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Cytotoxicity activity of rabbit antisera of riboflavin carrier protein (RCP) against HeLa cells, MCF-7 and A549 cancer cell lines

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ABSTRACT

In the present study, riboflavin carrier protein was purified from hen and spotted owlet (Athene brama) egg yolk and white. To determine and compare in-vitro cytotoxic activity of Rabbit antiserum against Riboflavin Carrier Protein (RCP) isolated from white as well as yolk of spotted owlet egg and hen eggs on HeLa cells, MCF-7 and A549 cancer cells. The anticancer activity of Riboflavin Carrier proteins (RCP) purified from egg-white of spotted owlet (Athene brama) antiserum was 16.531 %, egg-yolk antiserum was 11.231 % on HeLa cell lines. Spotted owlet egg yolk antiserum on HeLa cells showed less cytotoxic potential. Riboflavin Carrier proteins (RCP) purified from egg-white of spotted owlet antiserum cytotoxic effect was 25.171 %, egg-yolk antiserum was 20.104% and Hen egg-white antiserum was 26.737 %, egg-yolk antiserum was 24.024% on A549 cancer cells. The anticancer activity of Riboflavin Carrier proteins (RCP) purified from egg white of spotted owlet (Athene brama) antiserum was 18.922 %, egg-yolk antiserum was 32.842 % and Hen egg-white antiserum was 22.499 %, egg-yolk antiserum was 18.860 % on Breast cancer cells. Highest cytotoxic activity was seen in spotted egg yolk antiserum on Breast cancer cell lines and standard drug used was Doxorubicin (48.528 % of Cytotoxicity). IC_{50} values for A549 were 12.46 \pm 0.9 & 11.20 ± 0.8 for hen white, yolk and $11.64 \pm 0.9 \& 14.89 \pm 0.4$ for spotted owlet yolk and white. MCF-7 cancer cell lines showed IC_{50} values of 17.75 ± 0.8 & 21.18 ± 0.5 for hen white , yolk and 21.11 ± 0.7 & 12.16 ± 0.6 for spotted owlet egg white and yolk respectively and the IC 50 values of spotted owlet egg white and yolk on HeLa cancer cells are 24.16 \pm 0.5 & 35.57 \pm 0.6 , Doxorubicin 10.30 \pm 0.4. The antiserum against RCP binds to Riboflavin carrier protein and depletes riboflavin in the cancer cell lines which might have lead to cell growth inhibition

Key words: Riboflavin Carrier protein (RCP), antibodies (Antisera), HeLa cells, MCF-7 and A549 cancer cells, Cytotoxic activity.

INTRODUCTION

Vitamin binding proteins bind stoichimetrically and reversibly to vitamins with high affinity and receptor like specificity. The specific carrier proteins for fat soluble vitamins such as vitamin A and vitamin D have been identified in normal serum in all vertebrates [1, 2, 3 and 4]. Proteins binding to water soluble vitamins such as Riboflavin binding/carrier proteins [5, 6,7,8,9,10 and 11], vitamin B_{12} binding protein [12 and 13] and thiamin binding protein [14 and 15] have been demonstrated in the sera, egg white and yolk of the egg laying hens. Riboflavin binding protein (RfBP) is a phosphoglycoprotein, whose primary physiological function is to store riboflavin [16]. This carrier protein is essential for embryonic vitamin nutrition [17, 18, 19 and 20]. RfBP was isolated and purified for the first time from the yolk of 73 parrot eggs [21]. Riboflavin binding protein (RfBP) was isolated in India from peacock eggs (*Pavo cristatus*) [22]. Fertilized eggs of a homozygous recessive mutant chicken

with avian riboflavinuria were found to lack a functional RfBP [23]. In the liver, RfBP is synthesized and is secreted into the blood stream, where it complexes with riboflavin. The vitamin-protein complex is then deposited as part of the yolk in a developing oocyte. After ovulation, this mature oocyte passes down the oviduct where after it is secreted by the magnum region of the oviduct. The albumin of egg comes from the diet, during restricted riboflavin intake it can come from tissues provided liver flavin is above 50% of the normal, otherwise, egg laying stops. Studies on RCP structure from the avain species spotted owlet eggs were not earlier done. In order to understand the structure aspects and immunological characteristics of Riboflavin carrier protein (RCP) from spotted owlet. RCP was purified from spotted owlet (Athene brama) eggs. This was compared with hen (Gallus domesticus) RCP to understand the structural aspects, immunological characteristics, $alpha(\alpha)$ & $beta(\beta)$ percentage in secondary structure of single poly peptide chain Riboflavin carrier protein(RCP) and isolated RCP against serum antibodies cytotoxicity activity study of the two avian species which were phylogenitically distinct and evolutionarily different. The Riboflavin carrier protein from hen and spotted owlet egg Riboflavin carrier protein have been isolated and their structures compared by SDS-PAGE here ours experiment observed spotted owlet yolk Riboflavin carrier protein molecular weight is 3kDa difference and secondary structure mapping study, Alpha (α), Beta (β) percentage in Riboflavin carrier protein is different[24]. To determine and compare *in-vitro* cytotoxic activity of Rabbit antiserum against Riboflavin Carrier Protein (RCP) isolated from white as well as yolk of spotted owlet egg and hen eggs on HeLa cells, MCF-7 and A549 cancer cells. To determine the concentration of antisera which is more cytotoxic against different cancer cell lines, this study was undertaken (HeLa cells, MCF-7 and A549 cancer cells). No study was conducted using spotted owlet (Athene brama) eggs ever before with specific target towards the cytotoxic activity. For the first time, we have come out with the anticancer antibodies produced against Riboflavin carrier protein of spotted owlet egg white and yolk.

EXPERIMENTAL SECTION

DEAE Sepharose DEAE-Sepharose (098K1665) DCL6B100-50ML, wet bead size: 45-Y, suspension in 20% ethanol (Sigma –Aldrich) used in the present study was obtained from Amersham Pharmacia fine chemicals, Uppsala, Sweden and Sephadex G-100 was obtained from Sigma Chemical Company, St. Louis, USA. Bovine Serum Albumin, Acrylamide, N, N1-, Melthylene–bis acrylamide, N,N,N¹,N¹–Tetra methyl ethylenediamine and SDS were procured from Sd Fine Chem. Limited, Mumbai .MILLEX.GS Filter Unit 0.22µm MF –Millipore MCE Membrane. All other reagents used were of analytical grade. RPMI-1640 media (Himedia, Mumbai, India), Fetal bovine serum (Gibco,USA), Penicillin-G (Himedia, Mumbai, India), Streptomycin (Himedia, Mumbai, india),Amphotericin–B,Phosphate buffered saline (PBS) (Himedia, Mumbai,India), Trypsin (Typsin-EDTA[1X] in HBSS,Gibco; UK), Ethylenediamine tetra –acetic acid (EDTA) (Himedia, Mumbai,India), SDS lysis buffer (Himedia,Mumbai, India), MTT(3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide) (Himedia,Mumbai,India).

METHODS

Cytotoxicity potential of antiserum against RCP was studied on HeLa cell lines (human epitheloid cervix carcinoma), A549 (Lung) Cancer cell and MCF-7 (Breast cancer) cancer cells. Cell lines were purchased from NCCS (National centre for Cell sciences), Pune, India. All three cell lines were subcultured and were maintained at 37ºC at 5% CO2 in CO2 incubator. Cultures were continuously observed every 24 hrs under an inverted microscope to assess the degree of confluency and to confirm the absence of any bacterial and fungal contaminants. In-vitro study of cytotoxicity effect of antiserum of hen, spotted owlet egg yolk & white Riboflavin carrier protein (RCP) was assessed by MTT (3- (4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay. Cell lines were subcultured and 250µl of media (containing 10000cells) were transferred into 96 well plates and incubated for 24 hr. The media was removed and fresh media (100µl) was added. Rabbit produced antiserum of hen, spotted owlet egg yolk &white Riboflavin carrier protein (RCP) was added at different concentrations (2-14 µl) and then final volume was made to 200µl with the media and incubated for 48 hr. After incubation, media containing drug was removed. 20µl of MTT reagent (6mg/ml in PBS) was added to each well containing media and incubated for 3 hr at 37 °C under an atmosphere of 5% CO₂ until a purple precipitate was observed. Media was then removed. 200µl DMSO (MTT solvent) was added to dissolve the purple precipitate. Absorbance was read at 570 nm with a reference filter of 630 nm. Percentage cytotoxicity was calculated and used for finding the IC_{50} value of the concentration required for 50% cell death by produced antiserum of hen, spotted owlet egg yolk & white Riboflavin carrier protein (RCP).

RESULTS

4.1.1 Cytotoxicity studies

RPMI-1640 Medium was used for the maintenance of the cell lines. Sub-cultures were maintained in a deep freezer at -80^oC. Neubar slide (heamocytometer) was used for cell counting by trypan blue dye exclusion method. Results of the cytotoxicity with percentage inhibition are presented. Rabbit Riboflavin carrier protein (RCP) antibodies (Antisera) of rabbit showed more significant anticancer activity as compared to the positive control and standard drug i.e. Doxorubicin.

1. % of Viability = <u>Antisera (Test) Average Absorbance</u> X100 Rabbit Average Absorbance

2. % of Cytotoxicity = 100 - % of Viability

3. IC ₅₀ Value = 50 % of cytotoxicity X Con.c of compound (μ l = Protein)

4.1.2 Cell Morphology

In our investigation on HeLa cells, MCF-7 and A549 cancer cells show change in cell structure. Cells were incubated with rabbit serum and RCP antisera and morphological alteration were confirmed via microscope as show in Figures. 1, 2 after 24 hr of incubation with various concentrations of antisera. Many of the cells showed cytoplasmic shrinkage and loss of normal nuclear architecture and were found floating in the medium. As a result, the number of cytotoxic cells increased with antisera concentration, with highest having the most pronounced inhibitory effect on cell proliferation on rabbit serum than the control. The growth inhibitory activity of Rabbit antisera was more significant as shown in Figures. 3 to 12 and Tables. 4 to 13 show the percentage viability and percentage inhibition of the treated cell with different dose of antisera.

4.1.3 Weight to Molar Quantity (for proteins):

This program was used which helps us to convert the weight (weight concentration) in the molar quantity (molar concentration) and vice versa. Calculation web (site:http://www.molbiol.ru/eng/scripts/01_04.html.)



Figure 1: Morphological study of HeLa cells against Hen and Spotted Owlet Egg White &Yolk Riboflavin Carrier Protein (RCP) antisera, Normal Rabbit Serum and control



Figure 2: Viability of HeLa cells against Hen and Spotted Owlet Egg White &Yolk Riboflavin Carrier Protein (RCP) antiserum, Normal rabbit serum and control

1. CYTOTOXICITY AND VIABILITY (%) OF RIBOFLAVIN CARRIER PROTEIN ANTISERA FROM SPOTTED OWLET EGG WHITE ON A549 CANCER CELLS (LUNG CANCER CELL LINE), MCF-7 CANCER CELLS (BREAST CANCER CELL LINE) AND HeLa CANCER CELLS (HUMAN CERVICAL CANCER CELL LINE)

Table 1: Invitro cytotoxicity and viability (%) of RCP antisera of Spotted Owlet Egg White, Normal Rabbit Serum and Standard Drug Doxorubicin

S.No	Concentration (µl)	Absorbance (O.D)570nm	% Viability	% Cytotoxicity
1	Blank	0.98686	-	-
2	Normal Rabbit Serum(Control)	0.93459	100	-
3	2	0.8918	95.421	11.034
4	4	0.87389	93.505	6.494
5	6	0.83485	88.965	4.578
6	8	0.71409	76.406	23.593
7	10	0.71364	76.358	23.641
8	12	0.71168	76.148	23.851
9	14	0.69588	74.458	25.541
10	16	0.69422	74.280	25.719
11	Doxorubicin2µl(10µM)	0.5276	53.462	46.538

Table 2: In-vitro cytotoxicity and viability (%) of Riboflavin carrier protein (RCP) antisera of Spotted Owlet Egg yolk, Normal Rabbit Serum and Standard Drug Doxorubicin

S.No	Concentration	Absorbance	%	%
5410	(µl)	(O.D)570nm	Viability	Cytotoxicity
1	Blank	0.98686	-	-
2	Normal Rabbit Serum(Control)	0.93459	100	-
3	2	0.85929	91.942	8.057
4	4	0.85562	91.550	8.449
5	6	0.81312	87.008	12.991
6	8	0.79269	84.816	15.183
7	10	0.78046	83.508	16.491
8	12	0.7796	83.416	16.583
9	14	0.76614	81.976	18.023
10	16	0.7467	79.896	20.104
11	Doxorubicin	0.5276	53.462	46.38

Table 3: In-vitro cytotoxicity and viability (%) of RCP antisera of hen egg yolk, Normal Rabbit Serum and Standard Drug Doxorubicin

S No	Concentration	Absorbance	%	%
9.140	(µl)	(O.D)570nm	Viability	Cytotoxicity
1	Blank	0.98686	-	-
2	Normal Rabbit Serum (Control)	0.93459	100	-
3	2	0.83752	89.613	10.386
4	4	0.83172	88.993	11.007
5	6	0.79804	85.389	14.610
6	8	0.79592	85.162	14.837
7	10	0.79362	84.916	15.083
8	12	0.74771	80.004	19.996
9	14	0.7292	78.023	21.976
10	16	0.71006	75.975	24.024
11	Doxorubicin	0.5276	53.462	46.538

Table 4: In-vitro cytotoxicity and viability (%) of RCP antisera of hen egg white, Normal Rabbit Serum and Standard Drug Doxorubicin

S.No	Concentration (µl)	Absorbance (O.D)570nm	% Viability	% Cytotoxicity
1	Blank	0.98686	- 1	-
2	Normal Rabbit Serum(Control)	0.93459	100	-
3	2	0.94404	101.011	+1.011
4	4	0.88472	94.663	5.336
5	6	0.87021	93.111	6.888
6	8	0.79857	85.446	14.554
7	10	0.7641	81.757	18.242
8	12	0.7545	80.730	19.269
9	14	0.73387	78.523	21.476
10	16	0.6847	73.262	26.737
11	Doxorubicin	0.5276	53.462	46.538

Table 5: Invitro cytotoxicity and viability MCF-7 (Breast cancer cells) of RCP antisera of spotted owlet egg yolk, Normal Rabbit serum and standard Drug Doxorubicin

S. No	Concentration (µl)	Rabbit serum Absorbance at 570nm (Control)	% of Viability	Anti sera Absorbance (SOYA)	MCF-7 % viability	% Cytotoxicity
1	2	1.9745	100	1.6977	85.981	14.018
2	4	1.7141	100	1.4141	82.498	17.501
3	6	1.5916	100	1.261	79.228	20.771
4	8	1.4943	100	1.221	78.561	21.438
5	10	1.4304	100	1.0172	71.112	28.88
6	12	1.2916	100	0.8674	67.157	32.842
7	Doxorubicin	0.5891		-	43.99	56.01
8	Blank	1.3389		-	-	-



Figure 3: Graphical representation of Cytotoxicity and percentage of cell viability versus different concentration for Breast cancer cells exposed to the spotted owlet egg yolk RCP antisera, Normal Rabbit serum and standard Drug Doxorubicin

Table 6: Invitro cytotoxicity and viability (Breast cancer cells) of Riboflavin carrier protein (RCP) antisera of spotted owlet egg whit	te,
Normal Rabbit serum and standard Drug Doxorubicin	

S.No	Concentration (µl)	Rabbit serum Absorbance at 570nm (control)	% Viability	RCP Antisera Absorbance (SOWA)	MCF-7 % viability	% Cytotoxicity
1	2	1.9745	100	1.7585	89.060	10.939
2	4	1.7141	100	1.366	79.691	20.308
3	6	1.5916	100	1.2682	79.680	20.319
4	8	1.4943	100	1.1942	79.917	20.082
5	10	1.4304	100	1.1703	81.816	18.183
6	12	1.2916	100	1.0472	81.077	18.922
7	Doxorubicin	0.5891		-	43.909	56.01
8	Blank	1.3389		-	-	-



Figure 4: Graphical representation of cytotoxicity and percentage of cell viability versus different concentrations for Breast cancer cells exposed to the spotted owlet egg white RCP antisera, Normal Rabbit serum and standard Drug Doxorubicin

Table 7: In-vitro cytotoxicity and viability of Breast cancer cells against RCP antisera of hen egg yolk, Normal Rabbit serum and
standard Drug Doxorubicin

S.No	Concentration (µl)	Rabbit serum Absorbance at 570nm(Control)	% Viability	Antisera Absorbance (HYA)	MCF-7 % viability	% Cytotoxicity
1	2	1.9745	100	1.7276	87.495	12.504
2	4	1.7141	100	1.4733	85.951	14.048
3	6	1.5916	100	1.3279	83.431	16.568
4	8	1.4943	100	1.234	82.580	17.419
5	10	1.4304	100	1.178	82.354	17.645
6	12	1.2916	100	1.048	81.139	18.860
7	Doxorubicin	0.5891		-	43.99	56.01
8	Blank	1.3389		-	-	-



Figure 5: Graphical representation of cytotoxicity, percentage of cell viability versus different concentration for Breast cancer cells exposed to the hen egg yolk RCP antisera, Normal Rabbit serum and standard Drug Doxorubicin

Table 8: In-vitro cytotoxicity MCF-7 of RCP antisera hen egg white, Normal Rabbit serum and standard Drug Doxorubicin

S.No	Concentration (µl)	Rabbit serum Absorbance at 570nm (Control)	% Viability	Antisera Absorbance (HWA)	MCF-7 % viability	% Cytotoxicity
1	2	1.9745	100	1.6950	85.844	14.155
2	4	1.7141	100	1.5009	87.561	12.438
3	6	1.5916	100	1.3890	87.270	12.729
4	8	1.4943	100	1.2891	89.267	13.732
5	10	1.4304	100	1.194	83.473	16.526
6	12	1.2916	100	1.001	77.500	22.499
7	Doxorubicin	0.5891		-	43.99	56.01
8	Blank	1.33	39	-	-	-



Figure 6: Graphical representation of cytotoxicity and percentage of cell viability versus different concentrations for Breast cancer cells exposed to the hen egg white RCP antisera, Normal Rabbit serum and standard Drug Doxorubicin

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 Table 9: In-vitro cytotoxicity (HeLa cancer cells) of RCP antisera spotted owlet egg yolk, Normal Rabbit serum and standard Drug Doxorubicin

S. No	Concentration (µl)	Rabbit serum Absorbance at 570nm (control)	% Viability	Antisera Absorbance (SOYA)	HeLa cells % viability	% Cytotoxicity
1	2	1.9654	100	1.8983	96.585	3.414
2	4	1.9248	100	1.8128	95.220	4.779
3	6	1.7597	100	1.6525	93.908	6.091
4	8	1.7135	100	1.6037	93.592	6.407
5	10	1.6524	100	1.5253	92.3081	7.691
6	12	1.4451	100	1.2828	88.768	11.231
7	Doxorubicin	0.8284	-		51.472	48.528
8	Control	1.6094		-	-	-



Figure 7: Graphical representation of cytotoxicity and percentage of cell viability versus different concentration for HeLa cancer cells exposed to the spotted owlet egg yolk RCP antisera, Normal rabbit serum and standard Drug Doxorubicin

 Table 10: In-vitro cytotoxicity HeLa cancer cells of Riboflavin carrier protein (RCP) antisera of spotted owlet egg white, Normal rabbit serum and standard Drug Doxorubicin

S. No	Concentration (µl)	Rabbit serum Absorbance at 570nm (control)	% Viability	Antisera Absorbance (SOWA)	HeLa cells % viability	% Cytotoxicity
1	2	1.9654	100	1.8669	94.988	5.011
2	4	1.9248	100	1.808	93.931	6.0681
3	6	1.7597	100	1.4641	90.612	9.387
4	8	1.7135	100	1.4833	86.565	13.434
5	10	1.6524	100	1.4029	84.900	15.099
6	12	1.4451	100	1.2065	83.468	16.531
7	Doxorubicin	0.8284		-	51.472	48.528
8	Blank	1.609	94	-	-	-



Figure 8: Graphical representation of cytotoxicity and percentage of cell viability versus different concentration for HeLa cancer cells exposed to the spotted owlet egg white Riboflavin carrier protein antisera, Normal Rabbit serum and standard Drug Doxorubicin

2. RIBOFLAVIN CARRIER PROTEIN ANTISERA IC $_{50}$ VALUE OF SPOTTED OWLET EGG & HEN EGG WHITE AND YOLK ON A549 CANCER CELLS (LUNG CANCER CELL LINES), MCF-7 CANCER CELLS (BREAST CANCER CELL LINES) AND HELA CANCER CELLS (HUMAN CERVICAL CANCER CELL LINE)

Table 11: Riboflavin Carrier Protein antisera IC_{50} values of Spotted owlet egg and hen egg white, yolk on A549 Cancer Cells

S.No	Concentration (µl)	Sources of Antisera (RCP)	IC 50 Valves
1	16	Spotted owlet egg white (RCP)	11.64 ± 0.9
2	16	Spotted yolk egg yolk (RCP)	14.89 ± 0.4
3	16	Hen egg white (RCP)	12.46 ± 0.9
4	16	Hen egg yolk (RCP)	11.20 ± 0.8
5	2 μl(μM)	Doxorubicin	10.74 ± 0.3



Figure 9: Comparison of IC 50 Values of Riboflavin Carrier Protein spotted owlet egg and hen egg white, yolk on A549 Cancer Cells and standard Drug Doxorubicin

 Table 12: Riboflavin Carrier Protein Antisera IC 50 Values of spotted owlet egg and hen egg white, yolk on MCF-7 Cancer Cells and standard Drug Doxorubicin

S.N0	Concentration (µl)	Sources of Antisera (RCP)	IC 50 Valves
1	12	Spotted owlet egg white (RCP)	21.11 ± 0.7
2	12	Spotted yolk egg yolk (RCP)	12.16 ± 0.6
3	12	Hen egg yolk (RCP)	21.18 ± 0.5
4	12	Hen egg white (RCP)	17.75 ± 0.8
5	2 µl(µM)	Doxorubicin	8.92 ± 0.4



Figure 10: Comparison of IC 50 Values of Riboflavin Carrier Protein Antisera of spotted owlet egg white, yolk and hen white, yolk on the MCF-7 Cancer Cells and standard Drug Doxorubicin

Table 13: Riboflavin carrier protein (RCP) antiseraIC₅₀ Values of hen white & yolk HeLa Cancer Cells and standard Drug Doxorubicin







Figure 11: Comparison of IC 50 values Riboflavin Carrier Protein Antisera of spotted owlet egg white, yolk on HeLa Cancer Cells and standard Drug Doxorubicin



Figure 12: Cytotoxic Effect of Riboflavin Carrier Protein antisera On the Growth of Tumor cells

Homologous Riboflavin carrier protein are present in the egg of reptiles, birds and in the plasma of pregnant mammals [25]. In the later cases, RfBP has been shown to be necessary for the transfer of riboflavin from mother to foetus. Further, it was demonstrated by immunological interference with the functioning of the RCP/RfBP by passive immunization could result in the impairment of the trans placental transport of vitamin leading to acute embryonic vitamin deficiency who have reported significant elevation of serum RCP/RfBP levels in patients with breast cancer [26]. Earlier, Vaidya and colleagues [27] had reported a decrease in serum Rf levels in breast cancer patients baseline Rf serum levels were achieved after tamoxifen treatment. Hence, Riboflavin carrier protein (RCP) antisera was used to study the cytotoxic effect on the growth of tumor cells.

In this study, we have employed a dose dependent approach to evaluate the toxicity of the antiserum on A549, HeLa and MCF-7 Cancer cell lines at different concentrations (2, 4, 6, 8, 10, 12, 14 and 16µl). The in vitro screening of the antiserum (antibodies of Riboflavin carrier protein) showed potential cytotoxic activity against the HeLa, A549 and MCF-7 cancer cell lines. The antiserum produced against Riboflavin carrier protein from spotted owlet egg white as well as yolk and hen egg white & yolk have been collected from rabbit. The cytotoxic activity was carried out using A549 cancer cells (Lung Cancer cells) (Tables.1, 2, 3, 4) and MCF-7(Breast cancer cells) (Tables. 5, 6, 7, 8 and Figures.1, 2, 3, 4, 5, 6) and Human cervical cancer cell line (HeLa) (Figures.7, 8 & Tables. 9, 10). Reduction in absorbance was seen with antiserum against hen white & yolk as well as spotted owlet white and yolk, the cytotoxic activity was carried out using Human cervical cancer cell line (HeLa). The IC 50 values were calculated three cancer cells A549 cancer cells (Lung Cancer cells), MCF-7(Breast cancer cells) and Human cervical cancer cell line (HeLa) (Figure. 9, 10, 11 and Table. 11, 12, 13). Previous studied Folic acid Binding Protein (FBP) antisera [28] and Riboflavin Binding protein (RfBP) antisera invitro cytotoxic activity on Human cervical cancer cells lines [29]. Hence, in present investigations on Riboflavin carrier protein (RCP) antisera at different concentrations were carried out to test the cytotoxic activity against HeLa cells, MCF-7 and A549 cancer cells. The antiserum against RCP binds to Riboflavin carrier protein and depletes riboflavin in the cancer cell lines (Figure: 12) which might have lead to cell growth inhibition.

DISCUSSION

In this study, we have employed a dose dependent approach to evaluate the toxicity of the antiserum on HeLa, A549 and MCF-7 Cancer cell lines at different concentrations (2, 4, 6, 8, 10, 12, 14, 16µl). The in vitro screening of the antiserum (antibodies of Riboflavin carrier protein) showed potential cytotoxic activity against the HeLa, A549, and MCF-7 cancer cell lines. It is a novel approach in the area of biochemical as well as cancer research which will be useful in targeted therapy of cancer. Future studies like *in-vitro* evaluation by other methods like Brdu, Thymidine uptake inhibition and in vivo evaluation in nude mice etc, help in developing a new strategy of treating cancer effectively. Riboflavin binding protein (RfBP) was purified from spotted owlet (Athene brama) Egg- white, eggyolk and Hen (Gallus gallus) egg-white and egg-yolk. Riboflavin carrier protein (RCP) was isolated first time in India from spotted owlet (Athene brama). These proteins showed a single band on SDS gels and the molecular weight was 29,200 Da but spotted owlet egg yolk Riboflavin carrier proteins showed a single band on SDS gels and the molecular weight was approximately 3 kDa less than hen yolk RCP. Antiserum was raised against these RCP's in rabbit. These proteins are emulsified in Freund's complete adjuvant and injected subcutaneously at weekly intervals for 4 weeks into the rabbit at multiple sites. The rabbit antiserum was collected through the ear vein, 7 days after the final injection. This serum was analyzed by in-vitro method with HeLa (cervical cancer cell lines), A549 (Lung cancer) and MCF-7 (Breast cancer) cells. MTT [(3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide] measures the metabolic activity of the viable cells. The viable cell counting was done with trypan blue dye exclusion method. Hence, in present investigations on Riboflavin carrier protein (RCP) antisera at different concentrations were carried out to test the cytotoxic activity against HeLa cells, MCF-7 and A549 cancer cells. The anticancer activity of Riboflavin Carrier proteins (RCP) purified from egg-white of spotted owlet (Athene brama) antiserum was 16.531 %, egg-yolk antiserum was 11.231 % on HeLa cell lines. Spotted owlet egg yolk antiserum on HeLa cells showed less cytotoxic potential. Riboflavin Carrier proteins (RCP) purified from egg-white of spotted owlet (Athene brama) antiserum cytotoxic effect was 25.171 %, egg-yolk antiserum was 20.104% and Hen eggwhite antiserum was 26.737 %, egg-yolk antiserum was 24.024% on A549 cancer cells. The anticancer activity of Riboflavin Carrier proteins (RCP) purified from egg white of spotted owlet antiserum was 18.922 %, egg-yolk antiserum was 32.842 % and Hen egg-white antiserum was 22.499 %, egg-yolk antiserum was 18.860 % on Breast cancer cells. Highest cytotoxic activity was seen in spotted egg yolk antiserum on Breast cancer cell lines and standard drug used was Doxorubicin (48.528 % of Cytotoxicity). IC₅₀ values for A549 were 12.46 \pm 0.9 & 11.20 \pm 0.8 for hen white, yolk and 11.64 \pm 0.9 & 14.89 \pm 0.4 for spotted owlet yolk and white. MCF-7 cancer cell lines showed IC₅₀ values of $17.75 \pm 0.8 \& 21.18 \pm 0.5$ for hen white , yolk and $21.11 \pm 0.7 \& 12.16 \pm 0.6$ for spotted owlet egg white and yolk respectively and the IC₅₀ values of spotted owlet egg white and yolk on HeLa cancer cells are $24.16 \pm 0.5 \& 35.57 \pm 0.6$, Doxorubicin 10.30 ± 0.4 respectively. The antiserum against RCP must have binded to Riboflavin carrier protein. Due to this depletion of riboflavin in the cancer cell lines leading to cell growth inhibition must have taken place as RCP could not carry riboflavin. No studies were conducted using Spotted owlet Eggs ever before with a specific target towards the cytotoxic activity. We have come out with the anticancer antibodies produced against Riboflavin carrier Protein's of spotted owlet egg white, yolk and hen egg white & yolk.

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