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MTT BIO ASSAY OF LACTOFERRICIN AND TACHYPLESIN ON THREE CANCER CELL LINES

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ABSTRACT In the advanced Research investigation, anti-cancer agents are an exciting and important part of medicinal chemistry due to its prevalence in the population. In our present study, Tachyplesin and lactoferrin are antimicrobial peptides isolated from cow, buffalo and sheep has been shown to kill cancer cells. So we focused to evaluate the peptide as an antibiotic as preliminary work to provoke and further analyses by testing the effects against cancer cells. In the study, before working on cancer lines, preliminary testing has done by MTT assay. An MTT assay was characterized to count percent of cell viability with relation to increasing concentrations of peptides. IC50 of lactoferricin towards HTB9, MFC-7 and PC-3cell lines were calculated at 784.1ug/ml, 697.1 µg/ml and841.2 µg/ml concentrations of anticancer peptide respectively. IC50 was calculated for the activity of Tachyplesin on all cell lines and recorded to be 452.9 µg/ml, 203.4 µg/ml and 281.0 µg/ml for HTB9, MFC-7 and PC-3 cell lines respectively. Results proved and gave assurance to have viability control effects itself.

KEYWORDS : Lactoferrin, Tachyplesin, cancer cell lines -breast cancer cells (MFC-7), Prostate cancer cells (PC-3) and Renal cancer cells (HTB9).

INTRODUCTION:

Lactoferrin was first isolated by Sorensen and Sorensen from bovine milk in 1939 [Sorensen M., 1939]. The protein is naturally present in large amounts in milk colostrum. Lactoferrin have many biological functions like antimicrobial, antioxidant, antitumor, antitumor, and immunomodulatory activity [Gonzalez-Chavez SA.,2009]. The iron content in lactoferricin have ability to iron bound even at low pH is important, it shows effect at infection and inflammation site. Lactoferrin is good to inhibit various tumor cell lines growth by its oxidative stress, such as in the case of leukemia cell lines [YooYC ., 1997].

Lactoferricin is a protein molecule has been isolated from cow, buffalo, goat and m comparative studies have been done to differentiate between the various origins [Magnuson JS., 1990]. Even the tachyplesin isolated from different soure, they differ in their structural domains, which made different from one species to another. Many studies have used the bovine and human lactoferrins, but no studies have been done using buffalo lactoferrin. Very less investigation was done in bovine lactoferrin is done till now in buffullo. Thus made to study further the mechanism of targeting the protein in parasitic desieasies and its mode of action. [NamrataAnand., 2015]. So in our present study we determined to evaluate it further.

Tachyplesin 1 has been reported to have potential anticancer activity and was first identified in leukocytes [Hong Ding., 2014]. Tachyplesin I is having disulfide-stabilized β-hairpin antimicrobial peptide with 17 residues have been isolated from hemocytes of the horseshoe crab (*Tachypleustridentatus*). This peptide can inhibits the growth of Gram-negative and Gram-positive bacteria at particularly low concentrations [Doherty T., 2008]. A synthetic peptide from tachyplesin I has been determined to decrease the viability of tumor cells *in vitro* and *in vivo* following linkage to the integrin homing arginine-glycine-aspartic acid (RGD) domain [Chen Y ., 2001]. Several literature articles reported that tachylesin inhibits the proliferation of tumor cells, human hepatocarcinoma, prostate cancer and melanoma, including gastric adenocarcinoma [Li QF ., 2003]. Thus , tachyplesin I affects the viability of tumor cells. These findings encouraged us to find out that tachyplesin can be used to as an anti- cancer agent by its viability test.

MATERIALS AND METHODS:
MTT assay:

Metabolic activity can be evaluated by measuring the activity of a mitochondrial enzyme succinate dehydrogenase using MTT test. MTT is designed for the quantification of cytotoxic index in cell population using 96 well plate formats. This test is widely used in the in vitro evaluation of the cytotoxic potency of drugs. In the present study we applied the MTT test to evaluate the potency of lactoferricin extracted from bovine colostrum on breast cancer cells (MFC-7), Prostate cancer cells (PC-3) and Renal cancer cells (HTB9), After growth of the cells up to 70% confluence, different concentrations of LFBC from 100 to

1000 were considered for treatment of the cells. Cellviability is monitored for every 24 hrs for 3 days.

RESULTS AND DISCUSSION:

Lactoferricin B6 and tachyplesin were analysed for MTT assay as preliminary test to prove, as these peptides are having strong anti cancer activity.
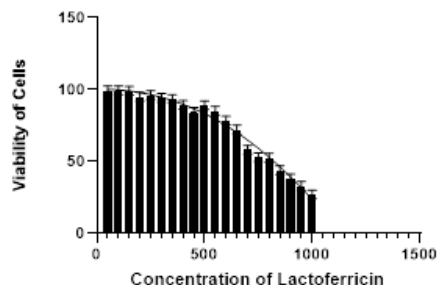
Table 1: table represents Cell Viability test by MTT assay of Lactoferricin from bovine colostrum on Renal cancer cell line (HTB9)

		24 Hours	48 Hours	72 Hours	Percentage Cell Viability		
					24 Hours	48 Hours	72 Hours
Control	PCM	0.973	0.982	1.121			
LFCB6K1	S1D1	0.998	0.936	1.128	99.445	99.410	99.583
	S1D2	0.919	1.005	1.127			
	S1D3	0.986	0.987	1.094			
	Average	0.96759985	0.976	1.116			
		0.968	0.976	1.116			
LFCB6K2	S2D1	0.993	0.969	1.128	99.54	97.23	99.112
	S2D2	0.923	0.978	1.139			
	S2D3	0.989	0.917	1.066			
	Average	0.969	0.955	1.111			
		0.969	0.955	1.111			
LFCB6K3	S3D1	0.897	0.988	1.135	99.13	98.52	97.94
	S3D2	0.993	0.998	1.027			
	S3D3	1.003	0.916	1.132			
	Average	0.965	0.967	1.098			
		0.965	0.967	1.098			
LFCB6K4	S1D1	0.955	0.921	1.087	95.159	97.810	96.001
	S1D2	0.879	0.989	1.087			
	S1D3	0.943	0.971	1.055			
	Average	0.92589707	0.960	1.076			
		0.926	0.960	1.076			
LFCB6K5	S2D1	0.959	0.958	1.059	96.131	96.114	93.107
	S2D2	0.892	0.967	1.070			
	S2D3	0.955	0.907	1.001			
	Average	0.935	0.944	1.044			
		0.935	0.944	1.044			
LFCB6K6	S3D1	0.853	0.950	1.073	94.292	94.737	92.598
	S3D2	0.945	0.960	0.971			
	S3D3	0.954	0.881	1.070			
	Average	0.917	0.930	1.038			

		24 Hours	48 Hours	72 Hours			
		0.917	0.930	1.038			
LFCB6K7	S1D1	0.938	0.850	1.019	93.453	90.218	89.992
	S1D2	0.863	0.912	1.018			
	S1D3	0.926	0.896	0.989			
	Average	0.90929769	0.886	1.009			
		0.909	0.886	1.009			
LFCB6K8	S2D1	0.891	0.857	1.000	89.342	85.99	87.913
	S2D2	0.829	0.865	1.011			
	S2D3	0.888	0.811	0.945			
	Average	0.869	0.844	0.986			
		0.869	0.844	0.986			
LFCB6K9	S3D1	0.765	0.804	0.968	84.52	80.133	83.501
	S3D2	0.847	0.812	0.875			
	S3D3	0.855	0.745	0.965			
	Average	0.822	0.787	0.936			
		0.822	0.787	0.936			
LFCB6K10	S1D1	0.896	0.791	0.907	89.231	83.993	80.124
	S1D2	0.824	0.849	0.907			
	S1D3	0.884	0.834	0.880			
	Average	0.86821763	0.825	0.898			
		0.868	0.825	0.898			
LFCB6K11	S2D1	0.851	0.821	0.895	85.321	82.433	78.691
	S2D2	0.791	0.829	0.905			
	S2D3	0.848	0.778	0.846			
	Average	0.830	0.809	0.882			
		0.830	0.809	0.882			
LFCB6K12	S3D1	0.710	0.715	0.764	78.431	71.329	65.933
	S3D2	0.786	0.723	0.691			
	S3D3	0.794	0.663	0.762			
	Average	0.763	0.700	0.739			
		0.763	0.700	0.739			
LFCB6K13	S1D1	0.725	0.623	0.696	72.234	66.135	61.456
	S1D2	0.667	0.669	0.696			
	S1D3	0.716	0.657	0.675			
	Average	0.70283682	0.649	0.689			
		0.703	0.649	0.689			
LFCB6K14	S2D1	0.581	0.552	0.641	58.239	55.392	56.349
	S2D2	0.540	0.557	0.648			
	S2D3	0.579	0.523	0.606			
	Average	0.567	0.544	0.632			
		0.567	0.544	0.632			
LFCB6K15	S3D1	0.479	0.484	0.520	52.941	48.23	44.88
	S3D2	0.531	0.489	0.470			
	S3D3	0.536	0.448	0.519			
	Average	0.515	0.474	0.503			
		0.515	0.474	0.503			
LFCB6K16	S1D1	0.528	0.464	0.477	52.561	49.239	42.150
	S1D2	0.486	0.498	0.477			
	S1D3	0.521	0.489	0.463			
	Average	0.51141853	0.484	0.473			
		0.511	0.484	0.473			
LFCB6K17	S2D1	0.438	0.408	0.412	43.89	40.95	36.19
	S2D2	0.407	0.412	0.416			
	S2D3	0.436	0.386	0.389			
	Average	0.427	0.402	0.406			
		0.427	0.402	0.406			
LFCB6K18	S3D1	0.346	0.360	0.412	38.23	35.94	35.58
	S3D2	0.383	0.364	0.373			
	S3D3	0.387	0.334	0.411			
	Average	0.372	0.353	0.399			
		0.372	0.353	0.399			
LFCB6K19	S1D1	0.330	0.316	0.335	32.910	33.581	29.540
	S1D2	0.304	0.340	0.334			
	S1D3	0.326	0.333	0.325			
	Average	0.3202143	0.330	0.331			
		0.320	0.330	0.331			

LFCB6K20	S2D1	0.269	0.229	0.200	26.93	23.013	17.594
	S2D2	0.250	0.232	0.202			
	S2D3	0.268	0.217	0.189			
	Average	0.262	0.226	0.197			
		0.262	0.226	0.197			

IC50 of Lactoferricin against HTB9 cell line

Graph 1: graph represents lactoferricin against HTB9 cell lines

Table 2: Table Cell Viability test by MTT assay of Lactoferricin from bovine colostrum on breast cancer cells (MFC-7)

		24 Hours	48 Hours	72 Hours	Percentage Cell Viability		
					24 Hours	48 Hours	7 2 Hours
Control	PCM	0.973	0.982	1.121			
LFCB6B1	S1D1	0.979	0.916	1.088	97.540	97.290	96.105
	S1D2	0.901	0.984	1.088			
	S1D3	0.967	0.966	1.056			
	Average	0.9490642	0.955	1.077			
		0.949	0.955	1.077			
LFCB6B2	S2D1	0.961	0.925	1.057	96.34	92.856	92.891
	S2D2	0.894	0.934	1.068			
	S2D3	0.957	0.876	0.999			
	Average	0.937	0.912	1.041			
		0.937	0.912	1.041			
LFCB6B3	S3D1	0.854	0.922	1.062	94.36	91.97	91.67
	S3D2	0.946	0.932	0.961			
	S3D3	0.955	0.855	1.060			
	Average	0.918	0.903	1.028			
		0.918	0.903	1.028			
LFCB6B4	S1D1	0.927	0.835	0.989	92.359	88.691	87.340
	S1D2	0.853	0.897	0.988			
	S1D3	0.915	0.881	0.960			
	Average	0.89865307	0.871	0.979			
		0.899	0.871	0.979			
LFCB6B5	S2D1	0.851	0.859	0.943	85.321	86.232	82.891
	S2D2	0.791	0.868	0.953			
	S2D3	0.848	0.814	0.891			
	Average	0.830	0.847	0.929			
		0.830	0.847	0.929			
LFCB6B6	S3D1	0.781	0.799	0.841	86.33	79.64	72.59
	S3D2	0.865	0.807	0.761			
	S3D3	0.874	0.740	0.839			
	Average	0.840	0.782	0.814			
		0.840	0.782	0.814			
LFCB6B7	S1D1	0.780	0.671	0.740	77.660	71.240	65.390
	S1D2	0.718	0.720	0.740			
	S1D3	0.770	0.707	0.719			
	Average	0.7556318	0.700	0.733			
		0.756	0.700	0.733			
LFCB6B8	S2D1	0.692	0.643	0.705	69.33	64.51	61.95
	S2D2	0.643	0.649	0.712			
	S2D3	0.689	0.609	0.666			
	Average	0.675	0.633	0.694			
		0.675	0.633	0.694			
LFCB6B9	S3D1	0.583	0.604	0.688	64.39	60.21	59.33
	S3D2	0.645	0.610	0.622			
	S3D3	0.652	0.560	0.686			
	Average	0.627	0.591	0.665			
		0.627	0.591	0.665			

LFCB6B10	S1D1	0.566	0.491	0.578	56.340	52.110	51.069
	S1D2	0.521	0.527	0.578			
	S1D3	0.558	0.518	0.561			
	Average	0.5481882	0.512	0.572			
		0.548	0.512	0.572			
LFCB6B11	S2D1	0.692	0.542	0.562	69.334	54.37	49.35
	S2D2	0.643	0.547	0.567			
	S2D3	0.689	0.513	0.531			
	Average	0.675	0.534	0.553			
		0.675	0.534	0.553			
LFCB6B12	S3D1	0.482	0.494	0.537	53.29	49.27	46.33
	S3D2	0.534	0.499	0.486			
	S3D3	0.539	0.458	0.536			
	Average	0.519	0.484	0.519			
		0.519	0.484	0.519			
LFCB6B13	S1D1	0.596	0.521	0.561	59.340	55.340	49.570
	S1D2	0.548	0.560	0.561			
	S1D3	0.588	0.550	0.545			
	Average	0.5773782	0.543	0.556			
		0.577	0.543	0.556			
LFCB6B14	S2D1	0.472	0.414	0.420	47.26	41.55	36.94
	S2D2	0.438	0.418	0.425			
	S2D3	0.470	0.392	0.397			
	Average	0.460	0.408	0.414			
		0.460	0.408	0.414			
LFCB6B15	S3D1	0.446	0.484	0.509	49.32	48.26	43.91
	S3D2	0.494	0.489	0.460			
	S3D3	0.499	0.449	0.508			
	Average	0.480	0.474	0.492			
		0.480	0.474	0.492			
LFCB6B16	S1D1	0.475	0.397	0.474	47.350	42.150	41.890
	S1D2	0.437	0.426	0.474			
	S1D3	0.469	0.419	0.460			
	Average	0.4607155	0.414	0.470			
		0.460	0.414	0.470			
LFCB6B17	S2D1	0.432	0.408	0.478	43.29	41	42.039
	S2D2	0.402	0.413	0.483			
	S2D3	0.430	0.387	0.452			
	Average	0.421	0.403	0.471			
		0.421	0.403	0.471			
LFCB6B18	S3D1	0.364	0.364	0.426	40.28	36.27	36.74
	S3D2	0.404	0.368	0.385			
	S3D3	0.408	0.337	0.425			
	Average	0.392	0.356	0.412			
		0.392	0.356	0.412			
LFCB6B19	S1D1	0.426	0.387	0.423	42.390	41.060	37.340
	S1D2	0.392	0.415	0.423			
	S1D3	0.420	0.408	0.410			
	Average	0.4124547	0.403	0.419			
		0.412	0.403	0.419			
LFCB6B20	S2D1	0.352	0.343	0.359	35.27	34.39	31.554
	S2D2	0.327	0.346	0.363			
	S2D3	0.350	0.324	0.339			
	Average	0.343	0.338	0.354			

Table 3: Table represents Cell Viability test by MTT assay of Lactoferricin from bovine colostrum on Prostate cancer cells (PC-3)

		24 Hours	48 Hours	72 Hours	Percentage Cell Viability		
					24 Hours	48 Hours	72 Hours
Control	PCM	0.973	0.982	1.121			
LFCB6P1	S1D1	0.966	0.907	1.079	96.250	96.270	95.240
	S1D2	0.889	0.974	1.078			
	S1D3	0.954	0.956	1.047			
	Average	0.9365125	0.945	1.068			
		0.937	0.945	1.068			
LFCB6P2	S2D1	0.940	0.926	1.036	94.26	92.99	91.05
	S2D2	0.874	0.936	1.047			
	S2D3	0.937	0.877	0.979			
	Average	0.917	0.913	1.021			
		0.917	0.913	1.021			
LFCB6P3	S3D1	0.872	0.905	1.057	96.347	90.24	91.22
	S3D2	0.966	0.915	0.956			
	S3D3	0.975	0.839	1.054			
	Average	0.937	0.886	1.023			
		0.937	0.886	1.023			
LFCB6P4	S1D1	0.897	0.795	0.933	89.340	84.390	82.410
	S1D2	0.825	0.853	0.933			
	S1D3	0.885	0.838	0.906			
	Average	0.8692782	0.829	0.924			
		0.869	0.829	0.924			
LFCB6P5	S2D1	0.927	0.859	0.959	92.891	86.232	84.265
	S2D2	0.862	0.868	0.969			
	S2D3	0.923	0.814	0.906			
	Average	0.904	0.847	0.945			
		0.904	0.847	0.945			
LFCB6P6	S3D1	0.736	0.804	0.946	81.347	80.22	81.671
	S3D2	0.815	0.813	0.856			
	S3D3	0.823	0.746	0.944			
	Average	0.792	0.788	0.916			
		0.792	0.788	0.916			
LFCB6P7	S1D1	0.857	0.765	0.920	85.340	81.260	81.250
	S1D2	0.789	0.822	0.920			
	S1D3	0.846	0.807	0.893			
	Average	0.8303582	0.798	0.911			
		0.830	0.798	0.911			
LFCB6P8	S2D1	0.791	0.747	0.814	79.324	74.96	71.53
	S2D2	0.736	0.754	0.822			
	S2D3	0.788	0.707	0.769			
	Average	0.772	0.736	0.802			
		0.772	0.736	0.802			
LFCB6P9	S3D1	0.673	0.755	0.839	74.32	75.26	72.43
	S3D2	0.745	0.763	0.759			
	S3D3	0.752	0.700	0.837			
	Average	0.723	0.739	0.812			
		0.723	0.739	0.812			
LFCB6P10	S1D1	0.797	0.671	0.740	79.350	71.250	65.370
	S1D2	0.733	0.721	0.740			
	S1D3	0.786	0.708	0.718			
	Average	0.7720755	0.700	0.733			
		0.772	0.700	0.733			
LFCB6P11	S2D1	0.752	0.700	0.676	75.339	70.25	59.37
	S2D2	0.699	0.707	0.683			
	S2D3	0.749	0.663	0.639			
	Average	0.733	0.690	0.666			
		0.733	0.690	0.666			
LFCB6P12	S3D1	0.676	0.685	0.710	74.656	68.34	61.25
	S3D2	0.748	0.693	0.642			
	S3D3	0.755	0.635	0.708			
	Average	0.726	0.671	0.687			
		0.726	0.671	0.687			
LFCB6P13	S1D1	0.722	0.504	0.276	71.940	53.490	24.390
	S1D2	0.665	0.541	0.276			
	S1D3	0.713	0.531	0.268			
	Average	0.6999762	0.525	0.273			
		0.700	0.525	0.273			

IC50 of Lactoferricin against MFC-7 cell line

(Y-axis: Viability of Cells, 0–150; X-axis: Concentration of Lactoferricin, 0–1500)
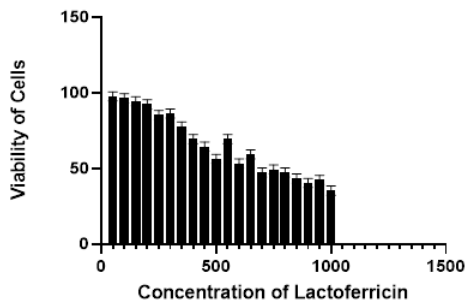
Graph 2: Graph represents lactoferricin against MFC-7 Cell lines.

LFCB6P14	S2D1	0.733	0.608	0.618	73.45	61.05	54.28
	S2D2	0.681	0.614	0.624			
	S2D3	0.730	0.576	0.584			
	Average	**0.715**	**0.600**	**0.608**			
		0.715	**0.600**	**0.608**			
LFCB6P15	S3D1	0.664	0.685	0.710	73.421	68.34	61.257
	S3D2	0.736	0.693	0.642			
	S3D3	0.743	0.635	0.708			
	Average	**0.714**	**0.671**	**0.687**			
		0.714	**0.671**	**0.687**			
LFCB6P16	S1D1	0.719	0.588	0.580	71.570	62.396	51.247
	S1D2	0.661	0.631	0.580			
	S1D3	0.709	0.620	0.563			
	Average	**0.6963761**	**0.613**	**0.574**			
		0.696	**0.613**	**0.574**			
LFCB6P17	S2D1	0.622	0.428	0.458	62.358	42.98	40.29
	S2D2	0.578	0.432	0.463			
	S2D3	0.620	0.406	0.433			
	Average	**0.607**	**0.422**	**0.452**			
		0.607	**0.422**	**0.452**			
LFCB6P18	S3D1	0.631	0.529	0.548	69.74	52.77	47.256
	S3D2	0.699	0.535	0.495			
	S3D3	0.706	0.491	0.546			
	Average	**0.679**	**0.518**	**0.530**			
		0.679	**0.518**	**0.530**			
LFCB6P19	S2D1	0.337	0.127	0.048	54.238	29.58	10.55
	S2D2	0.314	0.128	0.049			
	S2D3	0.336	0.120	0.046			
	Average	**0.329**	**0.125**	**0.048**			
		0.329	**0.125**	**0.048**			
LFCB6P20	S3D1	0.233	0.112	0.083	41.33	25.964	17.68
	S3D2	0.258	0.113	0.075			
	S3D3	0.261	0.104	0.082			
	Average	**0.251**	**0.110**	**0.080**			
		0.251	**0.110**	**0.080**			

IC50 of Lactoferricin against PC-3 cell line
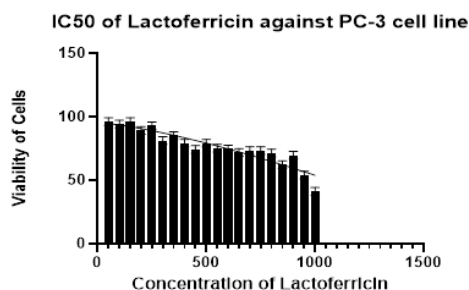
Graph 3: graph represents Lactoferricin aginst PC-3 cell lines

DISCUSSION:

Natural anti-cancer peptides like lactoferricin have been isolated from cow, sheep and buffalo. Previous research article reported that, lactoferricin have anti-cancer activity by performing MTT assay as a preliminary test.

Metabolic activity can be evaluated by measuring the activity of a mitochondrial enzyme succinate dehydrogenase using MTT test. MTT is designed for the quantification of cytotoxic index in cell population using 96 well plate formats. This test is widely used in the in vitro evaluation of the cytotoxic potency of drugs. In the present study we applied the MTT test to evaluate the potency of lactoferricin extracted from bovine colostrum on breast cancer cells (MFC-7), Prostate cancer cells (PC-3) and Renal cancer cells (HTB9), After growth of the cells up to 70% confluence, different concentrations of LFBC from 100 to 1000 were considered for treatment of the cells. Cell viability is monitored for every 24 hrs for 3 days. All concentrations of the anticancer peptide were able to inhibit the cancer cell lines proliferation. IC50 towards HTB9, MFC-7 and PC-3cell lines were calculated at 784.1 µg/ml ,697.1 µg/ml and841.2 µg/ml concentrations of anticancer peptide respectively. .It has shown efficient activity on viability of cancer cell lines. It is confirmed that lactoferricin has an anti cancerous capability.

Table 4: table represents Cell Viability test by MTT assay of Tachyplesin on Renal cancer cell line (HTB9)

		24 Hours	48 Hours	72 Hours	Percentage Cell Viability		
					24 Hours	48 Hours	72 Hours
Control	PCM	0.823	0.782	0.234			
TCHK1	S1D1	0.820	0.689	0.233	98.534	94.860	97.523
	S1D2	0.784	0.771	0.233			
	S1D3	0.829	0.765	0.219			
	Average	**0.81093482**	**0.742**	**0.228**			
		0.811	**0.742**	**0.228**			
TCHK2	S2D1	0.808	0.752	0.217	95.723	93.92	90.544
	S2D2	0.743	0.775	0.213			
	S2D3	0.812	0.676	0.205			
	Average	**0.788**	**0.734**	**0.212**			
		0.788	**0.734**	**0.212**			
TCHK3	S3D1	0.770	0.748	0.188	97.456	92.796	78.563
	S3D2	0.812	0.734	0.179			
	S3D3	0.826	0.694	0.184			
	Average	**0.802**	**0.726**	**0.184**			
		0.803	**0.726**	**0.184**			
TCHK4	S1D1	1.808	0.757	0.940	86.721	80.342	80.642
	S1D2	1.539	0.805	0.931			
	S1D3	1.786	0.805	0.841			
	Average	**1.71100533**	**0.789**	**0.904**			
		1.711	**0.789**	**0.904**			
TCHK5	S2D1	1.472	0.699	0.819	72.765	67.462	70.565
	S2D2	1.340	0.679	0.803			
	S2D3	1.495	0.610	0.751			
	Average	**1.436**	**0.662**	**0.791**			
		1.436	**0.662**	**0.791**			
TCHK6	S3D1	1.677	0.748	0.732	89.454	75.348	63.793
	S3D2	1.818	0.749	0.697			
	S3D3	1.800	0.723	0.716			
	Average	**1.765**	**0.740**	**0.715**			
		1.765	**0.740**	**0.715**			
TCHK7	S1D1	0.699	0.547	0.611	75.863	73.912	61.990
	S1D2	0.630	0.594	0.610			
	S1D3	0.704	0.606	0.557			
	Average	**0.67745659**	**0.582**	**0.593**			
		0.677	**0.582**	**0.593**			
TCHK8	S2D1	0.759	0.455	0.417	83.654	54.76	42.765
	S2D2	0.735	0.442	0.419			
	S2D3	0.748	0.397	0.388			
	Average	**0.747**	**0.432**	**0.409**			
		0.747	**0.432**	**0.408**			
TCHK9	S3D1	0.730	0.651	0.817	84.52	80.133	83.501
	S3D2	0.759	0.645	0.761			
	S3D3	0.777	0.598	0.815			
	Average	**0.755**	**0.631**	**0.798**			
		0.755	**0.631**	**0.798**			
TCHK10	S1D1	0.659	0.390	0.241	74.792	49.450	29.795
	S1D2	0.594	0.414	0.241			
	S1D3	0.663	0.415	0.220			
	Average	**0.63872368**	**0.406**	**0.234**			
		0.639	**0.406**	**0.234**			
TCHK11	S2D1	0.748	0.584	0.245	86.244	69.342	29.88
	S2D2	0.621	0.601	0.238			
	S2D3	0.841	0.525	0.220			
	Average	**0.737**	**0.570**	**0.234**			
		0.737	**0.570**	**0.234**			
TCHK12	S3D1	0.638	0.455	0.310	79.465	52.71	38.641
	S3D2	0.709	0.438	0.280			
	S3D3	0.689	0.405	0.318			
	Average	**0.679**	**0.433**	**0.303**			
		0.679	**0.433**	**0.303**			
TCHK13	S1D1	0.651	0.516	0.405	81.654	60.342	42.754
	S1D2	0.599	0.554	0.404			
	S1D3	0.643	0.544	0.393			
	Average	**0.63118542**	**0.538**	**0.401**			
		0.631	**0.538**	**0.401**			

TCHK14	S2D1	0.592	0.511	0.309	75.452	55.392	32.16
	S2D2	0.568	0.506	0.309			
	S2D3	0.590	0.465	0.286			
	Average	0.583	0.494	0.301			
		0.583	0.494	0.301			
TCHK15	S3D1	0.503	0.471	0.393	69.436	49.7723	40.96
	S3D2	0.557	0.454	0.367			
	S3D3	0.546	0.406	0.395			
	Average	0.537	0.444	0.384			
		0.536	0.444	0.385			
TCHK16	S1D1	0.562	0.464	0.249	75.951	63.751	39.621
	S1D2	0.413	0.519	0.246			
	S1D3	0.555	0.515	0.202			
	Average	0.51115023	0.499	0.233			
		0.510	0.499	0.233			
TCHK17	S2D1	0.568	0.447	0.265	79.593	53.62	41.6
	S2D2	0.487	0.434	0.246			
	S2D3	0.552	0.378	0.222			
	Average	0.536	0.420	0.244			
		0.536	0.420	0.244			
TCHK18	S3D1	0.444	0.421	0.228	73.961	50.157	35.58
	S3D2	0.532	0.433	0.189			
	S3D3	0.518	0.325	0.211			
	Average	0.498	0.393	0.209			
		0.498	0.393	0.209			
TCHK19	S1D1	0.573	0.280	0.112	64.762	39.289	10.549
	S1D2	0.515	0.327	0.108			
	S1D3	0.600	0.321	0.095			
	Average	0.5666675	0.308	0.105			
		0.564	0.309	0.105			
TCHK20	S2D1	0.455	0.175	0.100	49.773	20.959	9.453
	S2D2	0.398	0.172	0.101			
	S2D3	0.458	0.148	0.081			
	Average	0.436	0.165	0.094			
		0.437	0.165	0.094			

Table 5: table represents cell viability of tachyplesin against MFC-7 cell lines

		24 Hours	48 Hours	72 Hours	Percentage Cell Viability		
TCKB1	S1D1	0.948	0.683	0.256	97.250	83.170	62.960
	S1D2	0.807	0.725	0.253			
	S1D3	0.937	0.725	0.229			
	Average	0.8976175	0.711	0.246			
		0.898	0.711	0.246			
TCHB2	S1D1	0.851	0.434	0.114	91.860	64.930	44.920
	S1D2	0.767	0.471	0.114			
	S1D3	0.856	0.481	0.104			
	Average	0.824551436	0.462	0.111			
		0.825	0.462	0.111			
TCKB3	S3D1	0.836	0.645	0.167	94.341	73.125	41.83
	S3D2	0.882	0.633	0.159			
	S3D3	0.897	0.598	0.164			
	Average	0.871	0.625	0.164			
		0.871	0.625	0.164			
TCKB4	S1D1	2.022	0.703	0.720	96.990	74.610	61.730
	S1D2	1.721	0.747	0.712			
	S1D3	1.998	0.747	0.644			
	Average	1.9136127	0.733	0.692			
		1.914	0.733	0.692			
TCKB5	S1D1	1.806	0.506	0.387	89.340	71.990	53.720
	S1D2	1.538	0.538	0.383			
	S1D3	1.785	0.538	0.346			
	Average	1.709621586	0.527	0.372			
		1.710	0.527	0.372			
TCKB6	S3D1	1.403	0.559	0.456	73.55	55.23	39.71
	S3D2	1.460	0.554	0.424			
	S3D3	1.495	0.514	0.455			
	Average	1.451	0.542	0.445			
		1.452	0.542	0.445			
TCKB7	S1D1	0.816	0.558	0.544	88.520	73.910	55.230
	S1D2	0.735	0.594	0.544			
	S1D3	0.821	0.595	0.496			
	Average	0.7904836	0.582	0.528			
		0.791	0.582	0.528			
TCKB8	S3D1	1.222	0.477	0.282	91.65	84.33	60.41
	S3D2	1.325	0.477	0.268			
	S3D3	1.312	0.461	0.276			
	Average	1.286	0.472	0.275			
		1.286	0.472	0.275			
TCKB9	S3D1	0.746	0.625	0.581	86.41	76.9	59.33
	S3D2	0.776	0.619	0.541			
	S3D3	0.795	0.574	0.579			
	Average	0.772	0.606	0.567			
		0.772	0.606	0.567			
TCKB10	S1D1	0.775	0.501	0.241	87.990	63.650	29.795
	S1D2	0.699	0.533	0.241			
	S1D3	0.780	0.534	0.220			
	Average	0.7514346	0.523	0.234			
		0.752	0.523	0.234			
TCKB11	S3D1	0.591	0.512	0.260	84.23	77.96	43.81
	S3D2	0.657	0.493	0.235			
	S3D3	0.638	0.455	0.267			
	Average	0.629	0.487	0.254			
		0.629	0.487	0.254			
TCKB12	S3D1	0.633	0.535	0.276	78.88	61.94	34.41
	S3D2	0.704	0.515	0.250			
	S3D3	0.684	0.476	0.284			
	Average	0.674	0.509	0.270			
		0.674	0.509	0.270			
TCKB13	S2D1	0.527	0.386	0.182	81.95	70.34	62.95
	S2D2	0.438	0.397	0.177			
	S2D3	0.592	0.347	0.163			
	Average	0.519	0.376	0.174			
		0.519	0.376	0.174			
TCKB14	S1D1	0.451	0.210	0.080	78.930	59.990	42.850
	S1D2	0.331	0.235	0.079			
	S1D3	0.445	0.233	0.065			
	Average	0.409584664	0.226	0.075			
		0.409	0.226	0.074			
TCKB15	S3D1	0.522	0.634	0.412	71.95	66.93	42.99
	S3D2	0.577	0.610	0.385			
	S3D3	0.566	0.546	0.415			
	Average	0.556	0.597	0.403			
		0.555	0.597	0.404			
TCKB16	S1D1	0.587	0.392	0.258	79.340	53.890	41.060
	S1D2	0.432	0.439	0.255			
	S1D3	0.580	0.435	0.210			
	Average	0.5339582	0.422	0.241			
		0.533	0.422	0.241			
TCKB17	S3D1	0.361	0.339	0.139	73.86	50.43	32.99
	S3D2	0.400	0.327	0.130			
	S3D3	0.392	0.292	0.140			
	Average	0.385	0.320	0.136			
		0.385	0.319	0.136			
TCKB18	S2D1	0.441	0.247	0.069	79.05	54.97	26.75
	S2D2	0.385	0.242	0.069			
	S2D3	0.443	0.209	0.055			
	Average	0.421	0.232	0.064			
		0.423	0.233	0.064			
TCKB19	S1D1	0.616	0.314	0.308	69.550	43.960	28.990
	S1D2	0.554	0.366	0.297			
	S1D3	0.644	0.359	0.262			
	Average	0.6085625	0.345	0.288			
		0.606	0.346	0.289			
TCKB20	S1D1	0.500	0.201	0.121	81.540	63.820	39.120
	S1D2	0.449	0.234	0.116			
	S1D3	0.523	0.230	0.103			
	Average	0.493740753	0.221	0.113			
		0.491	0.221	0.113			
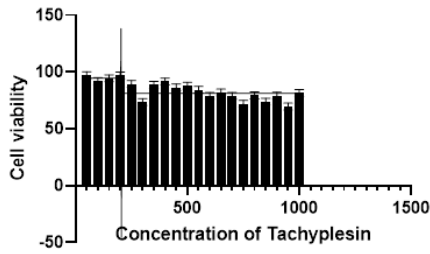
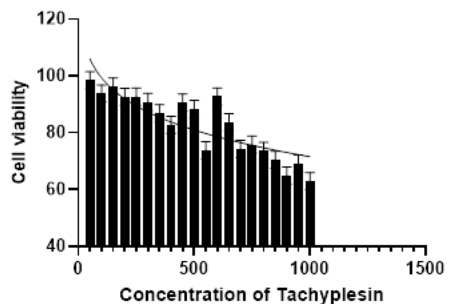**Graph 4: graph represents Tachyplesin against MFC-7 Cell line**

Table 6: table represents Tachyplesin cell viability against PC-3 cell line

		24 Hours	48 Hours	72 Hours	Percentage Cell Viability		
					24 Hours	48 Hours	72 Hours
Control	PCM	0.923	0.855	0.391			
TCHP1	S1D1	0.938	0.627	0.256	98.470	76.420	63.670
	S1D2	0.845	0.666	0.256			
	S1D3	0.944	0.667	0.234			
	Average	0.9088781	0.653	0.249			
		0.909	0.653	0.249			
TCHP2	S1D1	0.901	0.533	0.206	93.820	84.990	79.440
	S1D2	0.767	0.566	0.204			
	S1D3	0.890	0.566	0.184			
	Average	0.852794704	0.555	0.198			
		0.853	0.555	0.198			
TCHP3	S1D1	0.848	0.418	0.148	96.330	80.160	72.660
	S1D2	0.764	0.454	0.148			
	S1D3	0.853	0.464	0.135			
	Average	0.821497139	0.445	0.144			
		0.821	0.445	0.144			
TCHP4	S3D1	0.801	0.456	0.126	92.65	82.96	59.77
	S3D2	0.845	0.448	0.120			
	S3D3	0.860	0.423	0.123			
	Average	0.835	0.442	0.123			
		0.835	0.442	0.123			
TCHP5	S1D1	0.799	0.351	0.101	92.730	82.660	79.510
	S1D2	0.720	0.373	0.101			
	S1D3	0.804	0.373	0.092			
	Average	0.774448528	0.366	0.098			
		0.775	0.366	0.098			
TCHP6	S3D1	1.733	0.848	0.733	90.83	83.77	63.91
	S3D2	1.803	0.841	0.683			
	S3D3	1.846	0.779	0.732			
	Average	1.792	0.823	0.716			
		1.793	0.823	0.716			
TCHP7	S3D1	0.659	0.250	0.067	86.83	70.62	64.71
	S3D2	0.715	0.251	0.064			
	S3D3	0.708	0.242	0.065			
	Average	0.694	0.248	0.065			
		0.694	0.248	0.065			
TCHP8	S3D1	1.388	0.690	0.465	82.851	78.91	61.95
	S3D2	1.444	0.684	0.433			
	S3D3	1.479	0.634	0.464			
	Average	1.436	0.669	0.454			
		1.437	0.669	0.454			
TCHP9	S3D1	0.783	0.613	0.614	90.63	75.42	62.75
	S3D2	0.814	0.607	0.572			
	S3D3	0.834	0.563	0.613			
	Average	0.809	0.594	0.600			
		0.810	0.594	0.600			
TCHP 10	S1D1	0.779	0.590	0.492	88.390	74.860	60.880
	S1D2	0.702	0.627	0.491			
	S1D3	0.784	0.628	0.449			
	Average	0.7548506	0.615	0.477			
		0.755	0.615	0.477			
TCHP 11	S3D1	0.543	0.406	0.334	73.81	62.95	53.17
	S3D2	0.604	0.390	0.302			
	S3D3	0.586	0.361	0.343			
	Average	0.578	0.386	0.327			
		0.578	0.386	0.327			
TCHP 12	S3D1	0.745	0.716	0.512	92.77	82.95	63.76
	S3D2	0.828	0.690	0.462			
	S3D3	0.804	0.637	0.525			
	Average	0.792	0.682	0.500			
		0.792	0.681	0.500			
TCHP 13	S3D1	0.427	0.317	0.186	83.75	74.29	54.21
	S3D2	0.475	0.305	0.168			
	S3D3	0.462	0.282	0.191			
	Average	0.455	0.301	0.181			
		0.455	0.301	0.181			
TCHP 14	S1D1	0.372	0.169	0.096	74.290	60.330	49.640
	S1D2	0.273	0.189	0.095			
	S1D3	0.367	0.187	0.078			
	Average	0.337837727	0.182	0.090			
		0.337	0.182	0.090			
TCHP 15	S3D1	0.550	0.598	0.460	75.82	63.22	47.92
	S3D2	0.608	0.576	0.429			
	S3D3	0.597	0.516	0.462			
	Average	0.586	0.564	0.449			
		0.585	0.564	0.450			
TCHP 16	S3D1	0.256	0.106	0.039	73.61	59.37	39.16
	S3D2	0.284	0.102	0.036			
	S3D3	0.278	0.092	0.039			
	Average	0.274	0.100	0.038			
		0.273	0.100	0.038			
TCHP 17	S1D1	0.195	0.054	0.016	70.510	59.390	39.410
	S1D2	0.175	0.063	0.015			
	S1D3	0.204	0.062	0.014			
	Average	0.192544759	0.059	0.015			
		0.192	0.060	0.015			
TCHP 18	S1D1	0.126	0.029	0.006	64.990	52.740	35.910
	S1D2	0.113	0.033	0.006			
	S1D3	0.132	0.033	0.005			
	Average	0.124509165	0.031	0.005			
		0.124	0.032	0.005			
TCHP 19	S3D1	0.126	0.030	0.006	69.16	51.88	36.9
	S3D2	0.140	0.029	0.006			
	S3D3	0.137	0.026	0.006			
	Average	0.135	0.028	0.006			
		0.135	0.028	0.006			
TCHP 20	S2D1	0.576	0.386	0.230	62.95	46.21	21.77
	S2D2	0.503	0.379	0.233			
	S2D3	0.579	0.327	0.186			
	Average	0.551	0.363	0.216			
		0.552	0.364	0.216			

Graph 5: Graph reprents Tachyplesin against PC-3 cell line

DISCUSSION:

Tachyplesin an anticancer peptide isolated from hemocytes of the horseshoe crab (*Tachypleustridentatus*). The literature reported, tachylplesin to have anti-cancer activity, which is a natural source. In our study, Tachyplesin peptide was analyzed its activity by viability of

cell count through MTT assay. To evaluate the cytotoxic activity of Tachyplesin on three cell lines Renal cancer cell lines (HTB9), breast cancer cell lines (MFC-7) and prostate cancer cell lines (PC-3) MTT assay was performed. The dosage of Tachyplesin ranges from 50-1000 µg/ml with an increment of 50 µg/ml and incubated for 72 hours while readings were taken for every 24 hours. Cell viability of the cells was recorded as the anticancer peptide showed activity in a concentration dependent manner. At all concentrations of the anticancer peptide showed activity against the cell lines by inhibiting their growth. IC50 was calculated for the activity of tachyplesin on all cell lines and recorded to be 452.9 µg/ml, 203.4 µg/ml and 281.0 µg/ml for HTB9, MFC-7 and PC-3 cell lines respectively. The results evaluated, a good source of anti cancer agent and it can further to be characterized.

CONCLUSION:
The presence of iron in lactoferrin plays an important role in enhancing the various activities taking place inside these cells. This work provides a lot of information about targeting lactoferrin against many parasitic infections which can rule out the exact pathways for inhibition of diseases. Lactoferricin MTT assay results reported to be a good anticancer peptide, controlled the viability of three cell lines. All concentrations of the anticancer peptide were able to inhibit the cancer cell lines proliferation. IC50 towards HTB9, MFC-7 and PC-3cell lines were calculated at 784.1 µg/ml ,697.1 µg/ml and841.2 µg/ml concentrations of anticancer peptide respectively.

Several research articles have revealed the analysis of carcinogens and the development of neurosurgery, chemotherapy and radiotherapy. In the present study, we demonstrated tachyplesin against viability test as preliminary test for cancer activity. The variable concentrations proved to control viable count high at 72hs interval period of time. IC50 was calculated for the activity of tachyplesin on all cell lines and recorded to be 452.9 µg/ml, 203.4 µg/ml and 281.0 µg/ml for HTB9, MFC-7 and PC-3 cell lines respectively.

Both these peptide molecules proved to be anti cancer peptides by MTTassay. Our research progress the research work on indepth on anti cancerous activity in further analysis. This research work, would help in the better treatment of cancer.

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