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To estimate the effect of papaya, kiwi, and synthetic vitamin C on gingival health during experimental gingivitis: A randomized clinical trial

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## Abstract

**Aims:** To estimate the effect of papaya, kiwi, and synthetic vitamin C on gingival health during experimental gingivitis

**Settings and Design:** A triple blind randomized clinical trial was designed.

**Methods and Material:** Out of ninety-nine participants, only eighty were allotted randomly to four groups and were daily supplemented with either 400 g papaya (group 1), 270 g kiwi (group 2), 200 mg synthetic vitamin C-Limcee tablet (group 3), or water (group 4). All the participants were provided with a thorough scaling and supplementation during the pre-experimental period which was for 21 days and was instructed for maintaining oral hygiene. After that, the experimental gingivitis period was

commenced including the supplements for 21 days, with a minimal amount of dietary fruits/vegetable intake throughout the study period. The parameters evaluated were plaque index, gingival index, and bleeding index at baseline (day 0), day 7, day 14, and day 21 of experimental gingivitis.

**Results:** The values of plaque and the gingival index were increased in all four groups but group 1 showed significantly less value followed by groups 2 and 3 when compared with the control group (group 4).

**Conclusions:** The study showed that daily consumption of 400 g papaya, 270 g kiwi, or 200 mg synthetic vitamin C has an inhibitory effect during experimental gingivitis as compared to the control group.

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**Keywords:** Vitamin C, supplementation, experimental gingivitis, papaya

# Introduction

It is a well-known fact that nutrients depletion gives rise to malnutrition.<sup>[1,2]</sup> Vitamin C also known as ascorbic acid (water-soluble vitamin) is a rich source of various fruits and vegetables.<sup>[1]</sup> An antioxidant is one of its properties that plays an important role during the synthesis of collagen fibres. Its insufficient consumption results in collagen destruction and can lead to a state of vitamin C deficiency <sup>[1]</sup>, which is inimical to both general as well as to periodontal health. So, the present study was mainly focused on the role of vitamin C in gingival health.

The daily requirement of vitamin C is 90 mg for men and 75 mg for women respectively (Institute of medicine US, 2000).<sup>[3]</sup> But there is an evidence-based study by Frei et al. (2012) in which he concluded that consumption of 200 mg/day of vitamin C help to maximize the benefits of vitamin's potential health and lowers the risk of unfavorable health effect.<sup>[4]</sup>

In the study of vitamin C supplementation, it can be given either in a form of synthetic vitamin C (tablet) or by fruits (natural sources). The advantage of a tablet form of vitamin C is that its pharmacokinetics can be studied easily and required effects can be gained from the given dose of vitamin C but when it is in a form of natural sources, rich in fruits/vegetables provides many other micronutrients and phytochemicals that may impact for example the bioavailability of vitamin C.<sup>[5]</sup>

Both Papaya and kiwi fruit contains an excessive dietary source of vitamin C, as papaya contains 63 mg, and green kiwi fruits contain 92 mg of vitamin C/100g fruit whereas e.g. mango contains 36 mg and orange contain 53 mg of vitamin C/100 g fruit <sup>[6]</sup> and also have many properties like anti-inflammatory, antimicrobial, anticancer, and antioxidant.<sup>[7,8]</sup>

There are very few studies in the literature which have been carried out to study the effect of vitamin C on the development of gingival inflammation. Therefore, the present study investigated the effect of papaya, kiwi, and synthetic vitamin C on the development of gingival inflammation during experimental gingivitis

#### Material and methods

The triple-blind (participants, examiner, and assessor) randomized control trials were planned in this present experimental study according to the CONSORT statement (http://wwwconsort-statement.org/). The study examined four groups, group 1 (papaya fruit), group 2 (kiwi fruit), group 3 (synthetic vitamin C), and group 4 (control) during the development of gingival inflammation. The experimental period was of 4 weeks with an assessment conducted at baseline (day 0), day 7, day 14, and day 21. The study design was approved by the Ethical Committee of university. Before proceeding for the study, participants were explained in detail about the study design, and then they were asked to sign a written informed consent form.

A total of 99 participants were screened in the department of periodontology. The screened participants were all undergraduate dental students, who were staying in the hostel which was within college premises where the study was conducted. Age group between 18-25 years, Nonsmokers and  $\leq 4$  mm periodontal pocket depth were included in the study. Any type of systemic diseases, which can be detrimental to periodontal health, any medication (e.g. NSAIDs, analgesic or antibiotic) within 6 weeks before the study, history of drug abuse, lower jaw teeth present with a carious lesion, untreated crowns, or having orthodontic appliances, Pregnant women and lactating mother and Physically/mentally handicaps were excluded.

Two examiners were blinded throughout the experimental gingivitis period. Clinical parameters were evaluated by a

single-blinded examiner (AK) and conceal allocation was performed by a double-blinded examiner (MB) to determine the group subject.

The following clinical measurement was performed by a single-blind examiner (AK) during the experiment period:

- Plaque index (Silness and Loe, 1964)<sup>[9]</sup>
- Gingival index (Loe and Silness, 1963)<sup>[9]</sup>
- Gingival Bleeding index (Ainamo and Bay, 1975)<sup>[10]</sup>

A total of 80 participants were registered in the study. The groups were allocated by using a block randomization method with a selected block size of four ('ABBA') and a computer-generated list was used for the sequence allocation which was performed by the blinded examiner (MB). To obscure the subject allocation, it was sealed in an opaque envelope, and to decide for the group allocation, envelopes were opened and were assigned to Group 1, Group 2, Group 3, and Group 4. Each group comprised of 20 participants. As all the four groups were coded differently, it was difficult for the examiner and assessor to determine to what group the participants were assigned and the participants were blind about which group (control or study) they were registered. The coding system of the groups was disclosed as soon as the study's data analyses were done.

## **Study procedures**

The pre-experimental period for 21 days was included in the present study under which all the participants received thorough scaling and supplementation. Participants were allocated randomly into four groups, under which they were provided with the supplements. As for supplements, group 1 (papaya), group 2(kiwi), group 3 (synthetic vitamin C- Limcee tablet), and group 4 (control group) a glass of water (it was not revealed what was mixed in water) was given. In a glass of water, fruit flavour was mixed and given in a form of juice. The provided papaya and kiwi were brought from the fresh fruits market in Jaipur. 400 g and 270 g of papaya and kiwi were given to the participants which contain 250 mg of Vitamin C.<sup>[8,11]</sup>

All the participants were under the supervision of the examiner (MB) during the supplementation which was provided to them till the end of the study period. Participants were allowed to consume little fruits and vegetables as much as it can be possible and had to be noted in a diary in terms of type, amount (pieces/bowl), and preparation (raw/cooked). The whole of the supplementation records was given to the research assistant at end of each week (MB). At the end of the study, the total amount of vitamin C consumed by all the participants during the entire 6 weeks was calculated by means of the National Nutrients Database for Standard Reference (USDA, 2016)<sup>[12]</sup>.

After 21 days of pre-experimental study, the period of experimental gingivitis started and the supplements which were provided to the groups were continued till the end of the study. The participants were asked to abstain from any type of oral hygiene measures for 21 days in the lower jaw of all teeth. While brushing the teeth in the upper jaw and to avoid cleaning of teeth in the lower jaw, the participants were instructed to cover the lower teeth with a customized soft acrylic guard.

The clinical parameters were examined in all the 80 participants on day 0 (baseline), day 7, day 14, and day 21 by the examiner (AK) who was blinded about the group allocations throughout the experimental gingivitis periods.

All the participants received thorough oral prophylaxis after the experimental study period was over and was also allowed to resume their habitual oral hygiene and were released from dietary restriction.

The statistician was blinded as to what group the participants were allocated. Data obtained was compiled on an MS Office Excel Sheet (v 2010, Microsoft Redmond Campus, Redmond, Washington, United States). Data were subjected to statistical analysis using the Statistical Package for social sciences (SPSS v 21.0, IBM). Descriptive statistics like frequencies and percentages for categorical data, mean & SD for numerical data has been depicted. The normality of numerical data was checked using the Shapiro-Wilk test & was found that the data did not follow a normal curve; hence non-parametric tests have been used for comparisons.

The primary outcomes were gingival index and secondary outcomes were plaque index and bleeding index. Intergroup comparison (>2 groups) was done using Kruskal Wallis ANOVA followed by pairwise comparison using the Mann Whitney U test. The intragroup comparison was done using Friedman's (for >2 observations) followed by pairwise comparison using Wilcoxon Signed rank test. A comparison of frequencies of categories of variables with groups was done using the chi-square test. The individual mean value for plaque index and gingival index were calculated. The bleeding index was calculated in a score form. And the amount of daily dietary vitamin C intake during 6 weeks experiment period was also analyzed. P-values (<0.05, <0.01) were considered statistically significant.

#### Results

A total of 99 patients were evaluated for eligibility, and out of that 89 participants met the inclusion criteria. Among these 89 participants, 9 refused to participate; thus 80 participants were randomized and equally allocated into four groups. All the participants (n=80) completed the study (CONSORT flow diagram for study design, Figure 1)

On Intergroup group comparison the values of plaque index (PI) and gingival index (GI) showed statistically significant differences (p<0.01) at baseline, 7, 14, and 21 days, and a non-significant difference (p>0.05) was seen

at the baseline (day 0). Papaya group developed less plaque and gingival inflammation than the control group (Tables 1 and 2 and Figure 2 and 3).

Gingival Bleeding Index (GBI) showed a statistically significant difference (p<0.01) for the frequencies between the groups (**Table 3**). And the result of the amount of dietary vitamin C consumed by the 4 groups during 6 weeks of the experimental period is presented in (**Table 5**). It showed a statistically significant difference (p<0.001) in all the four groups.

On intragroup comparison the values of both PI and GI showed a statistically significant difference (p<0.01) in all the groups at various time intervals with higher values on day 21 and least on days 0, 7, and 14 in all the 4 groups (**Figure 4 and 5**). On pairwise intergroup comparison both PI and GI showed statistically significant differences (p<0.01) seen for the values between the groups on day 7, 14, and 21. (**Table 4**)

#### Discussion

It is well-known fact that vitamin C deficiency causes scurvy <sup>[13]</sup>, which is associated with gingival bleeding and tooth mobility <sup>[14]</sup> and is caused by altered collagen formation <sup>[15]</sup>, impaired connective tissue barrier formation, and fibroblast growth <sup>[16]</sup>. When the levels of serum ascorbate fall below 11  $\mu$ m, then it is diagnosed as scurvy whereas the normal range of ascorbate levels is 25-50  $\mu$ m<sup>1</sup>.

The present experimental study result showed that papaya, kiwi, and synthetic vitamin C supplementation can have an inhibitory effect on the development of gingival inflammation during experimental gingivitis in a group of non-smoking young adults. During experimental gingivitis, smokers were excluded because several studies demonstrated a reduced bleeding response in experimental trials <sup>[17]</sup>.

Vogel et al., in 1986<sup>[18]</sup> carried out the first study on the effect of vitamin C supplementation on experimental gingivitis and investigated that based on their result it was impossible to conclude that vitamin C supplementation had any positive effect in reduction of gingival inflammation. In that study a daily dose of vitamin C supplementation given was 1500 mg along with their daily dietary vitamin C intake amounting was almost 200 mg and as vitamin C is a water-soluble vitamin, the excess of vitamin C will be efficiently excreted and therefore further vitamin C supplementation will be highly unlikely to benefit<sup>[19]</sup>.

Another study of the experimental gingivitis model on vitamin supplementation by Amaliya in 2018 <sup>[20]</sup> estimated that consumption of vitamin C supplementation has a preventive effect on the development of experimental gingivitis as compared to the control group. The result showed a positive effect may be because the participants were requested to consume little fruits and vegetables.

There is one study in which untreated periodontitis population reported with daily supplementation of 200 mg vitamin C together with 100 mg citrus flavonoids and showed a better result destitute of regular dental care, also a reduction in both subgingival load as well as the serum CRP (C - reactive protein) levels and least inflammation. <sup>[21]</sup> Depletion of vitamin C result in gingival bleeding irrespective of oral hygiene performance. <sup>[22]</sup> The level of Vitamin C concentration was found low in both gingivitis <sup>[23,24]</sup> and periodontitis <sup>[25]</sup> patients.

The reduction of plaque and gingival inflammation may also be due to the local effect of papaya and kiwi components. It has been shown that proteolytic enzymes present in papaya (papain and chymopapain)<sup>[26]</sup> have shown immunomodulatory, antibacterial, and antiinflammatory activities. Proteolytic enzymes are present more in papaya than kiwi <sup>[27]</sup>. Due to which papaya group showed reduced inflammation as compared to other groups. Papaya was active against both gram-negative and gram-positive bacteria due to which the papaya group has shown reduced plaque development. Kiwi also has the properties of antioxidant, anti-inflammatory, and antibacterial properties which contain a higher amount of vitamin C than papaya.<sup>[6]</sup> Kiwi group has also shown a reduction in plaque and gingival inflammation as compared to vitamin C and control groups. Based on GI measurement, papaya and kiwi failed to show a significant increase in gingival inflammation. Papaya showed a better result as compared to other groups. It may be due to the greater preventive effect of papaya against the development of gingival inflammation than kiwi and synthetic vitamin C.

The advantage of the present study is that it is a tripleblind experimental study. In this study, all the participant were asked to consume little fruit and vegetables as possible or to limit the intake to 5-6 mg per day because the supplements which the groups were provided during the study was in a sparse quantity and higher increase in amount would have affected the study. This implies the present study was a kind of vitamin C depletion that was done in control group participants during the experimental period. Also, the risk of bias was low as follow up of the study was convenient because all the participants were staying in the hostels which were within the college campus premises where the study was conducted.

The limitation was that it could have been done in a large study sample size for getting more accurate mean values and also it is still not clear that whether vitamin C supplementation will have the same inflammatory preventive effect in smokers as well as those who follow the current RDA of vitamin C, i.e. 90 mg and 75 mg per day.

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# Legend Figures and Tables

Table 1: Intergroup comparison of plaque index at various time intervals

Day	Group 1 (Papaya)		Group 2 (Kiwi)		Group 3 (Vitamin C)			Group 4 (Control)			P Value of KW ANOVA		
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Medium	
0	.135	.149	.100	.140	.135	.100	.150	.153	.100	.105	.146	.050	0.639
7	.535	.220	.550	.690	.248	.700	.805	.228	.800	.960	.305	.900	0.000**
14	1.42	.289	1.450	1.55	.298	1.50	1.705	.243	1.70	1.90	.215	1.900	0.000**
21	1.95	.337	1.90	2.175	.291	2.15	2.275	.349	2.30	2.55	.291	2.550	0.000**

\*\*Statistically significant, P<0.01 statistically significant. SD-standard deviation

Table 2: Intergroup comparison of gingival index at various time interval

Day	Group 1 (Papaya)		Group 2 (Kiwi)		Group 3 (Vitamin C)			Group 4 (Control)			P Value of		
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Medium	KW ANOVA
0	.390	.311	.250	.430	.368	.250	.370	.348	.250	.320	.348	.200	0.678
7	.830	.319	.750	1.015	.385	.950	1.00	.369	.900	1.17	.379	1.05	0.032**
14	1.23	.329	1.10	1.385	.405	1.450	1.53	.372	1.450	1.93	.307	1.90	0.000**
21	1.81	.298	1.750	2.010	.346	1.950	2.150	.351	2.00	2.48	.348	2.450	0.000**

\*\*Statistically significant, P< 0.01 statistically significant. SD-standard deviation

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Day	Score	Group 1	Group 2 (Kiwi)	Group 3	Group 4 (Control)	Total	Chi Square Value	P Value Of Chi Square	
		(Papaya)		(Vitamin C)				Test	
0	0	15	16	17	16	64	0.625	0.891#	
	1	5	4	3	4	16			
7	0	16	14	12	7	49	9.427	0.024**	
	1	4	6	8	13	31			
14	0	12	10	8	3	33	9.233	0.026**	
	1	8	10	12	17	47	-		
21	0	12	8	5	1	26	14.815	0.002**	
	1	8	12	15	19	54			

# Table 3: Intergroup comparison of gingival bleeding index

\*\*Statistically significant, P<0.01 statistically significant, # non-significant, P>0.05. SD-standard deviation.

# Table 4: Pairwise intergroup comparison of values

Days	Papaya vs. Kiwi	Papaya vs. vitamin C	Papaya vs. control	Kiwi vs. vitamin C	Kiwi vs. control	Vitamin C vs. control			
	p value	p value	p value	p value	p value	p value			
Plaque I									
0	0.779	0.726	0.440	0.92	0.268	0.24			
7	0.07	0.001**	0.000**	0.086	0.004**	0.107			
14	0.269	0.004**	0.000**	0.048**	0.000**	0.015**			
21	0.038**	0.006**	0.000**	0.346	0.000**	0.018**			
Gingival	Gingival Index								
0	0.762	0.782	0.388	0.600	0.249	0.476			
7	0.130	0.134	0.005	0.989**	0.166	0.076			
14	0.178	0.027**	0.000**	0.370	0.000**	0.001**			
21	0.000**	0.006**	0.000**	0.226	0.000**	0.004**			

\*\*Statistically significant, P< 0.01 statistically significant. SD-standard deviation

Table 5: Daily mean dietary, dietary plus supplemented and total vitamin C intake during 6 week study period

variable	experimental groups								
	Group 1	Group 2	Group 3	Group 4	p-value				
	(Papaya)	(Kiwi)	(Vitamin C)	(Control)					
Dietary vitamin C (mg) intake per day	5.67	5.63	6.08	6.13	<0.001**				
Dietary + supplemented vitamin C (mg)	255.67	255.63	206.08	6.13	<0.001**				
intake per day									

\*\*Statistically significant, P< 0.01 statistically significant.





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Figure 1: CONSORT flow diagram for study design



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Figure 2: Graph showing difference between the groups with group 4 having the higher mean values for plaque index at baseline (day - 0) to day 21



Figure 3: Graph showing difference between the groups with group 4 having the higher mean values for gingival index at baseline (day - 0) to day 21



Figure 4: Line graph showing difference between the groups with group 4 having highest medium values for plaque index at various time intervals



Figure 5: Line graph showing difference between the groups with group 4 having highest medium values for gingival index at various time intervals