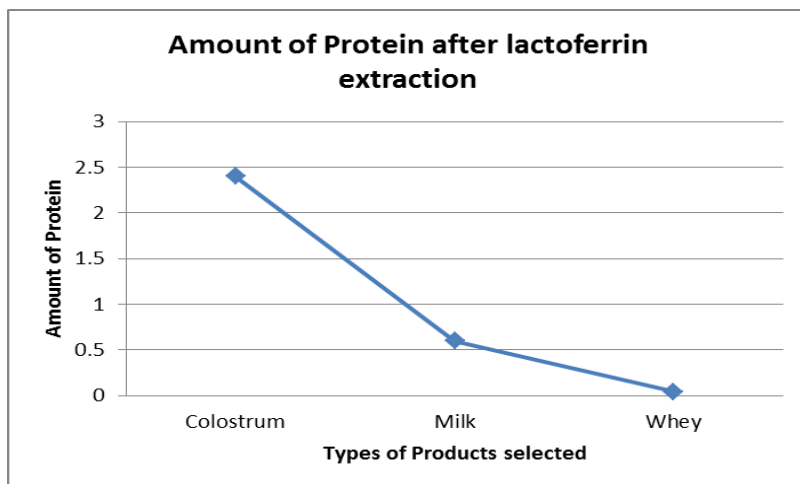
Cow	Type of Product	Colostrum	Milk	Whey
	Amount of Protein	2.1	0.32	0.056



Graph 2: Graph represents the lactoferrin exaction from cow.

Table 4: Table represents the concentration of protein from goat.

Goat	Type of Product	Colostrum	Milk	Whey
	Amount of Protein	2.4	0.596	0.043



Graph 3: Graph represents the lactoferrin exaction from goat.

## CONCLUSION

The research work was analysed to screen bovine lactoferrin from natural sources, include buffalo, cow and goat using milk, whey and colostrums. The AOP concentrations had been analysed and reported to have good level AOP in colostrums of buffalo, goat and cow compared to milk and whey. The results reported to have AOP in buffalo is 2.46 (colostrums), cow had 2.1 (colostrums) and in goat AOP 2.40 (colostrums).

In this context, anticancer peptides have been proved to be a resourceful strategy for the molecularly targeted cancer drug discovery and development process. Small molecules with an efficient tissue penetration and uptake by the heterogeneous cancer cells, endowed with intrinsic activity or synergizing with existing therapeutics, are expected to result in improved anticancer drugs with higher selectivity for neoplastic cells and reduced harmful effects over healthy tissues.

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