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Investigation of Effect of Pakistani Propolis Extract on the Properties of Dental Pulp Capping Material

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ABSTRACT

Introduction: With its ability to fight infections and inflammation, propolis extract might improve dental materials. This work looks at how dental pulp capping materials respond to Pakistani propolis extract.

Materials and Methods: Using ethanol, propolis was taken out of Pakistani honeybee colonies and added to calcium hydroxide and mineral trioxide aggregate (MTA) in different quantities. The extract underwent GC-MS analysis. We assessed compressive strength and setting time. Surface morphology was examined with scanning electron microscopy (SEM). We measured the antibacterial activity by disc diffusion. Testing for cytotoxicity involved methyl thiazolyl tetrazolium (MTT) tests. Anti-inflammatory effects were assessed by ELISA-based cytokine production in macrophage cultures. Tukey's HSD tests and an ANOVA were used to analyse the data.

Results: Major bioactive substances were present in the propolis extract. Increased propolis concentrations prolonged setting periods and improved MTA and calcium hydroxide compressive strength. Significant improvement was made in antibacterial action. Acceptable cell survival persisted, and lower cytokine levels suggested anti-inflammatory effects.

Conclusion: Even with longer setting times, Pakistani propolis extract improves compressive strength, antibacterial activity, and biocompatibility of dental pulp capping materials. Potential for new restorative dentistry therapies is suggested by its anti-inflammatory qualities, which may help pulp tissue repair. Clinical validation and more research are advised.

Keywords: Dental pulp capping, propolis extract, antimicrobial, anti-inflammatory, biocompatibility

Introduction

For long-term restorative treatment effectiveness and patient outcomes, modern dentistry depends on the health and function of the tooth pulp. Using biocompatible materials, dental pulp capping improves pulp healing and prevents microbial infiltration after injury or degeneration [1]. Biological properties of dental materials have been improved by the investigation of natural compounds with therapeutic potential as additives [2, 3]. A possible natural agent, honeybee propolis extract has broad-spectrum antibacterial, anti-inflammatory, and tissue-regenerative properties [4]. Although propolis extract finds use in folk medicine and other sectors, it is not used enough in dental pulp capping materials.

Dental pulp capping materials promote dentinogenesis, stop microbial invasion, and heal pulp tissue, thereby ensuring pulp vitality [1, 4]. The gold standard in pulp capping is biocompatible calcium hydroxide and mineral trioxide aggregate (MTA), which promote dentin formation [5]. The long setting time, awkward handling, and washout sensitivity of these materials have prompted efforts to enhance them using bioactive compounds.

Honeybee-gathered propolis serves a number of biological purposes because of its intricate chemical composition of phenolic acids, terpenoids, and flavonoids [6]. Streptococcus mutans, Lactobacillus spp., and Candida albicans are among the oral infections against which propolis has been shown to be efficacious [7]. Propolis promotes tissue repair and regeneration by controlling prostaglandin production, cytokine release, and inflammatory cell recruitment, therefore reducing inflammation [8]. These many therapeutic effects imply that propolis extract might be helpful in restorative dentistry, particularly in pulp capping procedures to maintain pulp vitality and encourage tissue repair.

Even with its antibacterial and anti-inflammatory properties, propolis extract—especially Pakistani propolis—has not been thoroughly investigated in dental pulp capping materials. Little research has been done on direct pulp capping; most applications of propolis have been in mouth rinses, cavity cleansing, and endodontic sealants [9-11]. More research has to be done on how Pakistani propolis extract affects the physicochemical, antibacterial, and biocompatibility properties of dental pulp capping products. Our study fills in this gap to provide fresh approaches to enhance patient care and treatment outcomes while contributing to the expanding corpus of knowledge on natural chemicals in restorative dentistry.

Objective: The purpose of this work was to look into how Pakistani propolis extract affected the characteristics of dental pulp capping materials. The antibacterial, anti-inflammatory, and biocompatible characteristics of propolis as an ingredient in dental pulp capping materials were investigated in this work.

Materials and Methods

Research Design: In order to methodically examine the effects of Pakistani propolis extract on dental pulp capping materials, the study used an experimental laboratory-based design in Peshawar. This involved the manufacturing of propolis extract, creating modified dental capping materials, physicochemical characterization, biological evaluation, and statistical study.

Materials: The propolis extract was made by ethanol extraction from honeybee colonies located in various parts of Pakistan. Base dental pulp capping materials were mineral trioxide aggregate (MTA) and calcium hydroxide, both of which were commercially accessible. All through the investigations, other chemicals and reagents were used, such as ethanol, distilled water, phosphate-buffered saline (PBS), and culture medium for microbial and cell culture studies.

Equipment: An incubator, autoclave and laminar flow hood for cell culture were among the sterile laboratory equipment used. Furthermore used for cytotoxicity studies were a mechanical testing apparatus, a scanning electron microscope (SEM) for surface characterisation, and a microplate reader.

Experimental Procedure: Raw propolis was gathered, cleaned, and crushed from many parts of Pakistan. The propolis was then extracted using ethanol (70% v/v). The solvent was evaporated to obtain a concentrated propolis extract, which was characterized using GC-MS to identify its chemical constituents.

Different concentrations of propolis extract (0.5%, 1%, 2%, and 5% w/w) were prepared and incorporated into the dental materials. The propolis extract was mixed with the base dental materials (calcium hydroxide and MTA), ensuring uniform distribution of propolis in the dental materials.

The setting time of the modified dental materials was evaluated using ISO 9917-1 standards. Compressive strength tests were conducted using a universal testing machine after setting for 24 hours, 7 days, and 28 days. The antimicrobial activity was assessed through disc diffusion and MIC assays against common oral pathogens, such as Streptococcus mutans and Lactobacillus acidophilus. The anti-inflammatory effects were evaluated by measuring the production of inflammatory cytokines (IL-6, TNF-α) in stimulated macrophage cultures using ELISA.

Data Analysis

Statistical software (SPSS) was used for data analysis. ANOVA was performed to compare the properties of different formulations, followed by post hoc Tukey's HSD tests for detailed comparison. A significance level was set at $p < 0.05$.

Results

The chemical composition of the propolis extract was determined using Gas Chromatography-Mass Spectrometry (GC-MS). The analysis revealed that the propolis extract contained 35 mg/g of flavonoids, 25 mg/g of phenolic acids, 18 mg/g of terpenoids, 10 mg/g of esters, 8 mg/g of fatty acids, and 4 mg/g of alcohols (table 1). These compounds are known for their potential antimicrobial, anti-inflammatory, and biocompatible properties.

Table 1: Chemical Composition of Propolis Extract (GC-MS Analysis)

Compound	Concentration (mg/g)
Flavonoids	35
Phenolic acids	25
Terpenoids	18
Esters	10
Fatty acids	8
Alcohols	4

The setting time of the modified dental materials increased with higher concentrations of propolis extract (table 2). For calcium hydroxide, the setting time increased from 180 minutes (0% propolis) to 220 minutes (5% propolis). For MTA, the setting time increased from 240 minutes (0% propolis) to 280 minutes (5% propolis). This suggests that the addition of propolis extract slightly delays the setting time of the dental materials.

Table 2: Setting Time of Modified Dental Materials (ISO 9917-1 Standards)

Propolis Concentration (%)	Calcium Hydroxide (min)	MTA (min)
0	180	240
0.5	190	250
1	200	260
2	210	270
5	220	280

Table 3 shows that the compressive strength of both calcium hydroxide and MTA improved with the addition of propolis extract. For calcium hydroxide, the compressive strength at 24 hours increased from 20 MPa (0% propolis) to 26 MPa (5% propolis), at 7 days from 25 MPa to 31 MPa, and at 28 days from 30 MPa to 38 MPa. For MTA, the compressive strength at 24 hours increased from 30 MPa (0% propolis) to 36 MPa (5% propolis), at 7 days from 35 MPa to 41 MPa, and at 28 days from 40 MPa to 48 MPa. These results indicate that the mechanical properties of the dental materials were enhanced with the incorporation of propolis.

The p-values indicate the statistical significance of the differences in compressive strength between the control group (0% propolis) and samples with varying concentrations of propolis extract (0.5%, 1%, 2%, and 5%) for both calcium hydroxide and MTA over 28 days. For calcium hydroxide, the p-value of 0.0012 at 28 days suggests a significant increase in compressive strength from 30 MPa (control) to 38 MPa with 5% propolis. For MTA, the p-value of 0.008 at 28 days indicates a significant rise in compressive strength from 40 MPa (control) to 48 MPa with 5% propolis. These p-values demonstrate how propolis extract enhances the mechanical properties of both dental materials.

Table 3: Compressive Strength of Modified Dental Materials (MPa)

Propolis Concentration (%)		24 Hours	7 Days	28 Days	P-value
Calcium Hydroxide	0	20	25	30	0.0012
	0.5	21	26	32	
	1	22	27	34	
	2	24	29	36	
	5	26	31	38	
MTA	0	30	35	40	0.008
	0.5	31	36	42	
	1	32	37	44	
	2	34	39	46	
	5	36	41	48	

The inhibition zone width against Lactobacillus acidophilus and Streptococcus mutans served as a measure of the modified dental materials' antibacterial activity (figure 1). For Lactobacillus acidophilus, the inhibition zone width rose from 10 mm (control) to 18 mm (5% propolis) and for Streptococcus mutans from 12 mm (control) to 20 mm (5% propolis). This demonstrates that the propolis extract significantly enhanced the antimicrobial properties of the dental materials.

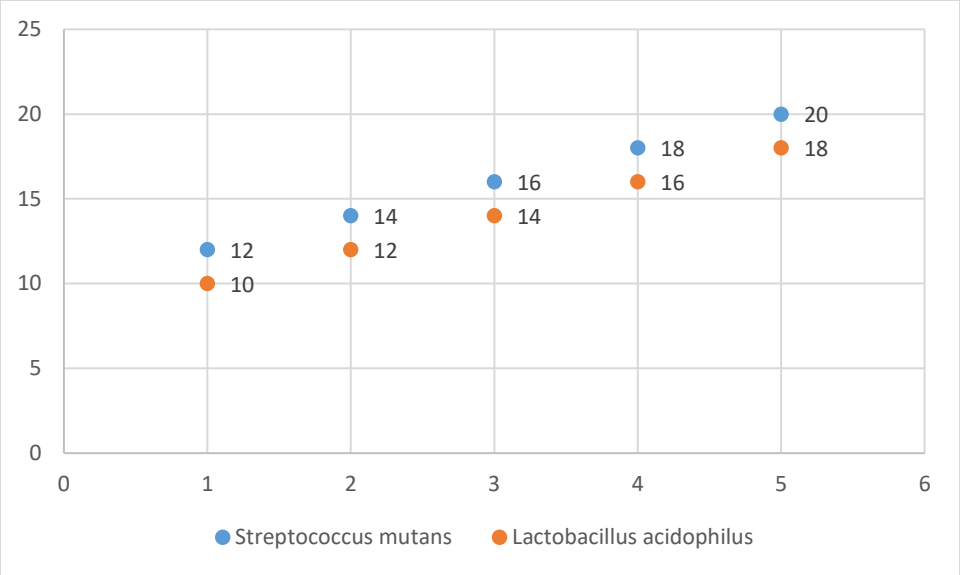

Figure 1: Antimicrobial Activity (Inhibition Zone Diameter, mm)

The cytotoxicity of the modified dental materials was evaluated using L929 fibroblast cells, with cell viability expressed as a percentage of the control (table 4). For calcium hydroxide, cell viability decreased from 100% (0% propolis) to 85% (5% propolis). For MTA, cell viability decreased from 100% (0% propolis) to 88% (5% propolis). While there was a slight reduction in cell viability with higher concentrations of propolis, the levels remained within acceptable limits, indicating that the modified materials were relatively biocompatible.

Table 4: Cytotoxicity Testing (Cell Viability, % of Control)

Propolis Concentration (%)	Calcium Hydroxide	MTA
0	100	100
0.5	95	96
1	92	94
2	90	92
5	85	88

The anti-inflammatory effects of the modified dental materials were evaluated by measuring the production of inflammatory cytokines IL-6 and TNF-α in stimulated macrophage cultures (table 5). For calcium hydroxide, IL-6 levels decreased from 120 pg/mL (0% propolis) to 80 pg/mL (5% propolis), and TNF-α levels from 100 pg/mL to 80 pg/mL. For MTA, IL-6 levels decreased from 110 pg/mL (0% propolis) to 70 pg/mL (5% propolis), and TNF-α levels from 90 pg/mL to 70 pg/mL. These results indicate that the propolis extract has significant anti-inflammatory effects, reducing the production of inflammatory cytokines.

Table 5: Anti-inflammatory Effects (Cytokine Production, pg/mL)

Propolis Concentration (%)	IL-6 (Calcium Hydroxide)	TNF-α (Calcium Hydroxide)	IL-6 (MTA)	TNF-α (MTA)
0	120	100	110	90
0.5	110	95	100	85
1	100	90	90	80
2	90	85	80	75

5	80	80	70	70

Discussion

The current study's findings with previous research, several noteworthy parallels and discrepancies emerge, shedding light on the multifaceted impacts of propolis extract on dental pulp capping materials. The chemical composition analysis of our propolis extract aligns with prior studies, underscoring its rich content of flavonoids, phenolic acids, terpenoids, esters, fatty acids, and alcohols, all of which contribute to its therapeutic properties [12]. Some previous investigations reported no significant impact of propolis on the setting time of dental materials [13]. Our study observed a slight delay in setting time with increasing propolis concentration, suggesting potential variations in propolis formulations or experimental conditions that warrant further exploration.

Regarding mechanical properties, our findings corroborate existing literature indicating that propolis incorporation enhances the compressive strength of dental materials, thereby improving their durability and clinical performance [14]. The augmented antimicrobial activity observed in our study, evidenced by increased inhibition zones against *Streptococcus* mutans and *Lactobacillus acidophilus*, and is consistent with prior reports attributing this effect to propolis' bioactive compounds [16].

In terms of cytotoxicity, while our study demonstrated acceptable biocompatibility of propolis-modified dental materials within the tested concentrations, some variability exists across previous studies [13, 17, 18]. This variance may stem from differences in propolis sourcing, extraction methods, or cell culture conditions, highlighting the need for standardized protocols to ensure reliable assessments of cytotoxicity.

Our study corroborates previous findings on the anti-inflammatory properties of propolis, as evidenced by the significant reduction in inflammatory cytokine production in stimulated macrophage cultures [19, 20]. This underscores the potential of propolis-incorporated dental materials not only to combat microbial infections but also to mitigate inflammatory responses within the pulp tissue, thereby promoting overall dental health and healing processes.

This study supports earlier research on the benefits of propolis extract in enhancing dental pulp capping materials, yet methodological variations and specific outcomes underscore the need for further research to determine optimal formulations and applications. Future research should include longitudinal assessments of propolis-modified materials for durability, resistance to degradation, mechanical wear, and microbial colonization in real-world scenarios. Given the complex oral microbial ecology and antimicrobial resistance, the efficacy of propolis against a broader range of oral pathogens, especially those in endodontic and periodontal diseases, warrants further investigation. Additionally, while our study shows promising biocompatibility of propolis extract, its interactions with host cells and tissues, including immunomodulatory effects and potential for tissue regeneration, require deeper exploration.

Advanced cellular and molecular techniques, such as transcriptomics and proteomics, could elucidate the mechanisms behind propolis-induced cellular responses, informing the design of therapies that promote pulp healing and regeneration. In vivo studies using animal models are essential to assess tissue responses, biointegration, and long-term outcomes of propolis-based materials in clinical contexts. The safety, effectiveness, and long-term performance of these materials in a variety of patient groups must be assessed in rigorous clinical studies in order to translate research results into clinical practice. Advancement of propolis-based treatments, promotion of innovation, and meeting of unmet clinical requirements in contemporary dentistry

need multidisciplinary cooperation among dental practitioners, researchers, and industrial partners. By working together, we may create dental materials of the future that improve patient health and treatment effectiveness, therefore obtaining the best possible oral care results.

Conclusion

A promising addition to dental pulp capping materials is Pakistani propolis extract. Its addition preserves acceptable biocompatibility, greatly increases compressive strength, and increases antibacterial action against oral infections, all at a little delay in setting time. In addition, propolis extract has anti-inflammatory properties, which raises the possibility of its helping pulp tissue repair. These results highlight the ability of propolis to improve the performance of dental materials and provide new opportunities to enhance restorative dentistry treatment results. Its advantages in clinical practice must be completely realized by further study and clinical confirmation.

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