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Original Article

Comparative Evaluation of the Anti-plaque and Anti-gingivitis Effects of Moringa plant and Fluoride Toothpastes among a Group of Children: A Randomized Controlled Study.

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Abstract

Objective: The present study assessed the anti-plaque and anti-gingivitis effects, the effect on salivary pH, and participant satisfaction of Moringa plant extract and Fluoride toothpaste among some children aged between 12 to 15 years. **Material and methods:** Sixty children were randomly divided into two equal groups: Group 1 (Moringa toothpaste) and Group 2 (Fluoride toothpaste). The assessment was completed using the gingival and plaque indices at baseline, three weeks, and six weeks. A digital pH meter was used to measure the salivary pH. The participants' satisfaction was evaluated by a questionnaire survey. **Results:** Upon completion of the 3-and 6-week follow-up, the gingival index scores significantly differ between the two groups with P-values of 6.82E-06 and 3.01E-08, respectively. The plaque index scores significantly changed with P-values equal to 0.00227 and 2.15E-05, respectively. A significant difference in salivary pH levels was demonstrated. The satisfaction survey revealed no statistically significant difference. **Conclusions:** Moringa toothpaste had an added effect on plaque, gingivitis, and salivary pH compared to Fluoride toothpaste, with no adverse reactions or difference in participants' satisfaction.

Keywords: Dental plaque, Fluoride toothpaste, gingivitis and Moringa plant

I. INTRODUCTION:

Oral diseases remain a significant public health concern despite numerous important advancements in dentistry. Gingivitis and dental caries are the most common oral disorders worldwide **(Deshpande *et al.*, 2021).**

Children and adolescents are affected by various periodontal diseases, including gingivitis, periodontitis, and periodontal diseases associated with systemic disorders. According to epidemiological research, adolescents and children in general exhibit gingivitis of varying severity. Furthermore, it is widely acknowledged that periodontal disease first manifests as gingivitis and that only a small number of people develop periodontitis **(Nadar *et al.*, 2020)**.

Gingivitis is normally observed as an inflammatory disorder started by dental plaque buildup and characterized by gingival redness and edema with the absence of loss of periodontal attachment. Gingivitis is usually

painless, infrequently leads to spontaneous bleeding, and is frequently characterized by minor clinical changes, subsequent in most patients being uninformed of the disease or unable to detect it **(Trombelli *et al.*, 2018).**

Although mechanical cleaning is the best method for preventing the colonization of microorganism species in the oral cavity, the effectiveness of cleaning is limited by patient compliance, improper application, and the presence of complex prostheses and/or appliances in the mouth. As a result, one of the oral care recommendations is using chemotherapeutic drugs to support oral cleanliness and offer antibacterial activity **(Alawamleh, 2021)**.

Combined with a toothbrush, toothpaste is a semisolid product used to remove dietary deposits from teeth. Several chemical preventative agents serve to decrease or prevent oral diseases and have positive effects on controlling plaque. However, some of these drugs have been linked to unfavorable impacts such as tooth discoloration and changed taste experience. Due to this, herbal toothpaste made from natural substances has become popular **(Duarte *et al.*, 2022).**

Herbal toothpaste has recently grown in acceptance, and studies have demonstrated that it effectively enhances dental and oral health. Numerous herbal products have antibacterial qualities that can stop acid generation by cariogenic bacteria and reduce their proliferation and development into cariogenic plaque **(Biria *et al.*, 2017).**

Moringa plant is a plant that is found all over the region of Indonesia and has many advantages. Moringa oleifera (M. oleifera) is distinguished by the presence of useful bioactive substances or phytochemicals such as phenolic acids, flavonoids, alkaloids, phytosterols, natural sugars, vitamins, minerals, and organic acids. Additionally, M. oleifera is an abundant source of minerals, tocopherols, carotenoids, polyunsaturated fatty acids, ascorbic acid, folate, and phenolics **(Mohanty *et al.*, 2021).**

This herb is also reported to have various pharmacological effects and, therefore, can treat various diseases, including oral disorders **(Nurul and Harun, 2020).**

Therefore, the present study aimed to evaluate the anti-plaque and anti-gingivitis properties of Moringa plant extract and Fluoride toothpaste among a cluster of children 12 to 15 years. In addition, the impact of the toothpaste on the salivary pH and participants' satisfaction regarding these kinds of toothpaste were assessed.

II. SUBJECTS AND METHODS

A. Study design

The current research was a 1:1 ratio parallel randomized clinical trial. The reporting of this study adhered to the CONSORT criteria. The title of this study and its registration number on clinicaltrials.gov are "Anti-Plaque and Anti-Gingivitis Effects of Moringa Plant Extract and Fluoride Toothpastes" and NCT05390099, respectively.

B. Sample size

To apply a two-sided statistical test, a power analysis was performed with adequate power. Based on the findings of **Maden et al., 2017** an alpha level of (0.05), a beta of (0.2), i.e., power=80%, and an effect size of (0.3) were adopted. After accounting for a potential 20% dropout rate, a predicted sample size of (n) of 60 cases was calculated.

C. Study setting

Children considered for suitability to participate in the study were recruited and examined from the outpatient clinics of the Pediatric Dentistry Department.

D. Eligibility criteria

- Inclusion criteria:

1. Children 12–15 years of age.
2. Children with gingival index score ≥1 and plaque index score ≥1.
3. Compliant children willing to participate.
4. Children whose guardians or parents provided their informed consent.

- Exclusion criteria:

1. Children wearing removable prosthesis or fixed or removable orthodontic appliances.
2. Children suffering from any systemic illness.
3. Children who were previously using mouthwashes.
4. Oral prophylaxis history within the previous six months.
5. Children using any other oral hygiene aids.

E. Ethical approval and informed consent

The Helsinki Declaration was followed in conducting the current research. The Ethics Committee of Scientific Research, Faculty of Dentistry, granted ethical approval with approval number 13221. After thoroughly explaining the process, advantages, and any drawbacks, the parent of the children signed an informed consent form. Verbal assent was also obtained from the children.

F. Randomization and allocation concealment

Simple randomization using the shuffled sealed opaque envelope method was used to divide 60 children randomly into two equal groups (n=30) with a 1:1 allocation ratio, where Group I received Moringa toothpaste and Group II received fluoride toothpaste (Signal, Unilever, UK).

G. Blinding

The participants, investigators, and statisticians were blinded to the allocation of toothpastes.

H. Outcomes

The results were recorded by two researchers who had undergone plaque and gingival scoring training and calibration. All the primary outcome measures were assessed at baseline, after three weeks and six weeks of treatment.

The primary outcome measures were scores of gingivitis and plaque using the gingival index GI (Loe and Sillness, 1963) and plaque index PI (Sillness and Loe, 1964), respectively. Secondary outcome measures included assessing the effect of both types of toothpaste on salivary pH levels and participants' satisfaction through a questionnaire.

I. Intervention

Preparation of Moringa extract

The extract was prepared following **Jwa, 2019**. Fifty grams of the powdered M. olifera leaves were soaked with 250 mL of deionized water, and the mixture was incubated for six hours with constant stirring. The obtained solution was filtered and dried using a rotary evaporator. The resulting powdered extract was kept in the freezer for further use.

Preparation of Toothpaste

Moringa toothpaste was formulated at the Pharmaceutical Technology Department, National Research Centre, according to **Gautam et al., 2020.**

Diagnosis and clinical examination

The personal, medical, and dental histories of the children were gathered and typed into a diagnostic chart. Under appropriate illumination conditions, a clinical examination was conducted using a mirror and probe.

The participants were randomly allocated to either the intervention (Moringa toothpaste) or control (Fluoride toothpaste) groups. Except for a coded identifying number, the packaging for both toothpastes was the

same. The clinic assistant was in charge of coding the toothpaste and distributing them to the participants. The examiners weren't informed about the toothpaste tube code until the data analysis was complete. A soft bristle toothbrush and toothpaste were given to each participant. For the following 42 days, they were instructed to abstain from using other dental hygiene tools. They were given a brushing method demonstration and instructed to use a pea-sized amount of toothpaste and the modified bass technique for two minutes, twice daily, under parental supervision.

During the intervention period, the participants were urged to practice regular oral hygiene and given strict instructions. The parents were given a printed schedule for six weeks and instructed to mark the form with a checkmark each time they brushed their teeth to ensure compliance.

J. Assessment of the outcomes

1. Gingival and plaque indices scores recording

The gingival index **(Loe and Sillness, 1963)** and plaque index **(Sillness and Loe, 1964)** were used to calculate the individuals' initial baseline gingival and plaque scores.

a- Gingival index **(Loe and Sillness, 1963)**

A score of 0 to 3 was given to each tooth's four gingival areas. Next, the GI for the tooth was calculated by adding the scores from the four tooth areas (buccal, lingual, mesial, and distal) and dividing the result by 4. The individual's GI was then calculated by summing the tooth indices and dividing the result by the total number of teeth examined.

b- Plaque index **(Sillness and Loe, 1964)**

Each tooth had its buccal, lingual, mesial, and distal four surfaces inspected. The PI for the tooth was then calculated by adding the scores from the four sections of the tooth and dividing them by four. By combining the indexes for each tooth and dividing by the total number of teeth analysed, the individual's PI was finally determined.

Participants were recalled again after three and six weeks intervals, and the gingival and plaque scores were reassessed by the indices mentioned.

2. Salivary pH recording

By asking the child to spit into a cup, baseline salivary samples were obtained, and the baseline salivary pH was determined using a digital pH metre. Children were instructed to drink 200 ml of apple juice, and then saliva samples were collected again from each child for pH measurement. The pH meter was neutralized after each measurement using distilled water to avoid contamination of the measured sample.

Each child was given a toothbrush and a pea-sized amount of Moringa toothpaste. Children were instructed to brush their teeth with the Moringa toothpaste, and then saliva samples were collected for pH measurement. The same steps were performed to assess the salivary pH after brushing with fluoride toothpaste. Blinding was implemented as the assessor was not the same investigator giving the patients the toothpaste.

3. Patient satisfaction survey

At the 6-week follow-up appointment, a survey based on Al-Hashedi et al., 2022 was conducted to gauge the participants' satisfaction with the toothpaste. The survey consisted of seven questions, with responses on a visual analog scale (VAS) ranging from not at all satisfied (score 0) to extremely satisfied (score 10).

K. Statistical analysis

The mean and standard deviation (SD) values of quantitative variables with a normal distribution were used to express the data. The student t-test was employed to see whether there were any significant differences between the two groups at various follow-up intervals.

Frequencies and percentages were used to describe qualitative data. The significance of the differences between the qualitative data was determined using the chi-square test. The cutoff for significance was set at P 0.05. The statistical package for social science (IBM®, SPSS® statistics for Windows computer software, version 20) was used to conduct the statistical analysis. By using MedCalc® to calculate Cohen's Kappa and the percent of agreement, the inter-examiner reliability was ascertained (statistical software version 16.8.4 for windows).

III. RESULTS

1. Demographic data

A total of 60 participants were enrolled in the study, and 30 children were allocated to each group. Only 57 participants completed the six weeks study period, as presented in Figure 1.

In the present study, children aged between 12-15 years with a mean age of 12.7 (±1.16) years in Group I and 12 (±1.31) in group II. No statistically significant difference was detected between both groups, with a P-value of 0.06571.

Regarding gender distribution, group I had 16 females (53.3%) and 14 males (46.7%), while group II had 17 females (56.7%) and 13 males (43.3%) with no significant difference (P-value= 0.96753).

There was no statistically significant difference between the groups concerning age and gender. Hence the groups were matched at the baseline.

2. Gingival and plaque scores

For all clinical measurements, the intra-examiner data were analyzed by calculating Cohen's κ test, which showed excellent agreement and significantly reliable results (κ = 0.92–0.96).

2.1. Group I

Regarding the gingival index scores, the mean GI scores at baseline, three weeks, and six weeks were1.25(±0.36),0.40(±0.37), and 0.11(±0.18), respectively. There was a statistically significant change in GI scores between the baseline and the three weeks (P-value= 5.55E-08) and between the 3 and 6 weeks (P-value= 0.00077).

Concerning the plaque index scores, the mean PI scores at baseline, three weeks, and six weeks were 1.23(±0.36),0.30(±0.31), and 0.10(±0.19), respectively. There was a statistically significant change in PI scores between the baseline and the three weeks (P-value= 1.85E-10) and between the 3 and 6 weeks (P-value= 0.01372). [Table 1]

2.2. Group II

Regarding the gingival index scores, the mean GI scores at baseline, three weeks, and six weeks were 1.5(±0.42),1.24(±0.41), and 1.20(±0.57), respectively. There was no statistically significant change in GI scores between the baseline and the three weeks (P-value= 0.09138) or between the three and six weeks (P-value= 0.83938).

Concerning the plaque index scores, the mean PI scores at baseline, three weeks, and six weeks were 1.30(±0.32), 0.84(±0.52), and 0.84(±0.57), respectively. There was a statistically significant change between the baseline and the three weeks (P-value= 0.00048). However, no statistically significant change in PI scores was displayed between the three and six weeks (P-value= 0.95578). [Table 2]

2.3. Comparison of gingival and plaque indices scores between both groups

Regarding the gingival index scores, the between-group comparison showed no significant difference in gingival scores between the two groups (P-value= 0.08963) at baseline. In contrast a significantly statistical change was shown in the 3 and 6 weeks follow

up with (P-value= 6.82E-06) and (P-value= 3.01E-08) respectively.

Concerning the plaque index scores, the between-group comparison showed no significant difference in plaque scores between the two groups (P-value= 0.56117) at baseline. In contrast a significantly statistical change was shown in the 3 and 6 weeks follow up with (P-value= 0.00227) and (P-value= 2.15E-05) respectively. [Table 3]

3. Salivary pH levels

The mean salivary pH levels at rest were (7.2 (±0.35) in Group I and 7.18(±0.31) in group II with no significant difference (P-value= 0.94114). After juice drinking, the mean salivary pH levels changed to 6.58 (±0.82) and 6.68 (±0.64)) in Group I and II respectively, with no significant difference (P-value= 0.81902). However, a significant difference in the salivary pH levels was shown between both groups after brushing (P-value= 0.00275), with the mean salivary pH levels changed to 7.91 (±0.27) and7.35 (±0.28) in Group I and II respectively. [Table 4]

4. Satisfaction

The mean values of the VAS scale for satisfaction questions between both groups are presented in [Table 5]. The patient satisfaction VAS survey revealed no statistically significant difference between both groups after six weeks of using the toothpaste. Throughout the experiment, no unfavorable reactions were noticed, and none of the subjects expressed any discomfort or sensitivity to the toothpaste.

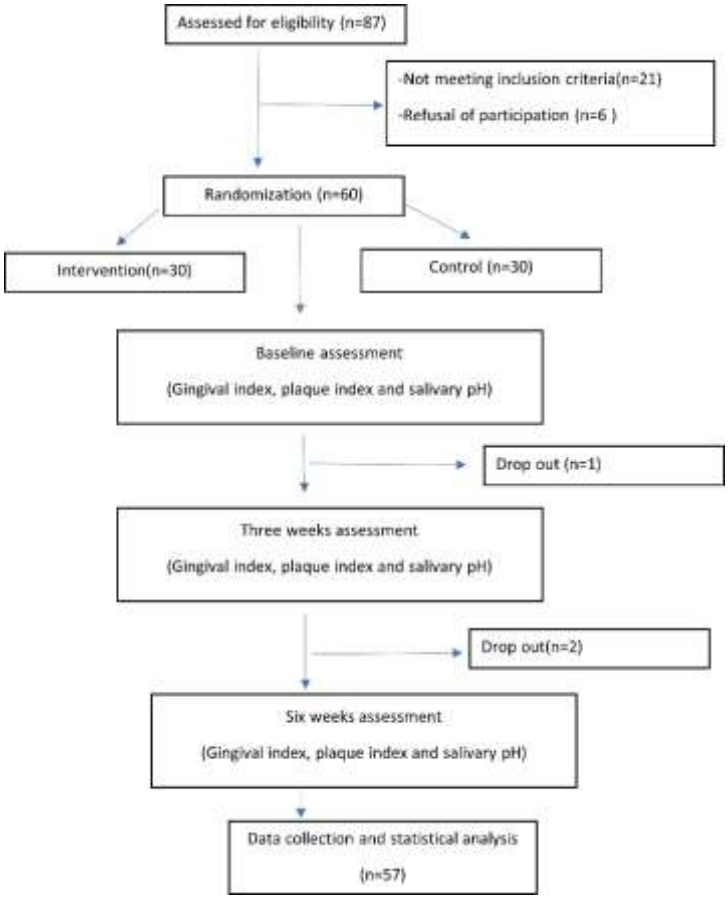

Figure (1): Participants flow chart.

Table 1: Mean Gingival index and Plaque index in Group I

	Gingival index			**Plaque index**		
	Baseline	3weeks	6weeks	Baseline	3weeks	6weeks
Mean (± SD)	1.25(±0.36)	0.40(±0.37)	0.11(±0.18)	1.23(±0.36)	0.30(±0.31)	0.10(±0.19)
P-value	5.55E-08	0.00077		1.85E-10	0.01372	

Table 2: Mean Gingival index and Plaque index in Group II

	Gingival index			**Plaque index**		
	Baseline	3weeks	6weeks	Baseline	3weeks	6weeks
Mean(±SD)	1.5(±0.42)	1.24(±0.41)	1.20(±0.57)	1.30(±0.32)	0.84(±0.52)	0.84(±0.57)
P-value	0.09138	0.83938		0.00048	0.95578	

Table 3: Comparison of Mean Gingival index and Plaque index in Both groups

Gingival index			
	Group I	Group II	P-value
Baseline	1.25 (±0.36)	1.5(±0.42)	0.08963
3weeks	0.40 (±0.37)	1.24(±0.41)	6.82E-06
6weeks	0.11 (±0.18)	1.20(±0.57)	3.01E-08
Plaque index			
	Group I	Fluoride	P-value
Baseline	1.23 (±0.36)	1.30 (±0.32)	0.56117
3weeks	0.30 (±0.31)	0.84 (±0.52)	0.00227
6weeks	0.10 (±0.19)	0.84 (±0.57)	2.15E-05

Table 4: Mean Salivary pH levels between both groups

	Group I	**Group II**	**P-value**
At rest	7.2 (±0.35)	7.18(±0.31)	0.94114
After juice	6.58 (±0.82)	6.68 (±0.64)	0.81902
After brushing	7.91 (±0.27)	7.35 (±0.28)	0.00275

Table 5: Mean VAS scale values for satisfaction questions between both groups

	Group I	**Group II**	**P-value**
Q1 How satisfied are you with the cleaning effect of the toothpaste?	9.7 (±0.48)	9.8 (±0.42)	0.62783
Q2 How satisfied are you with the toothpaste taste?	8.5 (±1.26)	9.3 (±0.82)	0.11178
Q3 How satisfied are you with the toothpaste texture?	8.5(±1.43)	9.1 (±0.87)	0.27354
Q4 How satisfied are you with the toothpaste consistency?	9.5 (±0.52)	9.4 (±0.51)	0.67331
Q5 How satisfied are you with the toothpaste stickiness?	9.1 (±1.10)	9 (±0.81)	0.82009
Q6 How satisfied are you with the toothpaste quality?	9(±0.81)	9.2 (±0.78)	0.58432
Q7 How is your overall satisfaction with the current toothpaste compared to other brands?	9.6 (±0.51)	9.5 (±0.52)	0.67331

IV. DISCUSSION:

Periodontitis is thought to start due to gingival inflammation brought on by the buildup of bacterial plaque. Gingivitis begins when dental plaque builds up over days or weeks without being disturbed or removed. Plaque-induced gingivitis, the most prevalent type of periodontal disease, has been demonstrated by epidemiologic data to be common in dentate populations of all ages **(Murakami *et al.*, 2018).**

A wide range of gingival infections can affect children and adolescents. According to epidemiological research, children and adolescents almost always have varying severity of gingivitis. Children must undergo a periodontal examination as part of their regular dental visits because early diagnosis is crucial for a successful course of therapy **(Pari *et al.*, 2014).**

One of the essential oral hygiene care strategies is using a toothbrush with Toothpaste to limit plaque accumulation and the risk of plaque-related diseases, including periodontitis and caries **(Nachu *et al.*, 2022).**

Since many herbal products have antibacterial qualities that reduce the development and growth of cariogenic bacterial plaque and prevent their acid generation, herbal toothpaste has recently grown in popularity. According to studies, non-herbal toothpaste is just as effective as herbal toothpaste at preventing plaque buildup and gingivitis **(Hosadurga *et al.*, 2018; Janakiram *et al.*, 2020; Duarte *et al.*, 2022).**

Clinical trials are essential to confirm any new product's efficacy rather than relying just on laboratory investigations to make this assumption **(Hosadurga *et al.*, 2018).**

Only one study assessed Moringa-based herbal Toothpaste on reducing plaque and gingivitis scores. Still, it was performed on an adult population **Duarte *et al.*, 2022** . To the best of our knowledge, no randomized controlled clinical trial assessed the effect of Moringa toothpaste on gingivitis and plaque in children. So, the present study aimed to evaluate and compare the anti-plaque and anti-gingivitis effects of moringa plant extract and fluoride toothpaste among a group of children aged between 12 to 15 years.

Children aged 12 to 15 were chosen for the current study. This age range almost encompasses the entire permanent dentition. Assessing plaque and gingivitis levels was more useful at this age since the permanent dentition had been exposed to the oral cavity for 3–9 years **(Nadar *et al.*, 2020).**

Brushing twice daily for at least two minutes with fluoride toothpaste, which has been widely adopted for decades and continues to be a benchmark for prevention treatments, is a crucial universal advice made by dental care professionals. The current study selected a commercially available and affordable fluoride toothpaste (Signal, Unilever, UK) as the positive control **(Valkenburg *et al.*, 2019).**

Group, I revealed a significant reduction in GI and PI scores between the baseline and the three weeks and between the 3 and 6 weeks. These results agree with **Duarte *et al.*, 2022** study that showed that the usage of the moringa-based dentifrice as compared to the miswak dentifrice resulted in a statistically significant decrease in mean gingival index scores and plaque index scores from baseline to day three. An explanation for this effect could be the antibacterial agents in Moringa that contain a profile of important antimicrobial agents. Previous studies have shown antimicrobial activity, which has been studied thoroughly in Moringa oleifera **(Raubilu *et al.*, 2020).**

In Group II, there was no statistically significant change in GI scores between the baseline and the three weeks, between the three weeks and six weeks, and in PI scores between the three weeks and six weeks. However, a statistically significant change in PI scores

between the baseline and the three weeks was shown. These findings partially agree with **Maden *et al.*, 2017 and Nadar *et al.*, 2020 ,** in which the reduction of gingival and plaque indices was statistically significant after 21 days.

The present study reported a significant statistical change in the 3 and 6 weeks follow-up in GI scores and PI scores between both groups, with more reduction in the mean scores in favor of Moringa toothpaste. The available literature revealed that no clinical studies had been reported that compared Moringa and fluoride toothpaste, so it was impossible to compare these findings with those of other studies. However, the presence of carotenoids in the moringa extract, which have an antioxidant effect and are also effective free-radical scavengers, as well as vitamin C in the moringa, can be credited for the strong anti-gingivitis and anti-plaque properties of moringa toothpaste. It might also be because of the flavonoids in the moringa extract, which could increase its antibacterial activity **Duarte *et al.*, 2022**. Toothpaste traditionally contains fluoride as the primary protective ingredient; however, fluoride has drawbacks because its primary mechanism of action does not target dental plaque **(Cummins, 2013)**.

In addition to the gingival and plaque indices evaluation, the effects of both types of Toothpaste on salivary pH levels were recorded. Saliva applies a vital effect on maturation, metabolism, and plaque initiation. In patients with periodontitis, exposing the gingiva to saliva with a neutral or more alkaline pH level enhances gingival repair. A lower pH level, on the other hand, might have a superficial necrotizing impact. Additionally, the pH level affects bacterial multiplication, including that of Porphyromonas gingivalis, which is linked to the aetiology of periodontal disorders **(Lăzureanu *et al.*, 2021; Koppolu *et al.*, 2022**).

In the current study, a significant difference in the salivary pH levels was shown between both groups after brushing with the Moringa toothpaste showing more increase in the pH levels. It's been observed that using herbal supplements caused the pH of total saliva to shift into the alkaline range. The increase in pH significantly after brushing with the toothpastes is consistent with **Soham *et al.*, *2015*** .

In addition to the previous study outcomes, another patient-reported outcome was assessed: the participants' satisfaction with using the toothpastes. One of the aspects of healthcare that has been examined the most is patient satisfaction. It has been proven to be a crucial indicator of the safety and quality of medical care. Pay for performance in healthcare heavily relies on patient happiness as an outcome indicator **(Voutilainen *et al.*, 2016).**

Throughout the trial, no unfavorable reactions were noticed, and none of the subjects expressed any discomfort or reaction to the toothpastes. After six weeks of using the toothpastes, the patient satisfaction survey found no statistically significant difference between the two groups. **Duarte *et al.*, 2022** indicated that patients used moringa toothpaste more consistently and enjoyed the taste. Animal studies using Moringa extracts have found a good level of safety. There were no negative consequences associated with investigations on humans **(Stohs and Hartman).**

- **Study limitations**

The study's advantages are the random selection of subjects, randomization procedure, concealed random allocation, and blinding of the statistician, participant, and researcher. These techniques minimize selection bias, allocation bias, and confounder bias.

However, the study has some drawbacks as the short study duration. Additionally, participants were provided tubes of toothpaste to use at home, making it challenging to know their adherence to the suggested tooth brushing protocol.

Future research is advised to assess how long-term use of toothpaste containing moringa extract affects people of different ages and at various intervals in reducing dental plaque and gingivitis.

V. CONCLUSIONS

Both Moringa and Fluoride toothpastes caused a reduction in the mean gingival and plaque indices scores. However, the reduction in gingival and plaque indices scores was significantly higher with Moringa toothpaste. Moringa toothpaste showed an additional effect on plaque and gingivitis compared to fluoride toothpaste. Moringa toothpaste resulted in a greater rise in salivary pH after drinking fruit juice. No adverse reactions to the toothpaste were observed during the trial, with no significant difference in participants' satisfaction.

LIST OF ABBREVIATIONS:

- Visual analog scale VAS
- Moringa oleifera M. oleifera
- Gingival index GI
- Plaque index PI

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