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RESEARCH ARTICLE

EVALUATION OF ANTIMICROBIAL ACTIVITY OF IgY AGAINST P. GINGIVALIS – AN INVITRO STUDY

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ABSTRACT

Background*:* Immunoglobulin Y (IgY) found in egg yolks performs similarly to mammalian IgG, but with distinct physicochemical characteristics and biological activity. An innovative form of immunotherapy with Immunoglobulin Y is used to promote passive immunity and is regarded as a secure substitute for antibiotics in the treatment of periodontitis. ***Aim****:* A study was conducted to determine the minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) of Ig Y against *P.gingivalis.* ***Material and Methods:*** *P.gingivalis* from stock bacteria was cultured and then incubated for 18 - 24 hours at 37°C. Colonies were added to 3 ml of liquid Brain Heart Infusion media, and they were then incubated at 37 °C for 18 hours. The turbidity of 0.5 McFarland standards, which is equal to 108 CFU/ml, was used to compare the bacterial suspension in BHI media. IgY that had been extracted and purified was tested at concentrations ranging from 5 mg/ml to 50 mg/ml. IgY was added to culture plates, and a clear zone—a sign that the addition had inhibited bacterial growth—was noticed. ***Results****:* The antimicrobial activity against *P.gingivalis* with a ZOI of 13-14mm at 320µg/mL was noted. MIC showed promising results above 360µg/mL 50% reduction and 380µg/mL 90% reduction. ***Conclusion****:* A Concentration of 320µg/mL of IgY is required to cause an inhibitory effect on *P.gingivalis.* IgY as LDD or as an adjunct to SRP can have a promising effect on the healing of periodontal diseases.

Key words: *Immunoglobulin Y, Minimum inhibitory concentration (MIC), P.gingivalis, Zone of inhibition (ZOI).*

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INTRODUCTION

The periodontal ligament, cementum, connective tissue, and alveolar bone are all destroyed by periodontitis, a chronic inflammatory disease of the supporting tissues of the teeth. It is the main cause of tooth loss in adults and is brought on by an overgrowth of gram-negative, primarily anaerobic bacteria at subgingival locations. (Yokoyama, 2007) *Porphyromonas gingivalis (P. gingivalis*), a Gram-negative anaerobe, is one of the many bacterial species linked to the onset of periodontitis and is considered a keystone pathogen in the cause of chronic periodontitis (Slots, 2000) *P. gingivalis* can bypass host defenses and penetrate periodontal tissues. As a result, the host's immunological and inflammatory responses are dysregulated (Bostanci, 2012) Therefore, *P.gingivalis*-specific therapy approaches may be effective to stop it from colonising the subgingival region. Egg yolks are a great source of immunoglobulin. To give the chick immunity, antibodies are transferred from the hen's blood into the egg yolk in avian eggs. Immunoglobulin Y (IgY), the name given to these egg yolk antibodies, performs a comparable function to mammalian IgG but differs in its physicochemical makeup and biological activity. Immunoglobulin Y is significantly different from mammalian IgG in terms of structure (Bostanci, 2012). The smallest concentration of a chemical, typically a medicine, required to stop bacterial growth is known as the minimum inhibitory concentration. In a lawn of cultivated organisms on a solid medium, a zone of inhibition is a region of growth inhibition brought on by the action of a chemical that inhibits growth, such as an antibiotic agent, which is present at the source. Due to its cost-effectiveness and safety as an alternative to antibiotics, the use of IgY as a novel modality of immunotherapy to bestow passive immunity has attracted growing interest. IgY's usage as a local therapeutic agent in the periodontal pocket would be facilitated if its efficiency against *P. gingivalis* could be established (Kovacs Nolan, 2012).

Aim of the Study: The study aims to evaluate IgY's efficiency in combating *P. gingivalis.*

Objectives of the Study:

- To determine the Minimum inhibitory concentration of IgY against *P. gingivalis*
- To measure the amount of Zone of Inhibition using the well diffusion method.

MATERIALS AND METHODS

Microbial sample preparation: In Bangalore's Laboratory of Microbiology, Dextrose Technologies Pvt ltd, *P. gingivalis* was resurrected from the stock bacterium. Before the study began, the microorganisms were subcultured and reidentified. On Blood agar media, the bacteria were grown before being incubated at 37°C for 18 to 24 hours. A bacteria colony was removed from the subculture media, added to 3 ml of liquid Brain Heart Infusion (BHI) media, and

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then cultured for 18 hours at 37°C in the incubator. Turbidity of 0.5 McFarland standards, or 108 CFU/ml, was used as a benchmark for the bacterial suspension that resulted in BHI media.

Extraction and purification of Immunoglobulin Y from egg yolk were carried out as described below.

1. The yolk is first delicately transferred to a "yolk spoon" to scrape off as much egg white as is practical after making a small fracture in the eggshell.
2. After rolling the yolk on a piece of filter paper to separate any residual egg white, the yolk layer is sliced using a lancet or other pipette-like tools. The egg volume is measured after the yolk has been added to a 50 ml tube.
3. The egg yolk is first mixed twice with PBS (phosphate buffered saline), and then 3.5% of the volume is added of polyethylene glycol and vortexed. Finally, the mixture is rolled in a rolling mixer for 10 minutes. The suspension is divided into two phases during this step of the extraction process. "Yolk solids and fatty substances" make up one phase, which is followed by a protein-rich fluid phase that includes IgY.
4. Centrifugation is performed on the tubes at 4 °C for 20 min (10,000 rpm, 13,000 x g, Heraeus Multifuge 3SR+, fixed angle, non-rotating rotor). The supernatant (V3) is transferred to a fresh tube after being put through a folded filter.
5. Add 8.5% PEG 6000 in gram to the tube, vortex it, and roll it on a rotating mixer, similar to the procedure mentioned in point 3 (estimated based on the increased volume).
6. Repeat the procedure mentioned in point 4 with the exception that you discard any extra fluid.
7. The pellet is carefully dissolved in 1ml of PBS using a glass stirrer and vortexed. Add PBS to a total volume of 10 ml. After the solution has been blended with 12% Polyethylene glycol 6000 it is processed as in step 3.
8. Repetition of point 6 and careful dissolution of the pellet in 800 µl Phosphate buffered saline. Transfer (pipette) the extract to a dialysis capsule after observing the air bubbles dissolve. Add it to the dialysis machine after rinsing the tube with 400 µl of PBS.
9. The extract is gently agitated with a magnetic stirrer while being dialyzed overnight in 1,600 ml of 0.1% saline. The saline is changed to PBS the following morning, and dialyzing continues for a further three hours.
10. The IgY is extracted from the dialysis chamber using a pipette and put into a 2ml tube. There are around 2 ml of extract in total.
11. The Lambert-Beer law is used to determine the protein level of the samples, with an absorbance value of 1.33 for IgYto show the quality of the preparation (80% pure) and is quantified with photometry at 280 nm (1:50 dilution with Phosphate buffered saline).

Method to determine Minimum Inhibitory Concentration: IgY was diluted into concentrations from 5mg/ml to 50mg/ml and taken in test tubes, then *P.gingivalis* is added into each test tube and the least concentration at which *P.gingivalis* is destroyed was checked with a spectrophotometer.

Well diffusion method: The extracted IgY was added to the culture plates using the well diffusion method, and the diameter of the clear zone, a sign of how successfully an antibacterial agent inhibited bacterial growth, was measured. It was possible to see the establishment of inhibitory zones around the pits by observing bacterial growth. Using a calliper, the diameter of the inhibition zones—clear spaces around the wells where no bacterial growth exists—was measured.

Statistical Analysis: Version 22.0 of the Statistical Package for Social Sciences (SPSS) for Windows was released in 2013. IBM Corp., Armonk, New York, were used for statistical analysis. The mean and standard deviation for quantitative variables, and frequency and proportions for categorical variables, were used in the descriptive analysis of all the explanatory and outcome parameters.

Statistical Inference: To compare the mean Zone of Inhibition and Minimum Inhibitory Concentration between Standard Drug & IgY against *P. Gingivalis* at various concentrations, the One-way ANOVA test, and LSD post hoc test were used.

RESULTS

Independent Student t-test results demonstrate that the mean Zone of Inhibition for *P. Gingivalis* was significantly higher inthe Triple Antibiotic group (23.67 ± 0.58) as compared to the IgY group (13.67 ± 0.58) with a mean difference of -10.00 (95% CI -11.31 to -8.69). At $P < 0.001$, the mean difference among the two groups was statistically significant. (Table 1). The antimicrobial activity against *P.gingivalis* with a ZOI of 13-14mm at 320µg/mL was noted. MIC showed promising results above 360µg/mL with a 50% reduction and at 380µg/mL there was a 90% reduction.

Table 1. Comparison of mean Zone of Inhibition for *P. Gingivalis* between IgY& Triple Antibiotic groups using Independent Student t Test. *(IgY: Immunoglobulin Y, CI: confidence interval, SD: Standard deviation)

Comparison of mean Zone of Inhibition for *P. Gingivalis* between IgY& Triple Antibiotic groups using Independent Student t Test								
Group	N	Mean	SD	Mean diff	95% CI of the diff		t	P Value
					Lower	Upper		
Ig Y	3	13.67	0.58	-10.00	-11.31	-8.69	-21.213	<0.001*
Triple antibiotic paste	3	23.67	0.58					

DISCUSSION

Immunotherapy against dental caries and periodontitis has included passive immunisation with antibodies from a variety of species. It is unnecessary to employ genetically modified organisms or bleed animals to create antibodies when using IgY for passive immunisation. The ability to acquire up to 40mg of IgY from a single egg makes it convenient and affordable to produce polyclonal antibodies in eggs (Smith, 2015). In the early stages of a chick's life, chicken immunoglobulin Y (IgY), which is contained in egg yolk, serves as the primary innate immune response against systemic infections. Important bacterial illnesses including *colibacillosis* and *salmonellosis* can cause early chick death (Majumder, 2020). However, it is thought that indigenous day-old chicks have a higher survival rate against such illnesses than farm-raised day-old chicks, which may be attributable to the IgY activity found in egg yolk. A 2019 study compared the levels of IgY in farm and village chicken eggs as well as the antibacterial activity of IgY in both egg types against *Salmonella* (Madushika, 2019). The antimicrobial resistance of extracted IgY against *Salmonella sp*. isolated from dead day-old chicks was assessed. By adjusting the turbidity of the broth to the 0.5 McFarland standard, the agar well diffusion test was used to determine the inhibition reactions of IgY. In samples from a farm and a hamlet, the extracted protein concentration was (7.35±0.92) and

(7.12±0.93) respectively. Village chicken eggs had a larger IgY output than farm chicken eggs, while statistically speaking, there was no difference between the two. Inhibition zones (mm) of IgY recovered from the farm and village chicken eggs were identical. Finally, IgY isolated from farm and village chicken eggs demonstrated a comparable antibacterial activity against *Salmonella.*

To ascertain the impact of IgY on the development of plaque bacteria, an in vitro study was carried out in 2016. Blood serum from Hysex Brown chickens was obtained and tested for IgY-specific *S. mutans* (Juni, 2016). The diffusion method was used to examine the antibacterial effects of IgY on the development of the oral bacteria *S. alpha, S. aureus,* and *S.mutans*. IgY did not affect the growth of *S. alpha* and *S. aureus*, while it had a favourable effect on *S. mutans*. Statistical analysis revealed considerable variations in how IgY serum affected *S. mutans'* inhibitory zones. This study is the first to document ZOI and MIC of IgY against *P.gingivalis,* to the best of the author's knowledge. As a result, it is impossible to accurately compare the findings of the current investigation to those of earlier investigations that have been published.

CONCLUSION

We can infer that an inhibitory impact on *P. gingivalis* requires an IgY concentration of at least 320 g/mL. At 320 g/mL, *P. gingivalis* with a Zone of Inhibition of 13–14 mm had antibacterial activity. Periodontal diseases may respond favourably to the use of IgY as a local drug delivery agent or as a supplement to scaling and root planing.

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