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Effect of Malabari goat lactoferrin on tumour growth kinetics in experimentally induced tumours in mice#

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Abstract

Lactoferrin, a minor whey protein, possesses a multitude of biological functions including antimicrobial, immunomodulatory and antitumour activities. The present study focuses on the antitumour activity of lactoferrin isolated from Malabari goats. Lactoferrin was isolated from Malabari goat colostrum by Cation exchange chromatography. The purity of the isolated protein was confirmed by SDS-PAGE and its identity was ascertained by Western Blotting. Solid tumours were induced in hind limbs of Swiss Albino mice using Daltons lymphoma ascites cells. Animals which developed the tumours were divided into groups and administered different doses of Malabari goat lactoferrin (MgLf) intratumourally as a single dose. The tumour positive animals were sacrificed on day 10 post treatment to study the effects of MgLf on tumour growth kinetics by assessing the tumour weight: body weight ratio and tumour volume inhibition of the slaughtered animals. It was found that MgLf @150µg significantly lowered the tumour weight: body weight ratio and tumour volume of the treated animals.

Keywords: *Lactoferrin, Malabari goat, Daltons ascites lymphoma, tumour weight: body weight ratio*

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Lactoferrin (Lf), a minor whey protein, is an iron-binding glycoprotein belonging to the family of transferrin. It has been attributed with antimicrobial, immunomodulatory, antiparasitic, anti-cancer and wound healing activities. Goat milk oligosaccharides and glycosylation pattern of goat Lf have been found to be very much similar to that of humans (Le Parc *et al.,* 2014). Due to its high digestibility, higher concentration of minerals like calcium and magnesium and immunological properties, goat milk is preferred as an alternative for infants and convalescents as a balanced nutrition. Among the established indigenous goat breeds of the country, Malabari breed of Kerala is known for good production performance and adaptability to hot humid conditions prevalent in the state. The antimicrobial potential of Lf of native goat breeds of Kerala *viz.* Attappady Black and Malabari was found to be significantly superior to that of crossbred goats (Vijayan *et al*., 2017). This study aims to furnish insights to the anticancer activity of Malabari goat Lf (MgLf) with respect to tumour growth kinetics.

Materials and methods

Isolation and purification of MgLf

Colostrum samples collected from newly kidded Malabari goats maintained at University Goat and Sheep Farm, Mannuthy, Thrissur, Kerala were pooled together and further processed to remove fat and casein (Chandran *et al.*, 2020). The whey obtained was subjected to ammonium sulphate precipitation to remove globulins. The remaining fraction containing proteins including Lf was separated out, dialysed against equilibration buffer and then loaded on to CM- Sephadex C-50 cation exchange chromatography column. The bound proteins were eluted with a step gradient of 0.4, 0.6 and 0.8 *M* NaCl. The eluted fractions that displayed high absorbance at 280 nm were pooled and analysed by 12 per cent SDS-PAGE to analyse the molecular weight of their components along with the standard (commercially available bovine Lf) and wide range protein marker. The identity of the isolated protein was finally confirmed as MgLf by Western blotting. The samples confirmed as lactoferrin were dialysed against several changes of distilled water, frozen using -120°C cold trap and then subjected to lyophilisation. The lyophilised samples were vacuum sealed and stored at -80°C till further use.

Solid tumour induction in mice and treatment with MgLf

Sixty healthy Swiss albino male mice aged between 4-6 weeks old were procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy. The animals were maintained in stress free condition adhering to the protocols of the CPCSEA and were allowed to acclimatize the environment for a period of two weeks before the start of the experiment. Daltons lymphoma ascites (DLA) cells were procured from Amala Cancer Research Centre, Thrissur. The cells were maintained continuously in ascitic fluid of stock adult mice by serial transplantation intraperitoneally @ 5 x 10^5 cells/ mouse as per the protocol followed by Thummar *et al*. (2016). For solid tumour induction, tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with phosphate-buffered saline (PBS) and viable DLA cells (1 x 10^6 cells/ mouse) suspended in 0.1 mL of PBS were injected subcutaneously into the right hind limb of Swiss albino mice. After 10 days, tumour development was assessed by determining tumour volume. Thirty tumour positive adult animals having a tumour mass size of 1 cm^3 or above were selected for the experiment and were randomly divided into five groups comprising of six animals each. The treatment was administered as follows:

Group	Treatment
I	Animals without any treatment
II	Animals treated with PBS intratumourally
III	Animals treated with 50µg MgLf as a single dose intratumourally
IV	Animals treated with 100µg MgLf as a single dose intratumourally
V	Animals treated with 150µg MgLf as a single dose intratumourally

Tumour mass development was assessed by calculating relative tumour volume and tumour volume inhibition. The volume of tumours was measured using vernier caliper on

the 3rd, 6th and 9th day post treatment. Tumour volume was calculated without including the height of the mass, so adjustment for the height errors was done by adopting the mathematical derivation given by Feldman *et al*. (2009). Further, relative tumour volume (RTV) and tumour volume inhibition (TVI) was calculated (Wei *et al.,* 2015). On the 10th day post treatment all the animals were weighed, humanely sacrificed and their tumour tissue excised to assess the tumour weight to body weight ratio.

The data generated from different parameters of the study were subjected to one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test. Analysis was performed by using SPSS software version 26.0. The differences among the groups were considered significant when P<0.05.

Results and discussion

Four eluted fractions *i.e.,* fraction numbers 45-49 eluted with 0.6M NaCl possessed highest OD_{280} values and were seen a single peak in the elution profile of the samples. These fractions were further used for confirmation of protein by SDS-PAGE. The presence of Lf in the eluted fractions was verified in terms of its molecular weight by 12 per cent SDS-PAGE. All the four fractions depicted a single band in the electrophoretogram at the same position as that of bovine Lf standard, the molecular weight of which was estimated as approximately 80kDa. Final confirmation of the eluted protein fractions as MgLf was done by Western blotting wherein prominent thick brown bands corresponding to the same position as that of standard could be observed.

The *in vivo* anticancer activity of MgLf was investigated in experimentally induced DLA solid tumour bearing mice. Relative tumour volume was measured to assess the progression or regression of tumours on days 0, 3, 6 and 9 of the trial (Table 1). Groups I (control) and II (vehicle control) showed up a significant increase in their tumour volume as days progressed (P<0.01) when compared to the treatment groups especially on the 6th and 9th day post treatment.

Means bearing different superscripts within a column differ significantly

Among the three treatment groups of mice, the relative tumour volume of Group V (mgLf- 150 µg) on 6th day had almost decreased to half when compared to its volume on the 3rd day post treatment and continued showing the same trend till the 9th day thereby yielding the maximum significant difference (P<0.01) from

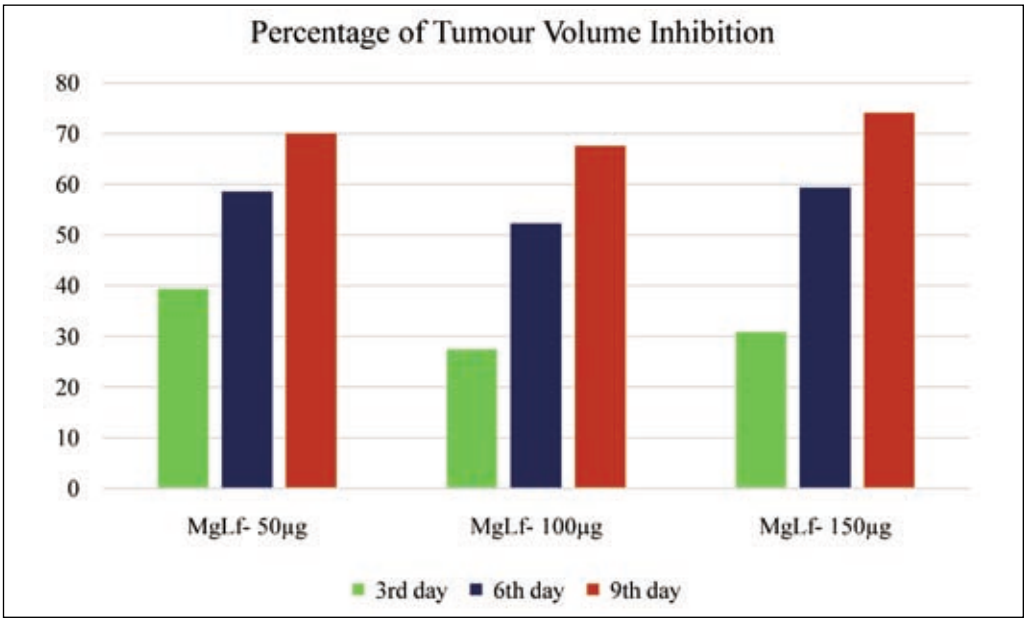

Fig. 1. Percentage tumour volume inhibition of different treatment groups of tumour induced mice

Table 1. Relative tumour volume of different groups of tumour induced mice

Experimental groups	Relative tumour volume (mm)-3rd day Mean ±S.E	Relative tumour volume (mm)-6th day Mean ±S.E	Relative tumour volume (mm)-9th day Mean ±S.E
Normal tumour control	1.20 ± 0.18^{ab}	1.59 ± 0.09^{a}	1.73 ± 0.19^{a}
P.B.S control	1.50 ± 0.15^{a}	1.69 ± 0.14^{a}	2.08 ± 0.25^{a}
MgLf- 50µg	0.91 ± 0.18^{b}	0.70 ± 0.08^{b}	0.62 ± 0.08^{a}
MgLf- 100µg	1.08 ± 0.09^{b}	0.81 ± 0.07^{b}	0.67 ± 0.05^{b}
MgLf- 150µg	1.03 ± 0.069^{b}	0.70 ± 0.09^{b}	0.53 ± 0.06^{b}

Table 2. Tumour weight: body weight ratio in different groups of tumour induced mice

Experimental groups	Tumour weight on 10th day post treatment	Tumour weight: body weight
Normal tumour control	1.51 ± 0.21^{a}	0.058 ± 0.009^{a}
P.B.S control	1.89 ± 0.90^{a}	0.068 ± 0.029^{a}
MgLf- 50µg	1.13 ± 0.33^{a}	0.039 ± 0.012^{a}
MgLf- 100µg	0.78 ± 0.062^{b}	0.031 ± 0.001^{a}
MgLf- 150µg	0.73 ± 0.05^{b}	0.022 ± 0.017^{a}

Means bearing different superscripts within a column differ significantly

Groups I and II. Group IV failed to achieve the same uniform tumour volume decrement as Group V but exhibited moderate difference ($P<0.01$) on the 6th and 9th day post treatment from Groups I and II. The amount of average tumour volume inhibition (TVI) was highest for Group V with 45 per cent and 67 per cent inhibition on 6th and 9th day respectively than the rest of the groups. Groups III and IV showed inhibition of 35 and 44 per cent respectively on 6th day whereas 59 and 62 per cent inhibition respectively was depicted on 9th day after treatment (Fig. 1).

Among the different groups, Group V showed a significantly low tumour weight: body weight ratio than the Groups III and IV that expressed almost similar values (Table 2).

The results of our study were in partial accordance with the first *in vivo* antitumour study on Lf by Bezault *et al*. (1994) in solid tumours of mice using intraperitoneally administered human Lf @ 100µg for consecutive three days. Optimal oral dose of 30–100 mg/kg of bovine Lf in BALB/c mice halted the spread and tumour growth of subcutaneously implanted highly metastatic colon carcinoma 26 (Iigo *et al*., 1999). Wolf *et al*. (2003) depicted that intratumoural injection of 250µg of Lf for a period of 2, 3 or 4 days remarkably had a significant tumour volume reduction effect of 50 per cent ($p < 0.03$) and 54 per cent ($p < 0.01$) respectively on both malignant head and neck squamous cell carcinoma as well as fibrosarcoma in immunodeficient and immunocompetent mice when compared to control group. Shimamura *et al*. (2004) depicted that intraperitoneally injected bovine Lf mediated IL-18 production by macrophages localized in the tumour significantly suppressed angiogenesis ($p<0.05$) of Lewis lung carcinoma @ 100 mg/kg whereas orally administered bLF achieved inhibition at dose of 600 mg/kg. Administration of Lf produced 62-75 per cent tumour inhibition of orthotopic tumours and 67-70 per cent inhibition in flank tumours (Wolf *et al*., 2007). These findings were almost compatible with the results of our work.

Conclusion

Malabari goat lactoferrin possesses significant antineoplastic activity in terms of reduction in tumour volume and tumour weight: body weight ratio of Dalton's ascites lymphoma induced solid tumours in mice. This emphasises the prospects of utilising the untapped potentials of lactoferrin in cancer therapy. Future studies on this multifaceted protein on different types of tumours are required to validate and accurately determine the correct dose rate and route of administration to utilise it as an anticancer agent.

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Conflicts of Interest

There were no conflicts of interest reported by the author (s).

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