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COMPARATIVE EVALUATION OF AZADIRACHTIN, VITAMIN C AND INSULIN LIKE GROWTH FACTOR 1 RELEASE IN TITANIUM PLATELET RICH FIBRIN INFUSED WITH NEEM AND TRIPHALA INDICA GEL EXTRACTS: AN INVITRO STUDY

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ABSTRACT

Background: Herbal extract gel addition to Titanium -Platelet Rich Fibrin (T-PRF) clots was started very recently. Their release from the T-PRF clots has not been performed. Hence present study aimed to evaluate the release of Azadirachtin (Az) from T-PRF+Neem gel group, Vitamin C release from T- PRF+ Triphala Indica group and Insulin like growth factor 1 (IGF 1) from T-PRF alone along with the mentioned test groups.

Methods and Materials: Present invitro study utilized 6 subject's blood and subjected to centrifugation. T-PRF clots were prepared and TI/ NE gel extracts were injected and compared with T-PRF alone to check the release of Vit C, Az and IGF 1 release at 6hours, 72 hours (3rd), 7th and 14th day. For statistical analysis, paired t test and ANOVA was performed. P value <0.05 was considered statistically significant.

Results: Az release there was decreased levels reported between 6 hrs and the 7th day and it was statistically significant (p = 0.045*). Whereas, all other time frame comparisons values were non-significant (p>0.05) Regarding Vit C release from T-PRF clot there was a decrease in the amount released when compared at 6hrs & 7th day (p=0.008*) and 72hrs and 7th day (p=0.037*). IGF 1 release in all the three groups and at different time frames was non-significant (p>0.05).

Conclusion: Added herbal extracts didn't alter the release of IGF 1 that proves the surety of GF release. T-PRF also showed the timely release of the Az and Vit C confirming the name of sustained drug delivery system (SDDS).

Key Words: Azadirachtin, Ascorbic Acid, Insulin like Growth Factor, Platelet Rich Fibrin, Periodontitis, Titanium

INTRODUCTION

Systemic antibiotics application has been a practice since ages for the control of infection pre and post treatments.[1] With the extensive usage of these medicines there was a development of side effects which is very difficult for doctors, researchers and patients to tackle the disease entity patterns and routes. Apart from this, there was development of resistance for bacteria, side effects to living beings led the researchers to develop newer delivery vehicles to release the drug at constant pace.[2] Materials like carbopolymer, polaxamer 123, collagen membranes, collagen plugs, microspheres etc have been used to deliver the drugs. But because of their increased incidence of antigenicity better biomaterials were searched.[3]

They came across platelet concentrates (PC) which were autologous and had a greater sustained capability of holding growth factors. They also had a three-dimensional fibrin structure which also hold platelets, white and red blood cells.[4] First generation PC like Platelet rich Plasma, Fibrin glue and Second-generation PC like Leukocyte Platelet Rich Fibrin (L-PRF), Advanced Platelet Rich Fibrin (A-PRF) were used regularly in different treatment strategies such as intra bony defects (IBD), gingival recession treatments, sinus augmentation procedures with and without adding bone graft materials.[5] Miron RJ et al.,[6] 2021 in their systematic review and meta-analysis concluded that PC's addition along with bone grafts helped in better treatment outcomes such as reduction in probing pocket depth, gain in CAL and improved bone fill and alveolar crest change. Hence, they have used this PRF for delivering the drug either antibiotics or anti-inflammatory drugs to tackle the post operative pain and infection.[7] Study done by Pollock D et al.,[8] 2019 utilized metronidazole, penicillin and clindamycin in the L-PRF and checked for antimicrobial efficacy for staphylococcus aureus and fusobacterium nucleatum where they concluded that PRF incorporation of antibiotics helped in greater antimicrobial efficacy than L-PRF alone. They also stated that 0.5ml drug injection into the L-PRF didn't alter the structural properties of it. Beyond that there was abnormalities noted for PRF structure. Pillai AK et al.,[9] 2021 incorporated diclofenac sodium pain killer into L-PRF and placed at the 3rd molar extraction sockets where they reported that test sites reported better than control group with reduced post-operative pain. Healing was also better in test sites.

But because of raised concerns regarding the possible silica contamination[10] in silica coated plastic tubes, breakage of tubes made of silica and shorter resorption time led to the search of better material that can be used.[11] This led to the introduction of Titanium Platelet Rich Fibrin (T-PRF) by Tunali M et al.,[12] 2013

where they prepared this in grade IV titanium tubes. They concluded that Titanium tubes had better haemo compatibility, titanium dioxide passivates itself within the tubes and activate the platelets similar to silica in glass tubes.[13] The fibrin structure that formed was thicker, denser, with superior fibrin border area. This was supported by later studies done by Chatterjee A et al.,[14] 2017, Mitra DK et al.,[15] 2019, Bhattacharya HS et al.,[16, 17] 2020 & 2022 where they concluded that T-PRF had better fibrin meshwork, superior cellular entrapment and better hold of growth factors than L-PRF. Tunali M et al.,[18] 2014 also concluded that T-PRF had a longer resorption time of 21 days when checked with rabbit's study. With this evidence, clinical trials regarding hard and soft tissue parameters were performed and achieved good results regarding the healing and improvement of PPD, CAL, bone fill, increased keratinized tissue width, improved mean root coverage and reduced recession depth & width.[19-21] Hence, Ercan E et al.,[22] 2022 used this T-PRF as sustained drug delivery system (SDDS) where they added doxycycline hydrochloride solution into T-PRF and studied regarding the drug kinetics where they concluded that T-PRF has a better with holding capacity and timely release of drug. They also showed good antimicrobial property against the staph aureus and pseudomonas aeruginosa bacteria. Recent study done by Gummaluri S S et al.,[23, 24] 2024, 2025 incorporated amoxicillin+ clavulanate gel, metronidazole and neem gels into T-PRF and checked for fibrin mesh work border and cellular entrapment. They concluded that T-PRF structure didn't alter and had a similar structure to that of T-PRF alone when checked for light and scanning electron microscopies.

With the increased hypersensitivity and resistance of antibiotics there was a gradual shift into herbal extracts. In the recent trends their utilization had gradually increased in the form of mouth washes, gels for topical applications in gingivitis and periodontitis condition. Extracts such as neem, aloe-vera, mangosteen, curcumin, Triphala indica, Matricharia chamomile, green tea catechins etc have been used as an adjunct therapy in treating gingival and periodontal diseases. Most of these extracts exhibit anti-oxidant, anti-inflammatory, antimicrobial, anti- carcinogenic, anti-cariogenic properties and help in reducing the bacterial load, post-operative inflammation which in turn improve the health of the tissues.[25] Studying the release of the components from the main drug at regular time interval, help in identifying the efficacious nature of the drug that would be released. This would enhance the development of newer treatment strategies. In the present study, there was

a utilization of Neem and Triphala indica extracts in the form of gels and incorporated into T-PRF.

Triphala, a blend of three plants consists of carotene, D-glucose and fructose, riboflavin empicol, chebulinic acid, vitamin C, arachidonic acid, linoleic acid, oleic, palmitic, and stearic acid, tannic acid, terchebin, and other essential components. These components help control cell death and lower lipid peroxidase activity, which indicates the antioxidant, anti-inflammatory, antimicrobial, and antiseptic qualities of TI.[26, 27] Whereas Neem consists of Azadirachtin, nimbin, nimbidin and some additional components such as flavonoids, alkaloids etc. showing antiseptic, insecticidal, antiulcer, astringent etc.[28] Studies were performed to check the efficacy of these herbal extracts on gingivitis or periodontitis conditions as topical application or as LDD and reported good results.[29, 30] These were not tried as SDDS by incorporating into T-PRF. Before performing a human trial, it is always beneficial to perform invitro substance release so that the biomaterial integrity and GF release hampering will be known. Study was performed according to null hypothesis that adding the Neem and Triphala gels to T-PRF clots will alter the release of Insulin like growth factor 1 and there will be no timely release of azadirachtin and vitamin C through the T-PRF clots. Hence present study aimed to evaluate the release of Azadirachtin (Az), Vitamin C (Vit C) and Insulin like Growth Factor 1 (IGF 1) release from T-PRF clots infused with Neem and Triphala indica gels to check T-PRF as sustained drug delivery system (SDDS).

MATERIALS AND METHOD

Study design and Patient Recruitment

Present study was a primitive invitro study where IGF 1, Vit C and Az releases were checked at various time frames. A total of 6 healthy (mean age of 26.4 years) were recruited in the study 3 males and 3 females from the out-patient department of periodontics Saveetha dental college and hospital, Chennai. Study was performed during the tenure of April 1st to May 10th 2025. Oral and written informed consent was obtained from the volunteers. Before performing the study, approval from the scientific review board was obtained and approval number as SRB/SDC/PhD/PERIO-2251/25/025. Study was performed according to the CRIS guidelines that report the invitro studies. Patient blood was drawn hence Helsinki declaration 1975 was also followed to maintain the reliability of the study and respecting the volunteers.

Inclusion and Exclusion Criteria

Subjects who were voluntarily willing to participate having greater than 2,00,000 platelets per cubic millimeters, not having any systemic disease, non-smokers, not under any sought of medication that would affect the blood and blood products were included in the study. Subjects who were not interested, pregnant and lactating females, any subjects that reported with some ailments after blood investigation were excluded from the study.

Herbal Extract gel Preparations:

Neem (NE) and Triphala indica (TI) Gels were procured from Perio Biologics ™ Lab, Hyderabad, Telangana, India. Briefly gels were prepared as follows- TI is a combination of three fruits Terminalia chebula (Haritaki), Terminalia bellrica (Bibhitaki) and Emblica officinalis (Amalaki). TI power is mixed with distilled water and left for few hours. Later mixture was strained to obtain a clear extract. Further this prepared extract was mixed with hydroxyethyl cellulose to obtain a homogenous mixture. glycerin & phenoxyethanol were added and pH was adjusted with proper stirring to obtain a final 22%w/v concentration of TI gel. While coming to neem gel preparation, neem leaves were boiled to prepared concentrated neem extract. Carbopol 943p was added to the neem extract to prepare the base gel. To this extract calcium chloride ($CaCl_2$) and Sodium Chloride (NaCl) were added to prepare the final 12% w/v concentrated NE gel. Both the gels were packed into syringes, subjected to stability testing and properly sterilized to use it further. All the raw materials were obtained from local ayurvedic shop vendors. Additional binder and gel preparation materials were procured from Sigma Aldrich Company.[31, 32]

PROCEDURE

T-PRF clots Preparation and herbal extracts injection

Based on Modified Tunali M et al., protocol of 3500rpm for 15 min with 400 x *g* force, blood of 15ml was drawn from antecubital vein (divided into 5 ml each) and transferred to medical grade titanium test tubes (Supra Alloys Company Camarillo USA) without any anticoagulant and subjected to centrifugation using (Remi R 8C, New Delhi, India). Clots were retrieved using sterile tweezers and transferred into kidney trays. Hence, a total of 18 T-PRF clots were retrieved and divided in to 3 groups each containing 6 clots (T-PRF+NE, T-PRF+TI and T-PRF alone. Prior to conduction of experiment, herbal extracts (NE and Az) were injected into the clots individually based on Ercan E et al.,[22] 2022, Gummaluri SS et al.,[23, 24] 2024 and 2025 protocol where gels were injected directly into clots without compressing them into membranes. Later these clots were macerated and that liquid was stored at -70°C. Further, at every time point 5ml of that macerated clot fluid from test pool and subjected to analysis

Regarding Azadirachtin release from T-PRF infused with NE, all the clots were macerated and approximately 5ml of the liquid was mixed (Figure 1) and shaken for 10-20 minutes with 5ml of HPLC grade methanol. The solution was then

filtered through 0.45 µm membrane filter. Accurately measured 1ml from above solution was further diluted with 10 ml with methanol. Accurately measured 4.0 mL portion of Azadirachtin extract solution was diluted to 10 mL with methanol so as to prepare concentration of 40 µg/ml. Equal volume (40 µL) of standard and sample solutions was injected separately after equilibrium of stationary phase. The HPLC method for estimating azadirachtin levels was used which involves reverse-phase chromatography with a C18 analytical column and a methanol-water mobile phase. The detection was done using a UV detector (HPLC©, Acidum India Pvt Ltd, Pune, India) at 222 nm, and the flow rate is maintained at 1 mL/min.[33]

While coming to Vit C release from T-PRF infused TI similar to the above procedure All the clots were macerated and approximately 5ml of the liquid was mixed and shaken for 10-20 minutes with 5ml of HPLC grade methanol. The solution was then filtered through 0.45 µm membrane filter. Reverse-phase C18 column is commonly used. Tissue fluids were deproteinized using metaphosphoric acid before injection into the HPLC system (HPLC©, Acidum India Pvt Ltd, Pune, India) and detection of Vit C was performed at 254 nm.[34] Though we used HPLC, the results were compared with a standard Vit c kit, Ascorbic Acid Test Kit, Model ASC-1, Hach India, New Delhi (Figure 2 and 3). Regarding IGF 1 release enzyme linked immunosorbent assay (ELISA) test (Miltenyi Biotec, Inc, Auburn, CA, USA) was used and performed according to manufacture instructions. The sensitivity and evaluation range for parameter was- human IGF- 1:(0.94 ng/ml & 1.56-100ng/ml). At time frames of 6 hours, 72 hours, 7th and 14th day, Vit C (µmol/L); Az (µg/ml) and IGF 1 (ng/ml) release was checked. Further data was retrieved using ELISA plate reader (for IGF 1), UV detectors were used for Vit C and Az release. Absorbance values were calculated and release output was tabulated into the Microsoft excel spreadsheet.

STATISTICAL ANALYSIS

Using statistical package for social sciences (SPSS) version 22 IBM Pvt Ltd, Chicago, Illinois, USA, study analysis was performed. Data were expressed in mean and standard deviations. Paired t test was performed to check the time wise comparisons for T-PRF+NE (Az release) and T-PRF+TI (Vit C) release. For IGF 1 levels assessment for T-PRF+NE, T-PRF+ Az and T-PRF alone groups paired T-Test was used to check for time variations. Whereas one way ANOVA was used for IGF 1 level comparison for all the three groups at different time frames of 6hrs, 72 hrs, 7th and 14th day. A p value of <0.05 was considered statistically significant.

Figure 1: Depict the Neem Test Extract solution used in the present study

Figure 2. Depict the Vitamin Reference Solution used in the study

Figure 3. Depict the image of Vitamin C kit used in the study

Table 1. Depict the comparative analysis of Azadirachtin levels in T-PRF + Neem at different time intervals.

		T-PRF + Neem			
TIME		**Azadirachtin µg/mL**			
		Mean ± SD	**Mean ± SD**	**mean Difference**	**P-Value**
6hrs	**72hrs**	42.37 ± 5.93	43.35 ± 9.28	-0.98	0.870#
6hrs	**7th DAY**	42.37 ± 5.93	38.12 ± 6.05	4.24	0.045*
6hrs	**14th DAY**	42.37 ± 5.93	44.12 ± 7.79	-1.75	0.722#
72hrs	**7th DAY**	43.35 ± 9.28	38.12 ± 6.05	5.23	0.440#
72hrs	**14th DAY**	43.35 ± 9.28	44.12 ± 7.79	-0.77	0.829#
7th DAY	**14th DAY**	38.12 ± 6.05	44.12 ± 7.79	-5.99	0.273#
TIME		**IGF1 (ng/mL)**			
		Mean ± SD	**Mean ±SD**	**mean Difference**	**P-Value**
6hrs	**72hrs**	15.58 ± 2.44	15.28 ± 2.95	0.31	0.787#
6hrs	**7th DAY**	15.58 ± 2.44	15.48 ± 2.87	0.11	0.948#
6hrs	**14th DAY**	15.58 ± 2.44	14.95 ± 2.33	0.63	0.686#
72hrs	**7th DAY**	15.28 ± 2.95	15.48 ± 2.87	-0.20	0.882#
72hrs	**14th DAY**	15.28 ± 2.96	14.95 ± 2.33	0.33	0.801#
7th DAY	**14th DAY**	15.48 ± 2.87	14.95 ± 2.33	0.53	0.640#

SD- Standard Deviation; µg- micro gram, mL- milli liters, ng-nano gram, * indicates statistical significance, # indicate non-significant, $p<0.05$ considered statistically significant

RESULTS

Regarding the Azadirachtin (Az) release there was decreased levels reported between 6 hrs and the 7th day and it was statistically significant ($p = 0.045$*). Whereas, all other time frame comparisons values were non-significant ($p>0.05$) indicating the stable release of Az. Further, when IGF 1 levels were assessed all the comparisons were non-significant (Table 1). Regarding Vit C release from T-PRF clot there was a decrease in the amount released when compared at 6hrs & 7^{th} day ($p=0.008$*) and 72hrs and 7^{th} day ($p=0.037$*). There was a marked significant reported from 7^{th} to 14 day which was statistically significant ($p=0.006$*) while remaining comparisons were non-significant ($p>0.05$). Further, when compared for IGF 1 release at different time frames they were non-significant (Table 2).

In T-PRF alone group there was non-significant release of the IGF 1 at all the time frames (Table 3). When overall comparisons were performed in all the three groups and at different time frames, IGF 1 release report was non-significant (Table 4 and Graph 1).

Table 2. Depict the Analysis of Vitamin C Levels in T-PRF + Triphala at different time intervals.

TIME		T-PRF+ Triphala Vit C µmol/l			
		Mean ± SD	Mean ± SD	mean Difference	P-Value
6hrs	72hrs	180.17 ± 21.71	172.83 ± 28.94	7.33	**0.191**#
6hrs	7th DAY	180.17 ± 21.71	151.28 ± 18.39	28.89	**0.008***
6hrs	14th DAY	180.17 ± 21.71	178.15 ± 15.92	2.01	**0.855**#
72hrs	7th DAY	172.83 ± 28.94	151.28 ± 18.39	21.56	**0.037***
72hrs	14th DAY	172.83 ± 28.94	178.15 ± 15.92	-5.32	**0.681**#
7th DAY	14th DAY	151.28 ± 18.39	178.15 ± 15.92	-26.88	**0.006***
TIME		T-PRF+ Triphala IGF1 (ng/mL)			
		Mean±SD	Mean± SD	mean Difference	P-Value
6hrs	72hrs	14.69 ± 2.25	15.49 ± 2.31	-0.80	**0.377**#
6hrs	7th DAY	14.69 ± 2.25	14.87 ± 2.64	-0.18	**0.636**#
6hrs	14th DAY	14.69 ± 2.25	16.35 ± 2.35	-1.66	**0.236**#
72hrs	7th DAY	15.49 ± 2.31	14.87 ± 2.64	0.62	**0.392**#
72hrs	14th DAY	15.49 ± 2.31	16.35 ± 2.35	-0.86	**0.428**#
7th DAY	14th DAY	14.87 ± 2.64	16.35 ± 2.35	-1.48	**0.264**#

SD- Standard Deviation; µg- micro gram, mL- milli liters, ng-nano gram, * indicates statistical significance, # indicate non-significant, p<0.05 considered statistically significant

Table 3. Depict the analysis of IGF1 Levels in T-PRF alone at different time intervals.

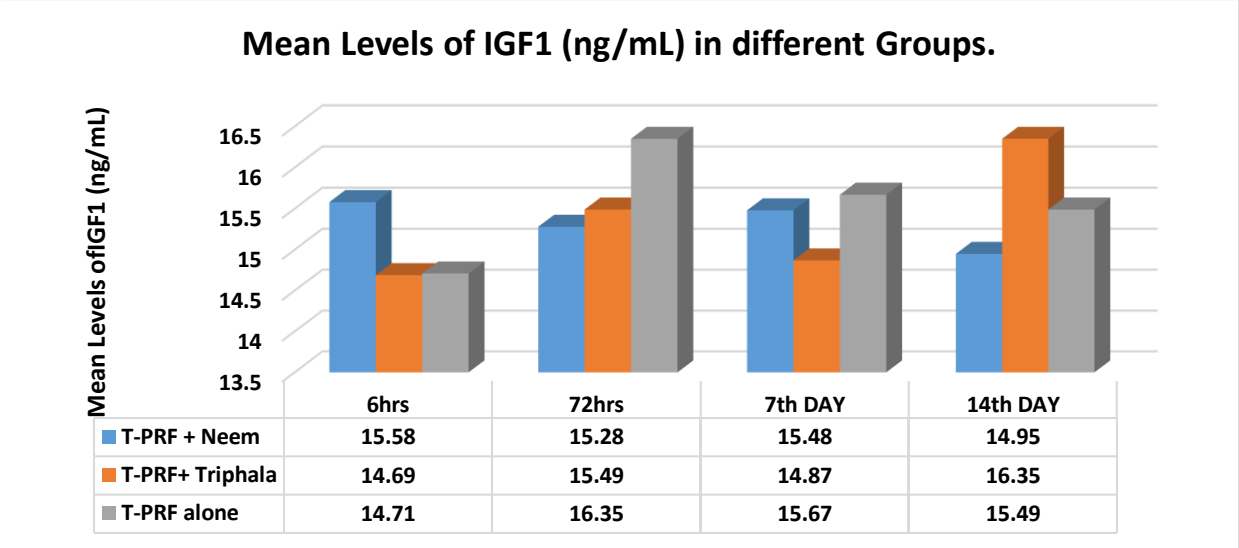
TIME		T-PRF alone IGF1 (ng/mL)			
		Mean ± SD	Mean ±SD	mean Difference	P-Value
6hrs	72hrs	14.71 ± 1.75	16.35 ± 2.06	-1.64	**0.305**#
6hrs	7th DAY	14.71 ± 1.75	15.67 ± 2.08	-0.96	**0.511**#
6hrs	14th DAY	14.71 ± 1.75	15.49 ± 1.39	-0.78	**0.488**#
72hrs	7th DAY	16.35 ± 2.06	15.67 ± 2.08	0.68	**0.328**#
72hrs	14th DAY	16.35 ± 2.06	15.49 ± 1.39	0.86	**0.319**#
7th DAY	14th DAY	15.67 ± 2.08	15.49 ± 1.39	0.18	**0.803**#

SD- Standard Deviation; µg- micro gram, mL- milli liters, ng-nano gram, * indicates statistical significance, # indicate non-significant, p<0.05 considered statistically significant

Table 4. Depict the comparison of IGF1 Across All Groups at Different Time Intervals using One-way ANOVA test.

	IGF1 (ng/mL)				
	T-PRF + Neem	T-PRF+ Triphala	T-PRF alone		
TIME	Mean±SD	Mean±SD	Mean±SD	F-Value	P-Value
6hrs	15.58 ± 2.44	14.69 ± 2.25	14.71 ± 1.75	0.334	0.722#
72hrs	15.28 ± 2.95	15.49 ± 2.31	16.35 ± 2.06	0.317	0.733#
7th DAY	15.48 ± 2.87	14.87 ± 2.64	15.67 ± 2.08	0.161	0.853#
14th DAY	14.95 ± 2.33	16.35 ± 2.35	15.49 ± 1.39	0.698	0.513#

IGF- insulin like growth factor, SD- standard deviation, # indicates non- significant



Graph 1 Depicts the overall mean levels of IGF 1 at different time frames for all the three groups

DISCUSSION

Present invitro study was performed mainly to assesses whether injecting TI and NE will hamper the release of growth factor from T-PRF and vice versa i.e. whether T-PRF will hold the extracts and release them timely. Vit C, Azadirachtin (Az) and IGF 1 release were checked was mainly because Vit C helps in proper wound healing and very much important for post translational modifications of proline and lysine proteins.[35] Az has antibacterial antifungal and anti-inflammatory properties that would combat the periodontitis causing bacteria.[28] Reason for assessing IGF 1 was mainly due to check whether apoptotic effect was happening due to the addition of extracts. Moreover, IGF 1 helps in wound healing, repair of soft and hard tissues by stimulating the cell proliferation and differentiation.[36] To the authors knowledge much number of studies were not performed for comparing the present study results. Hence existing other substance literature was compared to depict the present study results. Present study results were in accordance with study done by Pollock D et al.,[8] 2019 where they checked the incorporation of antibiotics in L-PRF helped in better outcomes regarding the antimicrobial efficacy and reduced post-operative infection. In the present study these herbal extracts (Az/ Vit C) release was also timely and didn't hamper the release of IGF 1 from T-PRF which indirectly indicate that T-PRF can be used as a SDDS. In Ercan E et al.,[22] 2022 study T-PRF clot timely released doxycycline antibiotic up to 7 days and also showed good antimicrobial property whereas similar release of Az and Vit C was reported from T-PRF was reported up to 14 days. This release will be helping to maintain good healing property (Vit C and IGF 1 release not hampered) as well as antimicrobial property (Az has anti-inflammatory and antimicrobial property) reducing the load of microbiota when explored in clinical studies.

The timely release of Vit C helps in good wound healing and better anti-inflammatory property which would enhance the treated site positively when used clinically. Thus, in the present study there was a check for Vit C release up to 14 days. This was supported by a systematic review done by Katariya C and Jayakumar N D[37] 2022 where they concluded that Vit C helped in improvement in clinical parameters such as probing pocket depth (PPD), clinical attachment level (CAL). It also activates the collagen synthesis by stimulating the procollagen m RNA and collagen transcription mechanism.

Study such as Ganvir MN et al.,[38] 2022 stated the use of Az chip (Neem chip) and Laser adjunct to non-surgical periodontal therapy (NSPT) they concluded that Az chip had shown better improvement in clinical parameters followed by laser and least by NSPT alone during the 1 month follow up. While coming to Heiman L et al.,[39] 2017 stated that neem leaf extract had a greater antioxidant property as well as antimicrobial property against Porphyromonas gingivalis (Pg) when interacted with red blood cells, lysozyme and bacteria. Hence, present study results may be comparable and confirmable as it showed the in-vitro release of Az from T-PRF up to 14th day which may be beneficial in the control of subgingival microbiota through improved anti-oxidant property. This also show the anti-inflammatory property. Regarding IGF 1 release there was no significant difference in all the groups at all the time frames this is in accordance with study done by Fukui N et al.,[40] 2015 where they stated that there no significance regarding the release of IGF 1 for L-PRF. They stated that IGF 1 is a multifunctional peptide that helps in osteogenic

differentiation of bone marrow mesenchymal stem cells (BMSC's) and human dental pulp stem cells (HDPSC's).[41] The levels of IGF 1 in the reported study was much higher than current study and this might be due to the amount of blood that was drawn for clot preparation varied. They also stated that levels of IGF 1 were almost similar to that of human blood.

Limitations of the present study might be smaller sample size and invitro study with shorter time releases of Az/ Vit C and IGF 1. Longer day release assessment with multiple growth factor release consideration in T-PRF as well as herbal extracts (TI/ NE) might have altered the results. Cell line studies, animal and huma clinical trials if performed after characterization with Scanning electron microscope (SEM) + Fourier Transmission Infrared Spectroscopy (FR-IR) can be helpful for proper establishment of T-PRF as SDDS of herbal extract.

CONCLUSION

Thus, within limitations present in-vitro study of T-PRF+TI and T-PRF+NE concludes that T-PRF can be used as SDDS and help in sustained release of herbal extract products such as Vit C, Az and IGF 1. Addition of herbal extracts didn't alter the release of growth factor. Further clinical trials with larger sample size should be performed to determine better conclusive outcomes.

DECLARATIONS

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Conflicts of interest and financial disclosures

The authors declare no conflict of interest and there was no external source of funding

Ethical approval

Approval for the conduction of the study was obtained from the Institutional Scientific Review Board (SRB/SDC/PhD/PERIO-2251/25/025).

Informed Consent

Verbal and written informed consent were obtained from healthy volunteers

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