

## Research Article

# Evaluation of the Effect of Royal Jelly Solution as a Subgingival Irrigant - A Randomised Controlled Clinical Trial

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## I N F O

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## A B S T R A C T

**Background:** Royal jelly is a bee's milky secretion extracted from a worker honey bee with potent antimicrobial, antioxidant, and anti-inflammatory properties. It is effective in periodontal disease.

**Methodology:** In this randomised controlled clinical trial, 30 chronic periodontitis patients with at least 10 teeth with probing pocket depth up to 5-7 mm were selected for the study by simple random sampling technique and were subdivided into three groups: Group 1 (n = 10; scaling and root planing (SRP) + royal jelly irrigation), Group 2 (n = 10; scaling and root planing + saline irrigation), Group 3 (n = 10; scaling and root planing + distilled water irrigation). Clinical periodontal parameters like plaque index, gingival index, and probing pocket depth were measured at baseline and after 45 days.

**Results:** Descriptive analysis was used for the distribution of variables in each group at both the baseline and 45th day for further statistical analysis and comparison. The clinical parameters compared within all three groups at baseline and 45th day by ANOVA were not statistically significant at different time intervals ( $p > 0.05$ ). For the royal jelly group, the results were statistically significant for all clinical indices at baseline and 45th day. It was found to be equally effective as saline irrigation ( $p < 0.005$ ) and more effective than distilled water ( $p > 0.005$ ) as analysed by paired t test.

**Conclusion:** Royal jelly agents, as an irrigant, can act as a better treatment modality in periodontal disease management due to their abundant clinical properties.

**Keywords:** Royal Jelly, Subgingival Irrigation, Saline, Distilled Water, Scaling and Root Planing

## Introduction

Periodontal disease is a chronic multifactorial disease caused by pathogenic microbiota in susceptible hosts characterised by the destruction of periodontal supporting tissues.<sup>1</sup> A subgingival pathogenic biofilm mainly consists of obligate and facultative anaerobic organisms, most cultured being *Aggregatibacter actinomycetemcomitans* (Aa), *Tannerella forsythia*, *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), and *Fusobacterium nucleatum* (Fn).<sup>1-3</sup>

Non-surgical periodontal therapy (NSPT) aims to alter subgingival microbial flora by removing soft and hard deposits from the root surfaces, thereby controlling periodontal disease progression and creating a healthy conducive subgingival environment. One of the NSPT techniques effective in plaque control is subgingival irrigation. The rationale behind subgingival irrigation includes reducing subgingival microbiota quantitatively to prevent the progression of periodontal disease. Irrigation systems are effective in penetrating inaccessible areas like deeper periodontal pockets, furcal zones, and root concavities to remove potential pathogens that penetrate dentin tubules and those residing in lacunae and concavities.<sup>4-7</sup>

Mechanical and chemical plaque control agents are effective in reducing microbial load and periodontal disease activity. Chemical plaque control agents which are being extensively studied involve antimicrobial agents, antiseptics, and anti-inflammatory drugs administered systemically or locally to reduce bacterial proliferation, with chlorhexidine being the gold standard.<sup>10</sup> Despite these chemical agents, currently, herbal products are gaining importance as local drug delivery agents in periodontal disease management because of their fewer side effects and better patient compliance.<sup>8-11</sup>

Royal jelly (RJ) is a newly studied product of apitherapy effective in reducing periodontal pathogens that could be a better alternative to other bee products. RJ, also called bee milk, is a creamy white viscous secretion obtained from the salivary glands of mandibular and hypopharyngeal regions of worker honey bees (*Apis mellifera*). It is used primarily for queen bees' growth.

Its characteristic features include a phenolic smell, yellowish-white colour, and a highly acidic nature (pH 3.5-4.5). Its components are water, proteins, carbohydrates, lipids, vitamins, hormones, and minerals. The antimicrobial components of RJ include RJ proteins, royalisin, jellenies, and enzymes like glucose oxidase and 10 HDA (hydroxy delta decenoic acid).<sup>12-14</sup>

The medicinal properties of RJ include detoxification of enzymes, acting as a biocatalyst in the cell regeneration process, antimicrobial effects (antibacterial and antifungal), anti-allergic property, lowering blood cholesterol level, and

enhanced effect in wound healing and growth acceleration. RJ is available as dietary supplement for cosmetic use.<sup>15,16</sup>

In vitro studies using RJ have proven its antimicrobial effect against periodontal pathogens and have shown that its efficacy is maximum at a bactericidal concentration of 50-100 µg/ml against *Porphyromonas gingivalis* and *Prevotella intermedia*. Hence this effective concentration was used in this study to find the royal jelly solution's clinical efficacy in periodontal disease management as a subgingival irrigating solution.

## Materials & Methods

This randomised controlled clinical study included 30 medically healthy individuals with chronic periodontitis undergoing periodontal therapy at the Department of Periodontics in Vivekananda Dental College for Women. Ethical clearance regarding the study was obtained from the Institutional Ethics Committee and the guidelines of the Helsinki Declaration were adhered to. Written informed consent was obtained from all patients. The study was performed from November 2021 to February 2021 for 3 months. The sample size was calculated by  $\alpha$  error fixed at < 5% ( $p < 0.005$ ). Based on this statistical calculation, the minimum sample size required was 10 participants in each group.

The patients were included in this study as per the following criteria: at least 10 teeth with two or more sites with a periodontal pocket depth of 5-7 mm; clinical attachment loss up to 3-4 mm; age 30-50 years old; good general health; and those who did not receive periodontal treatment in the last 6 months. Exclusion criteria included patients on antibiotic therapy within 3 months before or during the study; having systemic diseases; pregnancy or lactation; patients on drug therapy; and smokers or any form of tobacco users.

## Criteria for Grouping

After the baseline clinical measurements, the patients were divided into the following 3 groups by coin toss methods:

**Group A:** SRP + subgingival irrigation with royal jelly solution (n = 10)

**Group B:** SRP + subgingival irrigation with saline solution (n = 10)

**Group C:** SRP + subgingival irrigation with distilled water (n = 10)

Periodontal clinical examination was done by a single blinded examiner which included probing pocket depth (PPD) measured from the marginal gingiva to the bottom of the pocket in all 6 sites of the involved teeth using William's periodontal probe; Clinical attachment level (CAL) recorded as probing depth + recession fixed at cemento-enamel

junction using William's periodontal probe; Gingival index (GI)<sup>17</sup> and Plaque index (PI)<sup>18</sup>.

### Preparation of Royal Jelly Irrigant Solution and Irrigation

Royal jelly is commercially available in tablet form under the brand name Forever Royal Jelly containing 60 tablets. Each tablet consists of 0.6 g of this substance. A solution of an effective concentration of 60 µg/ml is made by crushing one tablet and dissolving it in 60 ml distilled water to obtain a higher effective antimicrobial concentration as proved in various in vitro studies.<sup>22,23</sup> Finally, the RJ irrigator solution is delivered as in-office irrigation using a portable irrigation device (Agaro portable water flosser).

### In-office Irrigation

Following oral prophylaxis, subgingival irrigation with this commercially available oral irrigator device was selected at custom mode for deep cleaning and gingival stimulation with water pressure between 10-90 psi and 1400 pulses per minute to reach maximum pocket depth. 10 to 15 ml of solution was delivered within 5 minutes at all sites.<sup>29</sup> Similarly, using the same modes of delivery system and specifications, distilled water and saline irrigation were given for the control groups. Local suction with a sterile surgical vacuum was avoided during the procedure. Oral hygiene instructions were given regarding brushing twice daily using modified bass technique and avoiding the use of any antimicrobial agents for at least a month. Patients were recalled after 45 days for clinical re-evaluation and

parameters were recorded and compared using statistical methods.

### Statistical Analysis

Analysis of variance (ANOVA) statistical method was used to compare variances across means of different groups. Paired t test was used for finding differences between plaque indices, gingival indices, and probing pocket depth variables at baseline and 45th day in each group.  $P < 0.005$  was considered statistically significant.

### Results

Thirty individuals were included in the study. Their follow-ups were done and clinical parameters were recorded. No side effects or discomfort were reported throughout the course of the study.

Descriptive analysis helped in equal distribution of the variables in each group at both baseline and 45th day for further statistical analysis and comparison. The mean and standard deviation were determined for different clinical indices for each group at baseline and after 45 days (Table 1).

Intragroup comparison, as shown in Table 2, was analysed by ANOVA (Analysis of variance). The clinical parameters compared within all three groups at baseline and 45th day were not statistically significant indicating their equal clinical efficacy at different time intervals ( $p > 0.005$ ). Mean square values were determined at baseline and after 45 days between and within the groups.

**Table 1. Descriptive Statistics**

Group	Index	Mean ± SD at Baseline	Mean ± SD at 45th Day
Distilled water	Plaque	2.399 ± 0.486	1.893 ± 0.491
	Gingival	2.280 ± 0.426	1.871 ± 0.489
	PPD	5.296 ± 0.324	5.025 ± 0.325
Saline	Plaque	2.772 ± 0.191	2.197 ± 0.244
	Gingival	2.567 ± 0.263	1.960 ± 0.226
	PPD	5.210 ± 0.428	4.802 ± 0.383
Royal jelly	Plaque	2.355 ± 0.319	1.779 ± 0.319
	Gingival	2.498 ± 0.280	1.763 ± 0.498
	PPD	5.601 ± 0.306	4.684 ± 0.440

**Table 2. Intragroup Comparison of Three Groups using ANOVA**

Time Interval	Index	F Value	Mean Square Value	p Value
Baseline (distilled water, saline, and royal jelly)	Plaque	5.328	Between groups: 0.520 Within groups: 0.098	0.011 (NS)
	Gingival	2.189	Between groups: 0.224 Within groups: 0.103	0.132 (NS)
	PPD	3.340	Between groups: 0.422 Within groups: 0.126	0.051 (NS)
45th day (distilled water, saline, and royal jelly)	Plaque	3.481	Between groups: 0.467 Within groups: 0.134	0.045 (NS)
	Gingival	0.551	Between groups: 0.097 Within groups: 0.176	0.582 (NS)
	PPD	2.032	Between groups: 0.300 Within groups: 0.148	0.151 (NS)

NS: Not significant; F value: ANOVA value

Table 3 shows the intergroup comparison between baseline and 45th day for 3 groups using paired t test analysing statistical significance between groups for different parameters. For the royal jelly group, the results were statistically significant for all clinical indices at baseline and 45th day. It was equally effective as saline irrigation ( $p < 0.005$ ) and more effective than distilled water ( $p > 0.005$ ).

PI and GI showed greater clinical improvements with mean  $\pm$  SD of  $1.779 \pm 0.319$  and  $1.763 \pm 0.498$  respectively as compared to baseline values in the royal jelly group and a statistically significant improvement as compared to saline

and distilled water irrigation groups. PPD was although statistically significant at baseline, its mean values on the 45th day were similar in all groups ( $5.025 \pm 0.325$ ,  $4.684 \pm 0.440$ , and  $4.802 \pm 0.383$  for distilled water, royal jelly, and saline group respectively).

The results of the study demonstrated that this new apitherapy product royal jelly was clinically effective in periodontal disease management as a subgingival irrigant at an effective concentration of  $60 \mu\text{/ml}$ . Its clinical effectiveness was more than distilled water and similar to that of saline.

**Table 3. Intergroup Comparison between Baseline and 45th Day for the Three Groups using Paired T Test**

Group	Parameter	Mean Difference	p-value
Distilled water (baseline and 45th day)	Plaque	-0.506	0.031
	Gingival	-0.409	0.051
	PPD	-0.280	0.058
Saline (baseline and 45th day)	Plaque	-0.573	0.001*
	Gingival	-0.600	0.001*
	PPD	-0.40	0.024
Royal jelly (baseline and 45th day)	Plaque	-0.580	0.0004*
	Gingival	-0.730	0.0002*
	PPD	-0.900	0.0001*

\*p value  $< 0.005$  - statistically significant

## Discussion

In recent years, many clinical trials have focused on natural therapies concerning their properties and the enormous advantages offered by them compared to synthetic drugs. Various experimental evidence has demonstrated the pharmacological activity of apitherapy products like propolis, raw honey, caffeic acid phenethyl ester, purified bee venom etc. They have anti-inflammatory, antimicrobial, and antioxidant activities which make them helpful in treating various oral bacterial diseases like gingivitis, periodontitis, and dental caries.<sup>19-21</sup> In this category, royal jelly, which is a bee milk secretion has been tested for its antimicrobial activity in various in vitro studies. However, there is no clinical study performed with royal jelly in periodontal disease management. Hence this current study proved the first clinical effect of royal jelly solution as a subgingival irrigant in chronic periodontitis patients with moderate probing depth.

Coutinho et al. 2021<sup>22</sup> in their in vitro study proved the antimicrobial efficacy of RJ against periodontopathic bacteria like Pg, Pi, Aa, and Fn at higher concentrations of 12.5 and 100 µg/ml, exhibiting the potency of RJ to reduce microbial load. In concordance with this study, we used RJ available in tablet form as a solution in an effective concentration of 60 µg/ml (0.6%) for irrigation purposes to reduce inflammation and plaque accumulation. Khosla et al.<sup>23</sup> in their in vitro study compared the efficacy of RJ against periodontal pathogens with chlorhexidine and reported more antibacterial sensitivity at higher concentrations of 50-100 µg/ml. Yamaguchi et al.<sup>24</sup> analysed the potent antimicrobial effect of RJ against the synergistic effect of *Streptococcus mutans* and *Porphyromonas gingivalis* in potentiating the activity of periodontal disease. In accordance with its antimicrobial role, this study compared royal jelly irrigation with 0.9% saline irrigation and obtained better improvement in all clinical parameters from baseline comparatively in both groups with significant p value (< 0.005).

Sricholpech et al.<sup>25</sup> proved the royal jelly efficacy on promoting periodontal ligament fibroblasts proliferation in tooth replantation procedures. With this concept of its uses in increasing periodontal ligament fibroblast viability and proliferation, this study found statistically insignificant ( $p > 0.05$ ) results in clinical improvement of probing pocket depth from baseline which was contradictory to these results since they used royal jelly solution at higher concentration of 500-900 µg/ml. The mean value for PPD decreased from  $5.601 \pm 0.306$  at baseline to  $4.684 \pm 0.440$  on the 45th day.

The osteoconductive and anti-inflammatory effect of royal jelly in periodontal ligament cells of clone 22 in mice was assessed by Yanagita et al.<sup>26</sup> They demonstrated the

enhancement of mRNA expression of osteogenic proteins and its anti-inflammatory role in reducing LPS induced IL-6 secretion by *Porphyromonas gingivalis*. In concordance with it, this study analysed royal jelly's anti-inflammatory role in terms of improvement in the gingival index (mean value increased from  $2.498 \pm 0.280$  to  $1.763 \pm 0.498$ ) proving its clinical effectiveness in reducing gingival inflammation ( $p$  value < 0.0002).

Nagarakanti et al.<sup>27</sup> and Jalaluddin et al.<sup>28</sup> in systematic reviews highlighted the role of subgingival irrigation in reducing the subgingival microbial population quantitatively that initiates and advances periodontal diseases, hence adjunctive use of chemotherapeutic agents may provide additional clinical benefits when compared to SRP alone. Mushtaq et al.<sup>29</sup> in a review article demonstrated that the purpose of various subgingival irrigants like water, normal saline, hydrogen peroxide, povidone iodine, ozonised water, chlorhexidine, metronidazole, tetracycline, sodium bicarbonate etc. for pocket irrigation is to non-specifically reduce the bacteria and their byproducts to alter microbial flora. In concordance with it, the results of the study demonstrated statistically significant clinical improvements from baseline with  $p < 0.005$  as demonstrated in Table 3.

Subgingival irrigation with 10% povidone-iodine solution by Kotsilkov et al.<sup>30</sup> showed statistically significant improvement in all periodontal clinical parameters evaluated in chronic periodontitis patients with superior reduction in probing depth, gain in clinical attachment and better reduction of the gingival inflammation in comparison with regions treated by SRP only. The results were in concordance with the values of the saline irrigation group in our study with mean values reduced to  $2.197 \pm 0.244$ ,  $1.960 \pm 0.226$ , and  $4.802 \pm 0.383$  for PI, GI and PPD respectively.

Andrade et al.<sup>31</sup> analysed the efficacy of subgingival irrigation with a solution of 20% Propolis and showed its effectiveness in all clinical parameters like probing pocket depth, gingival index, plaque index, and oral hygiene index up to 90 days. The mean difference for probing depth on the 45th day was  $1.42 \pm 1.37$  which is comparable to our study (mean difference of  $0.92 \pm 0.1$ ). Pandya et al.<sup>32</sup> demonstrated that subgingival irrigation with chlorhexidine (CHX) reduced gingival inflammatory changes and the amount of periodontopathogen microflora. The gingival index mean difference obtained for CHX is 1.001 which is comparable to our study (0.912). In concordance with the above studies, we obtained improvement in all clinical parameters up to 45 days from baseline which is statistically significant compared to distilled water and saline group ( $p < 0.005$ ). The effectiveness of subgingival irrigation with propolis extract and chlorhexidine as an adjunct to mechanical debridement was compared by Tejashvi Ashok Seth et al. 2022.<sup>33</sup> The study showed a statistically significant



reduction in PI, GI, and PPD from baseline to 30 days in both groups with mean values obtained as  $1.57 \pm 0.29$ ,  $1.12 \pm 0.30$  and  $3.65 \pm 1.18$  respectively. In accordance with these results, this study also observed a significant reduction of PI, GI and PPD from baseline up to 45 days with mean values of  $1.779 \pm 0.319$ ,  $1.763 \pm 0.498$ , and  $4.684 \pm 0.440$  respectively.

Severo et al.<sup>34</sup> analysed the presence of sucrose component in royal jelly and its role in reducing oxidative stress in patients with oral mucositis. They observed an increase in glutathione anti-oxidant levels with royal jelly for up to 14 days. Gevorgyan et al.<sup>35</sup> analysed royal jelly's mediated antimicrobial effect of green synthesized silver particles (GS-Ag). RJ-mediated GS Ag nanoparticles (NP) were prepared by the interaction of NPs with bacterial membranes. Here, the RJ served as an oxidising and reducing agent in green synthesis technology. In agreement with these properties explained in these studies, this clinical study has demonstrated the anti-oxidant, anti-bacterial and anti-inflammatory role by reduction of periodontal disease activity in terms of clinical parameters like PI, GI and PPD with a statistically significant reduction from baseline values ( $p < 0.005$ ).

The above-discussed studies have shown the uses of various irrigation agents and uses of royal jelly products in invitro studies. Based on this view, this study found the clinical efficacy of royal jelly solution in chronic periodontitis patients. Although its antibacterial and anti-inflammatory roles have been demonstrated, its relative ineffectiveness in pocket depth reduction is due to inadequate subgingival delivery of the agents through subgingival irrigation as they provide insufficient contact time in achieving effective concentrations between antimicrobial agents and target microbes.

## Conclusion

From the results of the study, it is evident that, royal jelly can reduce inflammation and plaque formation in chronic periodontitis patients and hence can be used as adjunct in periodontal therapy as chemical plaque control agent.

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**Conflict of Interest:** None

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