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Evaluating antimicrobial efficacy of bovine colostrum in patients suffering from periodontal disease: An invitro study

Maitri Ketan Pandya
Post Graduate Student, College of Dental Sciences and Research Centre, Manipur, Ahmedabad, India
*Corresponding author email: pandyamaitri1996@gmail.com

Anita Panchal
Professor& HOD, College of Dental Sciences and research centre, Manipur, Ahmedabad, India

Faizan Ather Khan
Post Graduate Student, College of Dental Sciences and Research Centre, Manipur, Ahmedabad, India

Rachana K. Pandya
Post Graduate Student, College of Dental Sciences and Research Centre, Manipur, Ahmedabad, India

Dinesh Khandhadia
(M.Sc. {Medical}, Fellow Researcher, Gujarat University), Ph. D. Scholar, Micro-Aid Diagnostic Laboratory, Ahmedabad, India

***Abstract*---**Purpose: Bovine Colostrum is the first milk, a mammary secretion produced by all cows for their babies during the first 24–48 hours after birth. Bovine Colostrum contains immunoglobulins and lactoferrin that help in bactericidal and bacteriostatic effects against microorganisms. The purpose of this study is to evaluate the efficacy of bovine colostrum at different concentrations in patients suffering from chronic periodontitis. Methodology: Plaque and calculus samples were obtained from 15 patients aging from 30 - 60 years. The collected samples were then poured on agar plates and different strains of Gram -ve bacteria were obtained. The streak plate method was adopted. For the anti-microbial susceptibility test, a solution of Bovine Colostrum Powder was prepared in Dimethyl sulfoxide (DMSO) at various concentrations (50 µg/ml, 100 µg/ml, and 200 µg/ml). Result: A minimal zone of inhibition was seen at 50 µg/ml with a mean of 3.8. Proper inhibition zones were seen in 100 µg/ml and 200 µg/ml with

means of 9.9 and 12.7 respectively. One-way ANOVA showed a statistically significant difference in the values between the groups ($p<0.01$). Tukey HSD Post Hoc Test showed a statistically significant difference in the values between all the pairs of groups ($p<0.01$). Conclusion: Bovine Colostrum shows significant antimicrobial activity against gram -ve bacteria and thus might help target bacterial infections.

***Keywords*---**bovine colostrum, anti-microbial activity, plaque, periodontitis.

Introduction

Colostrum, also known as first milk, is a mammary secretion produced by all mammals for their babies during the first 24–48 hours after birth.Newborn infants receive colostrum from their mothers during the first few hours after birth which creates the foundation of lifelong immunity. Bovine colostrum (BC) is a thick, sticky, yellowish liquid containing several antibodies at a higher concentration than that of ordinary milk. [1] Nutritional components [2], immunological factors [3], and growth factors [4] are the three main areas in which Bovine Colostrum components can be classified. Antiviral, antifungal, and antibacterial characteristics are present in bovine colostrum enabling it to destroy various infections. Bovine Colostrum shows activity against Candida albicans, Helicobacter pylori, five forms of Streptococci, and Cryptosporidium opportunistic infections. [5] Immunoglobulins, lactoferrin, and cytokines present in Bovine Colostrum have been shown to have potent antiviral properties. [6]

Periodontitis is defined as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation, and gingival recession, or both. [7] Periodontal disease is a complex infectious disease caused by a combination of bacterial infection and host response to bacterial challenge, and the disease is altered by environmental, acquired risk factors, and genetic susceptibility. The dental plaque represents an example of both a biofilm and a microbial community that displays emergent properties. [8] Our present study aimed to determine the antimicrobial activities of Bovine Colostrumat different concentrations using antimicrobial susceptibility tests by obtaining microorganisms from plaque and calculus in patients suffering from chronic periodontitis.

Materials and Method

The test sample (plaque and calculus) used in this study was obtained from 15 systemically healthy patients aging from 30 years to 60 years. Patients who had periodontitis Stage II Grade B were included in the study. Patients with any systemic history or patients who were on any medication and pregnant or lactating women were excluded from the study. Nutrient agar, MacConkey agar, blood Agar, and nutrient broth were supplied by HiMedia, and the Cooked Meat Medium with Glucose, Hemin, and vitamin K was also supplied by HiMedia.

Colostrum was obtained from Nutriherbs, a nutraceutical company based in New Delhi. Dimethyl sulphoxide was obtained from S D Fine-Chem Limited. Carrageenan (AR grade) was obtained from Sigma Aldrich. The samples were collected using a swab and were inserted in a cooked meat medium with Glucose, Hemin, and vitamin K at the bottom of the container (Figure 1).The samples were kept in the medium for the proper cultivation of organisms. This medium is useful as an enrichment broth for cultivating organisms from very small inoculations. This medium preserves the viability of organisms over a long period. The bacterial strains obtained from the medium were cultured on nutrient agar slants. The cultures were maintained by subculturing periodically and stored at a temperature of 4°C before use. The collected samples were then poured on Mc Conkey Agar, Blood Agar, and Mullen Hilton Agar (Figure 2). The agar plates were kept for incubation at a temperature of 37 °C for 24hrs.Different strains of Gram-negative bacteria were obtained. The streak plate method was chosen for the cultivation of bacteria. Solution of Bovine Colostrum Powder was prepared in Dimethyl sulfoxide (DMSO) at various concentrations (50 µg/ml, 100 µg/ml, and 200 µg/ml). The study was divided into 3 groups Group 1 (50 µg/ml), Group 2 (100 µg/ml), and Group 3 (200 µg/ml). Nutrient agar was autoclaved at 120°C, 15psi, and gram-ve bacterial strains were added into autoclaved nutrient agar, poured into the sterile petri dish, and allowed to set, all the procedures were performed in laminar airflow. Wells were made in the pre-solidified nutrient agar plate using a metal borer. The test samples were added to respective wells and kept for incubation at a temperature of 37 °C for 24 hrs. After 24 hours inhibitory zones were seen (Figure 3) and the data is shown in Table 1.

Figure 1. Cooked meat medium with Glucose, Hemin, and vitamin K

Figure 2. Mc Conkey Agar plate showing growth of Gram -ve bacteria

Figure 3. Inhibition zones obtained in different concentrations of Bovine colostrum

Statistical analysis

Normality of numerical data was checked using the Shapiro-Wilk test & was found that the data followed a normal curve; hence parametric tests have been used for comparisons. Intergroup comparison (>2 groups) was done using one-way ANOVA (Table 2) followed by pair wise comparison using a post hoc test (Table 3).

Result

A total of 15 samples were collected from systemically healthy patients suffering from chronic periodontitis. Bovine Colostrum showed potent antibacterial activity against bacteria found in plaque and calculus. Zones of inhibition were displayed by Bovine Colostrum at different concentrations as shown in Table 1. A minimal zone of inhibition was seen in Group 1(50 µg/ml) with a mean of 3.8. Proper inhibition zones were seen in Group 2(100 µg/ml) and Group 3(200 µg/ml) with means of 9.9 and 12.7 respectively. The mean of Group 1, Group 2, and Group 3 are 3.8, 9.9, and 12.7 with a mean ± SD of 0.52, 0.87, and 0.79 respectively and mean ± SE of 0.13, 0.22 and 0.20 respectively. One-way ANOVA showed a statistically significant difference in the values between the groups ($p<0.01$) as shown in Table 2.Tukey HSD Post Hoc Test showed a statistically significant difference in the values between all the pairs of groups ($p<0.01$) as shown in Table 3. This result suggests that Bovine Colostrum might help target bacterial infections.

Table 1
Values of the efficacy of Bovine Colostrum Powder mixed with DMSO at different Concentrations

BOVINE COLOSTRUM EFFICACY AT DIFFERENT CONCENTRATIONS			
Bacterial culture	Group 1 (50 µg/ml)	Group 2 (100 µg/ml)	Group 3 (200 µg/ml)
1	4.14mm	11.18 mm	13.46mm

2	3.57mm	8.98 mm	12.56mm
3	4.06mm	9.85mm	12.89mm
4	3.98mm	10.67mm	13.76mm
5	3.12mm	9.54mm	12.78mm
6	4.34mm	8.77mm	11.86mm
7	3.08mm	9.09mm	11.97mm
8	3.65mm	11.15mm	12.78mm
9	4.18mm	10.76mm	13.76mm
10	3.36mm	9.46mm	12.48mm
11	3.10mm	8.78mm	11.56mm
12	4.02mm	9.37mm	11.87mm
13	4.36mm	9.96mm	12.13mm
14	4.87mm	10.34mm	13.87mm
15	3.59mm	11.04mm	13.67mm

Table 2
Comparison of inhibition zones of antimicrobial susceptibility test using ONE WAY ANOVA

	Inhibition Zones									
Groups	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum	F value	the p-value of one way ANOVA
Group 1 (50 µg/ml)	15	3.8280	.52987	.13681	3.5346	4.1214	3.08	4.87	562.482	.000**
Group 2 (100 µg/ml)	15	9.9293	.87250	.22528	9.4462	10.4125	8.77	11.18		
Group 3 (200 µg/ml)	15	12.7600	.79054	.20412	12.3222	13.1978	11.56	13.87		

Table 3
Group Comparison of inhibition zones of antimicrobial susceptibility test using Tukey HSD Post Hoc tests

I Group	J Group	Mean Difference (I-J)	Std. Error	p-value
1	2	-6.10133*	.27219	.000**
	3	-8.93200*	.27219	.000**
2	3	-2.83067*	.27219	.000**

Discussion

The number of microbial agents that have developed resistance against various antibiotics is increasing, and antibiotic-induced resistance has become a major challenge in the medical world. [9] Nowadays, healthcare practitioners have been talking a great deal about the healing properties of BC, and colostrum-derived new food supplements are intended to boost the immune systems in both healthy and chronically ill patients.[10]. In this study, the objective was to obtain information on the antimicrobial activity of Bovine Colostrum in patients suffering from chronic periodontitis. Colostrum constituents from bovine sources are 100-fold to 1,000-fold more potent than that of human colostrum. [11] Periodontal disease is a chronic, degenerative disease that is localized on the gingiva, periodontal ligament, cementum, and alveolar bone. These diseases are usually associated with microbial infection due to the accumulation of plaque biofilm and calculus. The current concept concerning the etiology of periodontal disease considers 3 groups of factors that determine whether the active periodontal disease will occur: a susceptible host, the presence of pathogenic species, and the absence of so-called "beneficial bacteria". Gram-negative and motile organisms increase significantly by increasing the severity of the disease.[12,13]

Several suspected pathogens have been identified to be involved in destructive periodontal disease. The most important species of which are Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td), Fusobacterium nucleatum (Fn), Prevotella intermedia (Pi), Campylobacter rectus (Cr) and Eikenella corrodens (Ec). [14,15]
Bovine Colostrumcan inhibit the endotoxin secreted by the Gram-negative bacteria by neutralizing the lipopolysaccharides due to the presence of lactoferrin, thereby causing bacteriostatic and bactericidal effects. [16] This study shows us the effects of bovine colostrum at varying concentrations. A total of 15 samples were collected from systemically healthy patients suffering from chronic periodontitis. The samples were collected using a swab and were inserted in a cooked meat medium with Glucose, Hemin, and vitamin K at the bottom of the container. The bacterial strains obtained from the medium were cultured on nutrient agar slants. The cultures were maintained by sub culturing periodically and stored at a temperature of 4ºC before use. The collected samples were then poured on Mc Conkey Agar, Blood Agar, and Mullen Hilton Agar. The agar plates were kept for incubation at a temperature of 37 °C for 24hrs.Different strains of Gram-negative bacteria were obtained. The pour plate method was adopted for microbial activity. Solution of Bovine Colostrum Powder was prepared in Dimethyl sulfoxide (DMSO) at various concentrations (50 µg/ml, 100 µg/ml, and 200 µg/ml). Wells were made in the pre-solidified nutrient agar plate using a metal borer. The test samples were added to respective wells and kept for incubation at a temperature of 37 °C for 24hrs. After 24 hours inhibitory zones were seen. The minimal effect of Bovine Colostrum was seen in Group 1(50µg/ml). Proper inhibition zones were seen in Groups 2 and 3(100µg/ml and 200µg/ml). The mean of Group 1, Group 2, and Group 3 are 3.8, 9.9, and 12.7 with a mean ±SD of 0.52, 0.87, and 0.79 respectively and mean ± SE of 0.13, 0.22 and 0.20 respectively.

In a study done by Vuokko Loimaranta et. al., Colostral products from non-immunized cows (CP) and cows immunized with mutant streptococci (IP) were used as mouth rinses in a short-term human study. The acidogenic potential of the products was tested and found to be negligible in vivo before application to subsequent rinsing tests. At first, all the participants received a professional tooth cleaning, after which they rinsed with one of the solutions (IP; CP; water) three times per day for 3 days. After each rinsing period, the resting pH and decrease in plaque pH after the sucrose challenge were determined, the amount of plaque was estimated, and all available plaque was collected. No significant differences were recorded in the composition or the amounts of accumulated plaque. The resting pH values of plaques with low ``innate'' pH were increased after the IP rinsing period. Surprisingly, the lowest pH values after the sucrose challenge were recorded in IP plaques. The number of cultivable facultative flora or total streptococci was not affected by different rinsings, but the relative number of mutans streptococci significantly decreased after the IP rinsing period when compared to the CP period. Thus, the short-term rinsing indicates favorable effects of bovine immune whey on human dental plaque. [17]

In a recent study done by Yadav et al., antibacterial and anti-inflammatory properties of Colustrum were investigated in which antimicrobial activity was determined by the pour-plate method using five different strains of bacteria (Gram -ve and +ve), and carrageenan-induced rat paw edema method was used for the evaluation of anti-inflammatory activity in adult Wistar rats. Diclofenac was used as a standard anti-inflammatory drug, and amoxicillin was used as a standard antimicrobial agent. BC showed significant antimicrobial activity against Escherichia. coli, Staphylococcus. aureus, Proteus. vulgaris, Enterobacter. aerogenes and Salmonella. typhi. At 100 µg /mL of Bovine Colostrum, the inhibition zones were found to be 13mm, 11 mm, 12 mm, 12 mm, and 11 mm, respectively. The Bovine Colostrum zones were comparatively smaller than those of amoxicillin at 10 µg/mL. The percentage of edema inhibition was found to be 67.94% at the third hour, suggesting high anti-inflammatory activity of BC in rats. [18]

Conclusion

This study concluded that Bovine Colostrum has an antibacterial effect in-vitro against bacteria found in plaque and calculus. Bovine Colostrum with 200 µg/ml showed the highest zone of inhibition while Bovine Colostrum with 50 µg/ml showed a minimal zone of inhibition. Bovine Colostrum might be used as a local drug delivery agent in the treatment of chronic periodontitis in the future. However further studies should confirm preliminary results.

Conflict of interest: None

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