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IN VITRO NEUTRALIZATION OF VIRULENCE PROPERTIES OF STREPTOCOCCUS MUTANS USING CHICKEN EGGYOLK ANTIBODIES (IGY)

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ABSTRACT

Dental Caries is a cause global concern and India is no exception to this condition. The eruption of the teeth during the first few years leads to colonization by *Streptococcus mutans*. The present investigation was aimed to generate chicken eggyolk antibodies against antigens of *S.mutans* and to test their potential both *in vitro* to control the virulence of the cariogenic bacteria. The white leghorn chicken was immunized with each antigen, their eggs collected and the antibodies were purified. The titre of specific antibody was found to be 1:50000 on 56th Day. The protein concentration of the eggyolk was ~40.20±0.70 mg/ml and the total IgY concentration of eggyolk was ~20.74±0.38 mg/ml. Both anti-*S.mutans* IgY and anti-CA-GTF *S.mutans* IgY were able to prevent biofilm formation by *Streptococcus mutans* and to inhibit the sucrose dependant adherence of the bacteria to glass surfaces. Anti-CA-GTF *S.mutans* IgY was able to inhibit GTF activity of *S.mutans* higher than the control. The anti-CA-GTF and WC *S.mutans* IgY was able to neutralize *in vitro* the virulence properties of *S.mutans*.

KEY WORDS: Dental caries, IgY, *Streptococcus mutans*, prevention, *in vitro*.

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INTRODUCTION

General health described as complete state of physical, mental and social well-being[1] contributes to the social, economic and personal development and in essence to human progress. Oral health a vital component of overall health has a significant impact on general health, and healthy quality life[2]. Poor oral health causes pain, anxiety and impaired social functioning, deteriorates individual's physical and mental health cumulatively influencing the economic productivity of the individual and nation[3]. Dental diseases takes a share of 5 to 10% of total health care expenditure in developed countries and is still more worse in developing countries, and the cost of treatment of dental disease would probably exceed the available resources for health care[4]. Of these, dental caries and periodontal diseases are the two important oral health burden[5] which have been strongly related to socio economic factors with the higher incidence in deprived section of the population[6]. In children when left untreated results in pain, bacteremia, speech disability, and premature loss of tooth with the negative impact on chewing, nutrition, lack of weight gain, physical growth, self esteem, and harm to the permanent dentition[7]. Dental caries is a global health problem with a worldwide distribution afflicting all age groups from pediatric to geriatric population having a high predilection for young children. It has reached alarming epidemic proportions in the modern world. Globally, the prevalence of dental caries is 60–90% in school children and among adults is high as the disease affects nearly 100% of the population in the majority of countries. In 2004, WHO updated the epidemiological information available in the database. At present, the distribution and severity of dental caries vary in different parts of the world and within the same region or country. In developing countries, the prevalence of ECC differs according to the group examined, and a prevalence of up to 85% has been reported for disadvantaged groups[8]. In the Western world, the prevalence at 3 years of age was 19.9%, and strong associations were found with socioeconomic status and ethnicity[9]. In contrast, a decline in caries has been observed in most industrialized countries over the past 20 years or so. However, it must be emphasized that dental caries as a disease of children has not been eradicated, but only controlled to a certain degree. In Asia, the age standardized incidence rate per 100,000 population ranges from 0.7 in China to 4.6 in Thailand and 12.6 in India[10]. November, 2007 issue of the Journal of the American Dental Association *Health, Oral Health and Global increase in dental caries* reports "Caries levels for 12 year-olds in developing countries has been increasing constantly and this is particularly alarming owing to the fact that the developing countries represent most of our world."[11] This is largely due to the increasing consumption of sugars and inadequate exposure to fluorides.

Dental caries in rural populations in Tamil Nadu was 47.8% in 1973[12]. 49.8% in 2002 in 5 year old children[13]. Recent studies in India show early childhood caries incidence varying from 20 to 70%[14]. India, is a developing country facing many challenges in providing oral health care to its citizens. 72.2 % of the population reside in villages comprising of 40% children, with the predominance of disadvantaged and socially marginalized people (WHO) having a high prevalence of dental caries owing to their poor access to preventive dental care, inadequate use of fluoride and inadequate knowledge of oral hygiene[15]. Even though it appears that dental caries prevalence has declined with the measures taken such as use of systemic and topical fluorides, toothpastes, sealants, improvements in diet, oral health education and dental care in reality recent studies show increased prevalence due to certain contributing factors such as use of bottled water instead of fluoridated tap water, and dietary changes, shift in populations from rural to urban areas. The perception that dental caries is no longer a problem is disproved as

60% of the children and most of the adults are affected.

An urge for the development of more potent antimicrobial agents that target the suppression of the *Streptococcus mutans*, the etiological agent of dental caries and other pathogens is the need of the hour for dental caries. There are many reports suggesting the possibility of preventing dental caries by vaccination (active immunization) using antigens mutans streptococci whole cell or one of its cariogenic factors CA-GTF. Passive immunization has been explored considering the safety part of it. Chicken egg yolk antibodies, a natural harmless product for local passive immunization is still an underused resource and no study has been done with chicken egg yolk antibodies for dental caries in India while some studies have been conducted in Japan, China and Sweden. Chicken egg yolk has been recognized as an inexpensive alternative antibody source. The effectiveness of passive immunization with egg yolk immunoglobulin (IgY) against antigens of *S.mutans* can prevent the tooth decay by blocking the virulence properties of bacteria and preventing their pathogenesis. Hence, the present investigation is focused to generate chicken antibodies against *Streptococcus mutans*, which if found to be effective can be used for the mass application after long term clinical trial.

MATERIALS AND METHODS

i) Experimental animal used

White leghorn chickens (19 weeks old) were obtained from the poultry farm, Ayyampalayam with good health conditions and maintained in laboratory conditions with normal feeding. They were used for the generation of egg yolk antibodies (IgY) against *Streptococcus mutans* whole cell antigen, cell associated glucosyltransferase antigen and also for the preparation of control IgY.

ii) Preparation of antigen from standard Streptococcus mutans strain

Standard strain *Streptococcus mutans serotype c* (MTCC 497) purchased from Microbial Type of Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh India as a lyophilized culture was used for the study. The culture was confirmed based on microscopic, biochemical and adherence properties. The *Streptococcus mutans* c strain used as antigen was cultured for 24hours in brain-heart infusion broth (BHI broth) containing 5% sucrose at 37 °C under aerobic condition. This formalin killed whole cell antigen samples were kept frozen at -20 °C in aliquots until required after the concentration adjustment to 9 $x10^8$ CFU/ml[16]. Similarly, CA-GTF antigen was also prepared for immunization in chicken[17].

iii) Generation of anti-S.mutans IgY in White leg horn Hens

Antigen prepared were diluted using sterile saline to a cell concentration of 3 x 10^8 cells. 21 weeks old white leghorn chickens were immunized with streptococcal crude antigens for development of egg yolk antibodies. Chicken immunized with 0.5 ml of antigen immunized intramuscularly at four different sites of breast muscles (two sites per left and right) of the chicken. Two weeks after first injection chickens received 2 shots at an interval of 14 days with the same dose and route of administration. Similarly, the CA-GTF antigen was immunized along with the appropriate adjuvant to elicit antibody production in the chicken. The preimmune sera and postimmune sera were collected at specified time intervals during and after the various immunization schedules in chicken and checked for serum antibodies against *Streptococcus mutans* antigen by Slide agglutination and Micro Agglutination test. The eggs were collected and the egg yolk was separated from white. The egg yolk antibodies were purified according to the protocol described by Polson *et al*., (1980). This is a delipidation and precipitation procedure using PEG and ammonium sulphate. The crude anti-*S.mutans* IgY was further subjected to dialysis

and column chromatographic purification. The concentrated IgY was lyophilized and the freeze dried IgY powder was stored under refrigeration and used for further studies. The concentrated protein was then checked for protein estimation[18] antibody titer and the purity of IgY was analyzed by SDS-PAGE[19] and the IgY content was estimated[20].

iv) Determination of antibody titer by Indirect ELISA

The antibody titter of the serum and egg yolk antibodies generated against *Streptococcus mutans* whole cell antigens and CA-GTF were determined by Indirect ELISA[21]. The antigen dose used for coating was 150µl/well *Streptococcus mutans* whole cell antigen solution at a concentration of 10µg/ml (Reference value - 500µg is corresponding to $1x10^8$ CFU/ml[22]) using coating buffer (0.05M Carbonate bicarbonate buffer pH 9.6) and incubated at 4°C over night for binding. All samples were tested in triplicates. The same procedure was used for CA-GTF using CA-GTF as antigen and Anti CA-GTF as antibody.

v) In-vitro efficacy of Anti-Streptococcus mutans IgY

a) Inhibition of biofilm formation in polystyrene microtitre

Biofilm formation in polystyrene microtitre plate was assayed by crystal violet staining method. An overnight culture of *Streptococcus mutans* was diluted 1:100 in fresh BHI broth containing 1% sucrose and 100 *µ*l of aliquots of cell suspension, corresponding to 10^6 CFU were added to the wells of 96-well polystyrene microtiter plates. An equal volume of different concentrations (0 to 1 mg/ml) of filter sterilized anti-*Streptococcus mutans* IgY was added. After incubation for 72 hours at 37°C, media and unattached cells were decanted from the microtiter plates. The wells with adhered biofilms were fixed with formalin (37%, diluted 1:10) plus 2% sodium acetate, and each well was stained with 200 µL of 0.1% Crystal Violet for 15 min at room temperature. After two rinses with distilled water, bound dye was removed from the cells with 100 µL of ethanol/acetone in the proportion of 8:2. Plates were then set on a shaker for 5 min to allow full release of the dye. Biofilm formation was quantified by measuring optical density at 600 nm. Inhibitor-mediated reduction of biofilm formation was correlated to the value obtained without addition of IgY. All assays were run in triplicate, and the means ± SD of three independent experiments were calculated.

b) Inhibition of sucrose dependent adherence to glass surface by IgY[17]

Filter sterilized IgY (0 to 1 mg/ml) with antibody titre of 10^5 were dispensed into tubes 13 mm x 100mm.and then 3 ml of 1.5 fold concentrated BHI medium containing 1.5 % sucrose was added. 100µl of *Streptococcus mutans* standard strain MTCC 497 precultivated in BHI medium was added and allowed to decline at an angle of 30 degrees at 37 degree C for 18 hours. After static incubation the tube was gently rotated and the whole fluid containing the solution was discarded. Then 3 ml of 50mM phosphate buffer (pH 6.8) solution and the tube was again rotated and the resultant solution was discarded. 3 ml of 50mM phosphate buffer solution was added and subjected to ultrasonication procedure to prepare homogenous cell suspensions. The same procedure was run with nonimmune IgY instead of Immune IgY for control. Optical density of the suspensions was read at 600nm. All assays were run in triplicate, and the means ± SD of three independent experiments were calculated. The % of adherence was determined using the equation: The % of adherence was determined using the equation: (O.D. of the test / O.D. of non-treated control) x 100.

c) Saliva coated Hydroxyapatite adsorption inhibition assay[23]

Whole saliva was collected on ice from 1 donor after chewing sterile paraffin wax for 2 minutes, and it was clarified by centrifugation (8,500 *g,* 4 ° C, 10 min) [24] and filter sterilized with 0.22 µm Acrodisc syringe filter. Sterile Hydroxyapatite blocks 5 x 5 x 5 mm (IFGL, Bio ceramics Limited, India) were placed in a plastic tube and coated with filter-sterilized clarified human whole saliva for 2 h at 37°C with gentle shaking and air dried for 30

minutes. *Streptococcus mutans* standard strain MTCC 497 cultured for 20 hours at 37^0 C. was harvested by centrifugation, washed 3 times with 0.05M phosphate buffer (pH 7.2) and re suspended in the same buffer to give a bacterial suspension of 1.0 at 660 nm. Different concentrations of immune IgY were dissolved in phosphate buffer to get a final concentration of 1 to 2.5 mg/ml and 200ul of the solution was incubated with 2 ml of bacterial suspension ($1x10^{6/}$CFU/ml) for 30 minutes at 37^0C. The bacteria were collected by centrifugation, washed with Tris-HCL buffer and re suspended in 2 ml of the same buffer. Same procedure was repeated with preimmune IgY instead of immune IgY. Saliva coated HA blocks were immersed in a suspension of bacteria ($1x10^{6/}$CFU/ml) which were untreated or pretreated with immune or control IgY and incubated with bacteria for 90 minutes at 37°C with gentle agitation. The blocks were washed and transferred to a tube containing phosphate buffer. The bacteria adsorbed to the HA blocks were dispersed by sonication using three 30-second pulses at an output of 7 W. Bacterial suspension obtained was serially diluted and plated on Mitis Salivarius Agar. After incubation for 48 hours at 37°C the number of colony forming units was determined. Colony counts were expressed as a CFU per unit area of the specimens (cm^3).

d) CA-GTF activity inhibition assay by determining inhibition of water insoluble glucan synthesis by immune IgY

The CA-GTF enzyme 100 *µ*l was added to 100 *µ*l of Antibody to whole cell of *Streptococcus mutans* of different concentrations (0 to 1 mg/ml) in 0.1 ml of 0.5 M phosphate buffer pH 6.8 and incubated for 30 min at 37°C. After incubation 1.5 % sucrose and buffer was added to a final volume 0.5 ml and incubated for 2 h at 37°C. The reaction was terminated by cooling the reaction to 4 degree C. Then it was centrifuged at 1600xg and the water insoluble fraction obtained was washed twice with distilled water and suspended in 3 ml distilled water to obtain a water insoluble glucan fraction. Water insoluble glucan was dissolved in 0.5N NaOH and the glucose content quantified by phenol sulphuric acid method[25].

RESULTS AND DISCUSSION

i) Generation and Characterization of the anti-S.mutans IgY

Standard stain obtained from culture collection center was revived as per protocol and confirmed as Gram positive cocci in pairs and chains, Slightly α hemolytic, small, gray circular colonies on Blood agar and medium sized, raised convex pale blue glistening frosted glass appearing colonies on Mitis Salivarius agar supplemented with 20% sucrose. Formalin inactivated whole cell *S.mutans* antigen and Cell associated Glucosyltransferase antigen prepared from *Streptococcus mutans* were used for immunization. Characterization of CA-GTF antigen was done by SDS-PAGE. Activity of the crude GTF enzyme preparation was 2.4U/mg of protein with a molecular weight of 160kDa.

Figure 1
Characterization of CA-GTF antigen on SDS-PAGE

Lane 1: Protein Marker
Lane 2: *S.mutans* CA-GTF *antigen*
(M.wt. 160KDa)

21 weeks old white leghorn chickens were immunized with the two antigens intramuscularly at four different sites of breast muscles. The specificity of Anti-*Streptococcus mutans* antibodies for whole cell and CA-GTF antigens in the serum and egg yolk from immunized laying hens was determined by Rapid slide agglutination Test (RSA). Appearance of agglutination within 2minutes, when the antigen was mixed with the corresponding IgY on plastic strip, revealed that the antibody generated in the chicken serum and the purified IgY-extracts from eggs of immunized chicken were specific against to their respective antigens. The specific antibody level in the egg yolk was begun to appear in day 14 from the date of first immunization. The peak titre of IgY up to 1:2560 for *Streptococcus mutans* whole cell antigen till 150days as determined by Micro agglutination test (MAT).

Table 1
Titre of Anti-Streptococcus whole cell antigen-IgY in Egg Yolk by MAT

Days after Immunization	Two fold Dilution								
	1:10	1:20	1.40	1:80	1:160	1:320	1:640	1:1280	1:2560
0	+	+	-	-	-	-	-	-	-
7	+	+	-	-	-	-	-	-	-
21	+	+	+	+	+	-	-	-	-
35	+	+	+	+	+	+	+	+	-
49	+	+	+	+	+	+	+	+	+
63	+	+	+	+	+	+	+	+	+
77	+	+	+	+	+	+	+	+	+
91	+	+	+	+	+	+	+	+	+
105	+	+	+	+	+	+	+	+	+
119	+	+	+	+	+	+	+	+	+
133	+	+	+	+	+	+	+	+	+
150	+	+	+	+	+	+	+	+	+

+ : Presence of Agglutination; - No Agglutination

The purity of chicken egg yolk antibodies and their molecular weight as determined by Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis revealed a 180kDa protein band. The total protein concentration by Lowry *et al.,* (1951) method reached maximum of 40.61 ± 0.30 mg/ml on 119th day and 40.20 ± 0.70 mg/ml on 133rdday for anti-*S.mutans* IgY and anti-CA-GTF *S.mutans* IgY respectively. The total IgY concentration photometrically at 280nm with the extinction co-efficient of 1.33 for IgY was maximum of 20 .74 ± 0.38 mg/ml and 18.72 ± 0.38 mg/ml for anti-*S.mutans* IgY and anti-CA-GTF *S.mutans* IgY respectively.

Table 2
Protein and Total IgY concentration of IgY extract

Weeks after Immunization	Age of the Birds	Anti-whole cell *S. mutans*-IgY		Anti- CA-GTF *S. mutans*-IgY	
		Protein Conc. (mg/ml)	Total IgY (mg/ml)	Protein Conc. (mg/ml)	Total IgY (mg/ml)
1st	19	17.86 ± 0.78	6.77 ± 0.86	16.23 ± 0.32	5.77 ± 0.86
7th	25	33.44 ± 1.58	18.35 ± 0.57	36.25 ± 0.68	15.75 ± 57
14th	32	39.77 ± 0.76	20.82 ± 0.82	39.65 ± 0.81	17.42 ± 0.82
20th	38	40.61 ± 0.30	20 .74 ± 0.38	40.20 ± 0.70	18.72 ± 0.38

**** Values are mean of quadruple samples***

The specific antibody level in chicken serum and egg yolk against Anti-whole *cell Streptococcus mutans*, and Anti- CA-GTF *Streptococcus mutans* were determined by

Indirect ELISA as against whole cells and CA-GTF as antigen and mentioned as optical density (OD) at 450nm (ELISA value). The level of specific antibodies against whole cell *Streptococcus mutans* antigen and CA-GTF antigen in serum was increased after 1 week and slowly it reached the maximum titre on day 28th day from the date of initial immunization respectively. However, the specific antibody level in the egg yolk gradually increased and reached the peak on 49th day for of whole cell and CA-GTF antigen of *Streptococcus mutans* and the titre was maintained till 150 days. The maximum titre of antibody obtained was 50,000 for both the antigens.

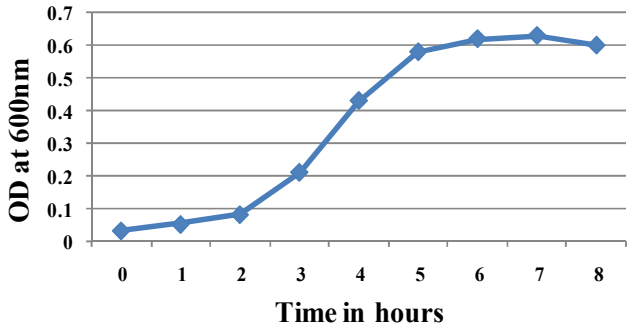
Table 3
Dynamics of Antibody Titres in Serum and Egg Yolk of Hens immunized with whole cell and CA-GTF antigen of Streptococcus mutans

Week	O.D at 450nm			
	Anti WC of *S.mutans*		Anti CA-GTF of *S.mutans*	
	Serum	Eggyolk	*Serum*	*Egg yolk*
0	0.5	0.3	0.5	0.2
7	2.7	3.5	2.6	2.9
14	2.6	3.3	2.4	3.1
21	2.6	3.3	2.4	3.1

ii) In vitro efficacy of Streptococcus mutans IgY
a) Growth Inhibition assay

In-vitro efficacy of anti-*Streptococcus mutans* IgY was determined to investigate whether the specific binding activity of anti- *Streptococcus mutans* IgY could inhibit the growth of *Streptococcus mutans* in a liquid medium. The Growth curve of *Streptococcus mutans* in liquid medium was plotted until the stationary phase reached. The growth of *Streptococcus mutans* showed a pattern of lag phase (0 to 2 hours), exponential phase (2 to 6 hours) and then stationary phase.

Figure 2
Growth Curve of Streptococcus mutans



Streptococcus mutans was grown in tryptic soy broth along with different concentrations of *Streptococcus mutans* specific IgY and non-specific IgY separately at 37ºC for 6 hours with shaking. Then samples were taken at 2 hours intervals to perform the growth inhibition assay by measuring the optical density. The growth of *Streptococcus mutans* when incubated with specific IgY or non-specific IgY was compared with the normal growth pattern of *S.mutans* in Tryptic-soy broth. A significant reduction in the growth of *Streptococcus mutans* was observed after 4 hours of incubation when grown with 25 µg of specific IgY. However, control IgY had no effect on bacterial growth.

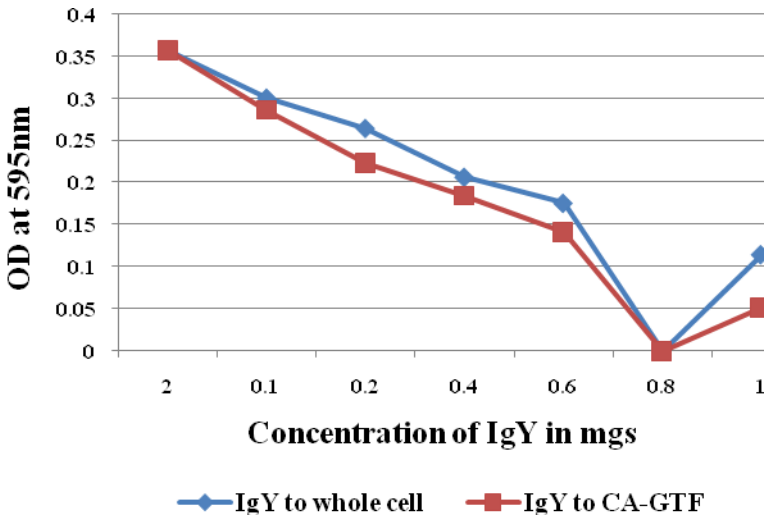
Table 4
Growth inhibition assay of Anti-Streptococcus mutans –IgY

Concentration of IgY to whole cell *Streptococcus mutans* in µg	O.D at 0 hours	O.D at the end of 2 hours	O.D at the end of 4 hours	O.D at the end of 6 hours	O.D at the end of 8 hours
5	0.25	0.165	0.379	0.379	0.39
10	0.25	0..104	0.245	0.245	0.2
15	0.25	0.038	0.113	0.113	0.12
20	0.25	0.031	0.09	0.09	0.1
25	0.25	0.029	0.068	0.068	0.07
30	0.25	0.027	0.053	0.053	0.058
Preimmune IgY 30 µg (control)	0.025	0.34	0.579	0.983	1.1
Amoxycillin 60 µg	0.025	0.025	0.025	0.025	0.025

b) Inhibition of biofilm formation by Anti-Streptococcus mutans–IgY

The efficacy of immune IgY in inhibiting biofilm formation prior to the application of *in vivo* models of dental caries and subsequent use in human trials. Biofilm formation in polystyrene microtitre plate in the presence of different concentrations (0 to 1 mg/ml) of filter sterilized anti-*streptococcus mutans* IgY and 1% sucrose containing BHI broth was assayed by crystal violet staining method quantified by measuring optical density at 595 nm. The Immune IgY was able to inhibit biofilm formation when compared to the control IgY.

Figure 3
Inhibition of Biofilm formation in polystyrene wells by immune IgY to whole cell and CA-GTF of Streptococcus mutans



c) Inhibition of sucrose dependent adherence to glass surface by IgY

Adhered material was subjected to ultrasonication and quantitated by measuring at 595nm. The % of adherence was determined using the equation: (O.D. of the test / O.D. of non-treated control) x 100. The inhibition of the adherence was highest in CA-GTF IgY when compared with whole cell IgY. The control IgY did not possess significant inhibitory effect on *S.mutans* adherence.

Table 5
Percentage of inhibition of adherence to glass surface by immune IgY to whole cell and CA-GTF of Streptococcus mutans

Concentration of IgY (in mgs)	% of inhibiton of adherence(Immune IgY to whole cell)	% of inhibiton of adherence(Immune IgY to CA-GTF)
0.1	10.8	24.4
0.2	21.1	40.3
0.4	32.2	63.2
0.6	45.5	72.7
0.8	58.4	87.6
1	69.6	98.3
Pre immune IgY 2mgs	4	4

d) Assay of Saliva coated Hydroxyapatite (S-HA) adsorption inhibition by IgY

Significant inhibition of bacterial attachment to S-HA was observed when *S.mutans* were treated with 2.5 mg of IgY to CA-GTF. Alternatively when S-HA was pretreated with 2.5mg of IgY to Whole cell-*S.mutans* inhibition was comparatively less but still showed significant inhibition. Inhibition increased with increasing concentration.

Table 6
Assay of Saliva coated Hydroxyapatite adsorption inhibition by IgY

Concentration of immune IgY in mgs	IgY to whole cell antigen		IgY to CA-GTF antigen	
	CFU/unit area of the specimen x 10^4	Percent inhibition	CFU/unit area of the specimen x 10^4	Percent inhibition
1	5.1	17.8	3.8	38.8
1.5	3.8	38.8	2.5	59.7
2	2.6	58.1	1.7	62.6
2.5	1.9	69.4	0.52	91.7
Pre immune IgY 2mgs	6.2 x 10^4			

e) Inhibition of CA-GTF activity by IgY by determination of glucan synthesis

Inhibition of water insoluble glucan synthesis by IgY against whole cell and CA-GTF of *Streptococcus mutans* in the presence of various concentrations of CA-GTF and 1.5% sucrose is shown below. Inhibition increased with increasing concentrations. 1mg of Immune IgY against CA-GTF showed 100% inhibition of glucan synthesis whereas the immune IgY against Whole cell *S.mutans* revealed 64% inhibition. The tests were done in triplicates.

Table 7
Effect of immune IgY on glucan synthesis in the presence of sucrose

Immune Igy Concentration in mgs	IgY to whole cell *Streptococcus mutans*		IgY to CA-GTF of *Streptococcus mutans*	
	Glucan synthesis µmol/minx10^2	Percent inhibition	Glucan synthesis µmol/minx10^2	Percent inhibition
0.1	4.74	14	4.14	24
0.2	4.66	22	3.01	43
0.4	3.71	38	2.27	62
0.6	2.29	45	1.32	78
0.8	2.5	57	0.60	90
1	2.15	64	0	100
Pre-immune IgY 2mgs	5.97	-	5.97	-

CONCLUSION

Dental caries is one of the most prevalent diseases which continue to plague most of the population in the world in spite of measures taken to contain this disease[26]. An additional concern is that epidemiological surveys have shown that tooth decay may be increasing again[27]. Dental procedures for prevention of caries is quiet uneconomical as quoted by Walter J. Loesche in the article "*Role of* Streptococcus mutans *in Human Dental Decay*", "This level of professionally delivered tooth debridement is so labor intensive that its cost would make it economically unavailable to most individuals." indicating the importance of finding economically viable alternatives for the prophylaxis or treatment of caries[29]. The results of this study provides a platform for the control of dental caries using antibodies prepared from chicken eggyolk (IgY) against the pathogenic cariogenic bacteria, *Streptococcus mutans.* It was revealed that by blocking the mode of action of the cariogenic *Streptococcus mutans,* it could be possible to prevent the initiation of dental caries in experimental animals challenged with the pathogen. With further human trials and testing, these antibodies specific against *S.mutans* could be used in an oral formulation to help in curbing the large incidence of this non-communicable and distressing condition of dental caries. The study could form a platform for further research on egg yolk antibodies and its commercial application against dental caries in India.

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