Dentistry Section

Comparative Evaluation of Efficacy of Three Different Herbal Toothpastes on Salivary Alkaline Phosphatase and Salivary Acid Phosphatase - A Randomized Controlled Trial

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ABSTRACT

Introduction: Very few researches in the past have tried to evaluate the effect of herbal toothpaste on saliva and salivary constituents like alkaline phosphatase and acid phosphatase which play an important role in maintaining oral health.

Aim: To evaluate and compare the effect of three different herbal toothpastes on Salivary Alkaline Phosphatase (ALP) and salivary Acid Phosphatase (ACP).

Material and Methods: The present study was a preliminary study conducted among 45 dental students (15 subjects in each group) in the age group of 19-21 years. Subjects in each group were randomly intervened with three different herbal toothpastes respectively (Group A – Patanjali Dant Kanti, Group B - Himalaya Complete Care and Group C – Vicco Vajradanti). Unstimulated saliva sample were collected before and after brushing and salivary ACP and salivary ALP levels were assessed at an interval of one week each for a period of four weeks starting from day one. Compiled data was analyzed using chi square test, paired t-test and ANOVA based on the nature of the obtained data.

Results: All the three toothpastes showed significant (p<0.001) reduction in ACP and ALP levels at each interval. For patanjali toothpaste, the mean reduction was in the range of 2.55 – 2.62 IU/L for ACP and 2.94 – 2.99 IU/L for ALP. For Himalaya toothpaste, the mean reduction was in the range of 1.39 – 1.47 IU/L for ACP and 1.55 – 1.61 IU/L for ALP. For Vicco toothpaste, the mean reduction was in the range of 2.46 – 2.50 IU/L for ACP and 2.64 – 2.77 IU/L for ALP. Patanjali and Vicco toothpaste were significantly effective in reducing the levels of salivary ACP and ALP more than Himalaya toothpaste (p<0.05).

Conclusion: Herbal toothpastes, especially Dant Kanti and Vicco Vajradanti, showed significant reduction in levels of ACP and ALP resulting in overall improvement towards the oral health.

INTRODUCTION

Optimal oral health requires proper oral hygiene measures. Oral hygiene measures comprise of mechanical methods, chemical methods or combination of both. Mechanical oral hygiene procedures seem to be the most effective form of all oral hygiene measures available. Mechanical oral hygiene aids include use of toothbrushes along with dentifrices and other interdental aids. Today, toothbrushing along with dentifrices is the most reliable means of preventing and controlling plaque formation at individual level [1].

Dentifrices are the routinely administered oral hygiene aid for cosmetic as well as therapeutic purpose. Widespread and regular use of the dentifrices serves as the most effective tool for prevention and control of oral diseases. The therapeutic effect of dentifrices is to reduce caries incidence, gingivitis, or tooth sensitivity. Dentifrices are marketed as toothpowders, toothpastes, and gels. Among dentifrices, toothpaste is the most common and accessible means of preventive oral health care tool available to Indian population. Available data from scientific literature shows that when used regularly, modern toothpastes can help prevent dental caries and limit the regrowth of dental plaque and gingivitis [2,3].

However, modern allopathic toothpastes contain some ingredients that can be harmful in large doses and can lead to allergic reactions in few individuals [4]. Therefore, people are moving to alternative herbal oral health care products that are safe and efficient. Consumers view that herbal products as safer alternative to those products containing chemicals; hence, get attracted towards herbal products. Natural/herbal dentifrices normally do not contain artificial substances such as sweeteners, colours, preservatives, etc., [5]. Instead they are formulated from naturally derived components. It is estimated that about 80% of people in developing countries still rely on plant derived traditional medicine for primary health care purpose [6]. Hence, it is the responsibility of dental professionals to provide patients with correct and current scientific information pertaining to these products. However, this can be difficult owing to the lack of professional agreement on the subject. Till date, there is lack of sufficient peer reviewed literature on the efficacies of herbal based toothpastes.

Salivary factors like salivary enzymes Alkaline Phosphatase (ALP) and salivary Acid Phosphatase (ACP) play an important role in maintaining oral health. Salivary enzyme such as ALP is a very important enzyme as it is part of normal turnover of periodontal ligament, root cementum, maintenance and bone homeostasis. Some studies have shown a remarkably increased activity of ALP in the acute phase of periodontal disease. ALP aggravates the bone loss; hence, periodontitis can progress rapidly [7]. ACP is closely associated with calculus formation [8]. Hence, determination of alkaline and acid phosphatase activity in saliva can be a helpful tool for diagnosis of periodontal diseases.

Literature addressing the potency or quality of herbal ingredients used in dental products is limited. Most of the available literature focuses mainly on antimicrobial properties of herbal toothpastes. Very few or none of them have tried to evaluate the effect of herbal toothpaste on saliva and salivary constituents which play an important role in maintaining oral health. The scientific literature regarding the effect of conventionally used as well as herbal toothpastes on salivary components like ALP and ACP are clearly lacking.

The three toothpastes used in the study i.e., Patanjali Dant-Kanti Dental Cream, Himalaya Complete Care Toothpaste and Vicco Vajradanti Toothpaste are the most commonly used and popular herbal toothpastes. Hence, they were selected for the present study and an attempt has been made to evaluate and compare the effect of three different herbal toothpastes on salivary ALP and ACP.

MATERIALS AND METHODS
The present study was a triple blind, interventional randomized parallel clinical trial conducted at ACPM Dental College and Hospital, Dhule, Maharashtra, India, in the month of August 2014 over a period of four weeks. It was carried out on a total of 45 subjects who were enrolled for BDS course (2nd and 3rd BDS students in the age group of 19-21years). The ethical clearance for the study was obtained from the Institutional Ethical Review Board and informed consent was obtained from all the participants prior to the study.
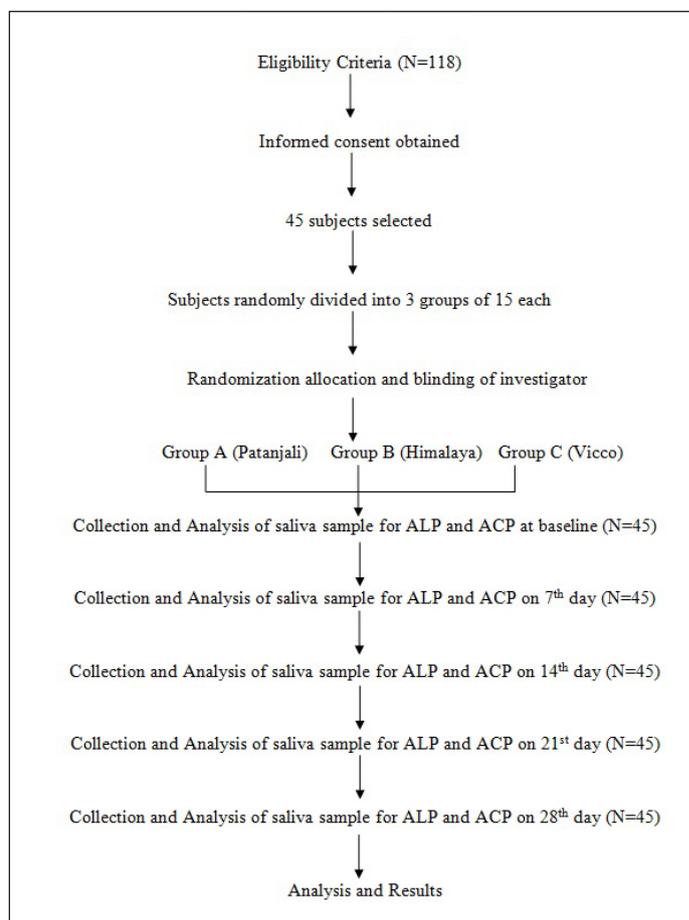
Materials and equipments used were three herbal toothpastes (Patanjali Dant-Kanti Dental Cream, Himalaya Complete Care Toothpaste and Vicco Vajradanti Toothpaste), sterile plastic containers, insulated containers, calibrated test tubes, ALP reagent kit, ACP reagent kit and Erba Manheim Automatic Analyzer.

Inclusion Criteria: Subjects in the age group of 19-21years with good general and periodontal health following routine oral hygiene procedures and those who agreed to comply with the study visits were included in the study.

Exclusion Criteria: Subjects suffering from systemic diseases, currently using antibiotics, subjects undergoing orthodontic treatment, subjects using oral hygiene aids other than toothbrush & toothpaste, subjects having adverse habits like smoking, alcohol consumption were excluded from the study.

Study Design [Table/Fig–1]: Since this was a preliminary study, it was decided to select 15 subjects in each group (For such preliminary studies the recommendation is a sample size of 12 per Group) [9]. All the under graduate dental students studying in 2nd and 3rd year and residing in the hostel of dental college who fulfilled the inclusion and exclusion criteria were invited to participate in the study. Among them, 45 subjects were randomly selected employing random sampling. Investigator not involved in examinations generated the random numbers. Total of 45 subjects were randomly divided into three groups of 15 each (Group A using Patanjali Dant-Kanti Dental Cream, Group B using Himalaya Complete Care Toothpaste and Group C using Vicco Vajradanti Toothpaste) employing random sampling using lottery method.

The present study employed a triple blinding procedure. The investigator was blinded and was unaware of the toothpaste allocation to the subjects of that particular group. A male assistant who was unknown to the principle investigator randomly allocated the toothpastes to three groups. The subjects were also blinded with respect to the particular toothpaste they used throughout the study period. All the study toothpastes were covered with white sticker labels indicating group name and caps of all the toothpastes were replaced with white caps of same uniform size to achieve blinding. Statistician was also kept blind regarding the groups while analyzing the data.

[Table/Fig-1]: Flow chart of study design.

Subjects were instructed to brush twice daily using the toothpaste which was given to them. Unstimulated saliva samples were collected before and after brushing and were transported to the laboratory where salivary ALP and salivary ACP levels were determined. The study was conducted over a period of 4 weeks on each subject and saliva samples were obtained at weekly intervals starting from day 1 i.e., on 1st day, 7th day, 14th day, 21st day and 28th day.

Saliva collection and analysis: The required quantity of saliva sample was collected before and after tooth-brushing procedure. Subjects were asked to abstain from eating and drinking for 1 hour before sample collection. Tooth-brushing along with the allotted toothpaste for each male participant was monitored by the investigator himself; while for female participants, it was monitored by a female investigator assistant throughout the study period.

The unstimulated salivary samples were collected from the subjects in morning between 7am to 7.30am before and after brushing. Subjects were made to sit in an upright position and saliva was collected by spitting method before and after brushing [10]. For each participant at least 10 ml of saliva was collected in sterile plastic container. The plastic containers with saliva were stored in an ice container maintained at $2\text{-}4^0C$ until analysis of salivary variables was performed. Each saliva sample was centrifuged at 3,000rpm for 10 min and clear supernatants were processed immediately for estimation of ALP and ACP. The enzyme activities in saliva were determined spectrophotometrically by the International Federation of Clinical Chemistry (IFCC) method on the Erba Manheim Automatic Analyzer using ALP reagent and ACP reagent as substrates [11].

STATISTICAL ANALYSIS
Data was analyzed using SPSS (Statistical Package for Social Sciences) statistical software version 16. Chi square test was used to compare gender-wise distribution. Mean and standard deviation of the obtained pre (before brushing) and post (after

brushing) mean levels of salivary ALP and ACP at 1st, 7th, 14th, 21st and 28th day were compared within group A, group B and group C by paired 't' test. Overall mean changes in the levels of Salivary ALP and ACP on 1st and 28th day within group A, group B and group C were compared by ANOVA test.

RESULTS

Chi square test showed no significant difference (p=0.913) in gender-wise distribution between Group A, B and C indicating a good match [Table/Fig-2].

All the three toothpastes showed reduction in salivary APL and ACP levels after brushing at each interval (1st, 7th, 14th, 21st and 28th day) and the difference in pre and post brushing levels was highly significant (p<0.001) in every group [Table/Fig-3-5].

When overall mean reduction for all the intervals was compared, group A toothpaste showed significantly more reduction (p<0.05) of ACP and ALP levels when compared to group B toothpaste. Similar result was seen when group B and group C toothpaste were compared where group C toothpaste was found to be more effective than group B toothpaste (p<0.05). However, no significant difference was seen in the efficacy of group A and group C toothpaste in reducing levels of ACP and ALP (p>0.05) [Table/Fig-6].

Gender	Group A	Group B	Group C	Total
Male	8 (53.3%)	8 (53.3%)	9(60%)	25(55.6%)
Female	7(46.7%)	7(46.7%)	6(40%)	20(44.4%)
Total	15(100%)	15(100%)	15(100%)	45(100%)
p-value	p>0.05			

[Table/Fig-2]: Gender-wise distribution between the three groups.
Chi-square test

Salivary Parameter	Interval	Pre-brushing Score	Post-brushing Score	Mean Difference	p-value
ACP	1st Day	7.72±1.4	5.15± 1.2	2.57±0.33	<0.001
	7th Day	7.71±1.4	5.15±1.3	2.56±0.35	<0.001
	14th Day	7.72±1.39	5.17±1.24	2.55±0.32	<0.001
	21st Day	7.67±1.42	5.12±1.25	2.55±0.30	<0.001
	28th Day	7.68±1.41	5.06±1.24	2.62±0.28	<0.001
ALP	1st Day	20.55±2.7	17.56±2.6	2.99±0.51	<0.001
	7th Day	20.46±2.7	17.51±2.6	2.95±0.50	<0.001
	14th Day	20.44±2.7	17.50±2.5	2.94±0.47	<0.001
	21st Day	20.39±2.6	17.40±2.6	2.99±0.51	<0.001
	28th Day	20.37±2.7	17.40±2.58	2.97±0.51	<0.001

[Table/Fig-3]: Mean change in pre brushing and post brushing levels of salivary acid phosphatase (IU/L) and alkaline phosphatase (IU/L) at each interval in group A.
IU/L – International Units per Liter; Paired 't' test

Salivary Parameter	Interval	Pre-brushing Score	Post-brushing Score	Mean Difference	p-value
ACP	1st Day	7.90±1.5	6.43±1.4	1.47±0.43	<0.001
	7th Day	7.89±1.46	6.44±1.4	1.45±0.45	<0.001
	14th Day	7.84±1.46	6.37±1.43	1.47±0.44	<0.001
	21st Day	7.83±1.44	6.37±1.45	1.46±0.46	<0.001
	28th Day	7.80±1.48	6.41±1.44	1.39±0.42	<0.001
ALP	1st Day	19.38±1.93	17.83±1.8	1.55±0.36	<0.001
	7th Day	19.33±1.90	17.77±1.92	1.56±0.33	<0.001
	14th Day	19.3±1.92	17.72±1.84	1.58±0.37	<0.001
	21st Day	19.29±1.91	17.68±1.90	1.61±0.31	<0.001
	28th Day	19.26±1.92	17.66±1.91	1.60±0.29	<0.001

[Table/Fig-4]: Mean change in pre brushing and post brushing levels of salivary acid phosphatase (IU/L) and alkaline phosphatase (IU/L) at each interval in group B.
IU/L – International Units per Liter; Paired 't' test

Salivary Parameter	Interval	Pre-brushing Score	Post-brushing Score	Mean Difference	p-value
ACP	1st Day	8.45±1.77	5.95±1.76	2.50±0.35	<0.001
	7th Day	8.28±1.78	5.81±1.77	2.46±0.35	<0.001
	14th Day	8.25±1.76	5.77±1.79	2.48±0.34	<0.001
	21st Day	8.21±1.78	5.75±1.76	2.46±0.36	<0.001
	28th Day	8.18±1.76	5.72±1.79	2.46±0.34	<0.001
ALP	1st Day	19.34±2.74	16.59±2.77	2.75±0.45	<0.001
	7th Day	19.29±2.72	16.55±2.77	2.74±0.44	<0.001
	14th Day	19.25±2.74	16.48±2.74	2.77±0.44	<0.001
	21st Day	19.23±2.78	16.46±2.79	2.77±0.44	<0.001
	28th Day	19.20±2.77	16.56±2.81	2.64±0.75	<0.001

[Table/Fig-5]: Mean change in pre brushing and post brushing levels of salivary acid phosphatase (IU/L) and alkaline phosphatase (IU/L) at each interval in group C.
IU/L – International Units per Liter; Paired 't' test

Groups	Mean reduction in ACP	Mean reduction in ALP
Group A	2.57	2.97
Group B	1.45	1.58
Group C	2.47	2.73
ANOVA	p<0.001	p<0.001
A vs B	p<0.05	p<0.05
B Vs C	p<0.05	p<0.05
A Vs C	p>0.05	p>0.05

[Table/Fig-6]: Comparison of mean difference in pre brushing and post brushing levels of salivary acid phosphatase (IU/L) and alkaline phosphatase (IU/L) for all the intervals.
IU/L – International Units per Liter

Groups	Mean difference in salivary acid phosphatase (IU/L)	Mean difference in salivary alkaline phosphatase (IU/L)
Group A	0.04	0.18
Group B	0.10	0.12
Group C	0.27	0.14
ANOVA	p<0.001	p<0.05

[Table/Fig-7]: Comparison of overall difference in the pre brushing levels of salivary acid phosphatase (IU/L) and alkaline phosphatase (IU/L) between first to last (28th) day in group A, group B and group C.
IU/L – International Units per Liter; ANOVA

In the present study, overall reduction in baseline (pre-brushing) values of salivary ACP on 1st day and 28th (last) day was 0.04 for group A, 0.10 for group B and 0.27 for group C. ANOVA test showed very highly statistically significant difference (p<0.001) in all the three groups [Table/Fig–7].

Overall reduction in baseline (pre-brushing) values of salivary ALP on 1st day and 28th (last) day was 0.18 for group A, 0.12 for group B and 0.14 for group C. ANOVA test showed statistically significant difference (p<0.050) in all the three groups [Table/Fig–7].

DISCUSSION

The present study was an in-vivo, three parallel groups and randomized clinical trial that evaluated the comparative efficacy of three different herbal toothpastes on salivary parameters i.e., ALP and ACP. From the results, it can be seen that there was reduction in salivary ALP and ACP levels after using each toothpaste. Vicco Vajradanti Toothpaste and Dant Kanti Dental Cream performed better than Himalaya Complete Care toothpaste. No clinically significant adverse reactions of the tested herbal toothpastes, were reported or observed, during the entire study period.

The present study employed only second and third year BDS students based on the availability during the actual study period and keeping in mind the future study compliance. The present study incorporated only herbal toothpaste because of increased public interest in the usage of alternative health care, including use of natural or herbal health care products. According to the

World Health Organization (WHO), up to 80% of the population in developing countries uses plants and its products as traditional medicine for primary health care. In the developed countries, 25% of the medical drugs are based on herbs and their derivatives [12]. Ease of availability, least side effects compared to conventional chemical toothpastes and low cost make the herbal preparations as the key player of all available therapies, especially in rural areas [13]. Available scientific literature shows that herbal toothpastes are as effective as conventional toothpastes [14,15]; but there is a dearth of scientific literature showing comparative efficacy of one herbal toothpaste over the other. Hence, only herbal toothpastes have been selected for the present study to assess the efficacy of one over another.

Composition of the three herbal toothpastes employed in the present study was:

1. Dant Kanti Dental Cream: Vajradanti, Pilu, Bakul, Babool, Akarkara (Spanish Chammomile), Majuphal, Karpur (camphor), Vidang, Neem, Clove.

2. Himalaya Toothpaste: Pomegranate, Tumburu, Babool, Triphala, Vidang, Nirgudi, Bhasma, Neem, Ajamoda Satva, Pilu, Bakula.

3. Vicco Vajradanti: Babool, Bakul, Jamun, Clove, Manjishtha, Bor, Walnut, Akkal-kadha, Liquorice, Cinnamon, Catechu, Patang, Harda, Vajradanti, Anantmul, Amla.

As seen from the composition, there is variation in the contents of the toothpastes which might have influenced the study results.

Unstimulated saliva was preferred over stimulated saliva in the present study, as unstimulated saliva is secreted by the salivary glands in normal physiological conditions. The unstimulated whole saliva is often preferred in the diagnosis of biomarkers, as stimulated saliva contains a diluted concentration of ingredients which are intended to be detected. The dilution of salivary sample may pose difficulties in detection of these ingredients and may result in inferior levels of these ingredients in the processed salivary sample [16].

ALP and ACP can act as good biomarkers for assessing periodontal disease. ALP aggravates the bone loss resulting in rapid progression of periodontitis and ACP is closely associated with salivary calculus formation. Also, there is lack of scientific literature regarding the effect of herbal toothpaste on these salivary parameters. Hence, these parameters were selected for the present study.

Salivary ALP and ACP showed reduction in all the three groups after brushing. Levels of ALP and ACP activities in saliva and GCF directly correlate with the intensity of inflammatory process of periodontal tissue and act as inflammatory markers in periodontal diseases. As the inflammation subsides, level of ALP and ACP in saliva also decreases [17,18]. In the present study, gingival inflammation present in some individuals might have come down due to various anti-inflammatory substances present in toothpastes such as pilu, akarkara, vidang, chamomile, babool, jamun, bor, liquorice, harda, neem etc [19-21] and thus, helped in reducing the levels of ALP and ACP in saliva.

Another reason for reduction in these salivary enzyme levels following use of herbal toothpaste may be inhibition of plaque bacteria. Plaque bacteria secrete ALP and ACP [18,22]. Activity of anti-bacterial ingredients present in herbal toothpaste resulted in inhibition of plaque bacteria and ultimately reduction in levels of acid and alkaline phosphatase in saliva.

Patanjali Dant Kanti and Vicco Vajradanti performed better than Himalaya Complete care toothpaste in terms of reduction of salivary ALP and ACP levels as these toothpastes contain more number of anti-inflammatory and anti-bacterial ingredients than Himalaya Complete Care toothpaste. Valid and accurate comparison of

results for salivary ALP and ACP in the present study could not be done with other scientific studies, as no previous research work has conducted to assess the efficacy of herbal toothpastes on these salivary parameters.

LIMITATION

1. Proper sample size for the study could not be determined due to lack of availability of data from previous studies. Hence, a preliminary study was conducted.

2. Control group was not incorporated in the present study and hence, comparison with routinely used fluoridated toothpastes was not done.

3. The levels of ALP and ACP were not compared with clinical parameters like gingival and plaque index.

RECOMMENDATION

Long term clinical trials taking larger samples with wider geographical representation need to be conducted to quantify the efficacy of herbal toothpaste. Studies should also be carried out to assess the combine effect of herbal toothpastes and herbal mouthwashes on saliva.

CONCLUSION

The herbal toothpastes possess significant ALP and ACP inhibitory activity and can prove to be an alternative to conventional toothpastes. Among the three tested toothpaste, Dant Kanti and Vicco Vajradanti were found to be more effective than Himalaya Complete Care toothpaste.

Conflict of Interest: None of the authors is associated with the herbal toothpaste companies used in the present trial.

REFERENCES

[1] Axelsson P, Odont D. Concept and practice of plaque-control. *Pediatric Dentistry.* 1981;3:101-13.

[2] Marinho VC, Higgins JP, Sheiham A, Logan S. Fluoride toothpastes for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev.* 2003;1:CD002278.

[3] Anand R. The effect of the supervised use of fluoridated toothpaste on the gingival health of children. *J Clin Diagn Res.* 2011;5(2):388-89.

[4] Francalanci S, Sertoli A, Giorgini S, Pigatto P, Santucci B, Valsecchi R. Multicentre study of allergic contact cheilitis from toothpastes. *Contact Dermatitis.* 2000;43(4):216-22.

[5] Goldstein BH, Epstein JB. Unconventional dentistry: Part IV. Unconventional dental practices and products. *J Can Dent Assoc.* 2000;66(10):564-68.

[6] Kamboj VP. General medicine. *Current Science.* 2000;78(1):35-51.

[7] Amitha R, Bhandary R, Thomas B, D'Souza SR, Kumari S. Alkaline phosphatase – a diagnostic marker of periodontitis in postmenopausal women – a biochemical study. *Nitte University Journal of Health Science.* 2013;3(4):71-73.

[8] Draus FJ, Tarbet WJ, Miklos FL. Salivary enzymes and calculus formation. *J Periodont Res* 1968;3:232-35.

[9] Julious SA. Sample size of 12 per group rule of thumb for a pilot study. *Pharmaceut Statist.* 2005; 4:287–91.

[10] Kaufman E, Lamster IB. The diagnostic applications of saliva - a review. *Crit Rev Oral Biol Med.* 2002;13(2):197-212.

[11] Dabra S, China K, Kaushik A. Salivary enzymes as diagnostic markers for detection of gingival/periodontal disease and their correlation with the severity of the disease. *J Indian Soc Periodontol.* 2012;16(3):358-64.

[12] Kumar G, Jalaluddin M, Rout P, Mohanty R, Dileep CL. Emerging trends of herbal care in dentistry. *J Clin Diagn Res.* 2013;7(8):1827-29.

[13] Singab AN, Youssef FS, Ashour ML. medicinal plants with potential antidiabetic activity and their assessment. *Med Aromat Plants.* 2014;3(1):1-12.

[14] Al-Kholani AI. Comparison between the efficacy of herbal and conventional dentifrices on established gingivitis. *Dent Res J (Isfahan).* 2011;8(2):57–63.

[15] Mohan Kumar KP, Priya NK, Madhushankari GS. Anti-cariogenic efficacy of herbal and conventional tooth pastes – a comparative in-vitro study. *J Int Oral Health.* 2013;5(2):8-13.

[16] Malamud D, Rodriguez-Chavez IR. Saliva as a diagnostic fluid. *Dent Clin North Am.* 2011;55(1):159–78.

[17] Dabra S, Singh P. Evaluating the levels of salivary alkaline and acid phosphatase activities as biochemical markers for periodontal disease: A case series. *Dent Res J (Isfahan)* 2012;9(1):41–45.

[18] Kunjappu JJ, Mathew VB, Hegde S, Kashyap R, Hosadurga R. Assessment of the alkaline phosphatase level in gingival crevicular fluid, as a biomarker to evaluate the effect of scaling and root planing on chronic periodontitis: An in vivo study. *J Oral Maxillofac Pathol.* 2012;16(1):54-57.

[19] Patki PS, Mazumdar M, Majumdar S, Chatterjee A. Evaluation of the safety and efficacy of complete care herbal toothpaste in controlling dental plaque, gingival bleeding and periodontal diseases. *J Homeop Ayurv Med.* 2013;2(2):1-5.

[20] Poureslami H. The Effects of Plant Extracts on Dental Plaque and Caries. In: Li MY, editor. Contemporary Approach to Dental Caries. Shanghai: Intech; 2012. pp. 395-402.

[21] Biswas G, Anup N, Acharya S, Kumawat H, Vishnani P, Tambi S. Evaluation of the efficacy of 0.2% chlorhexidine versus herbal oral rinse on plaque induced gingivitis-A randomized clinical trail. *IOSR Journal of Nursing and Health Science*. 2014;3(2):58-63.

[22] Dabra S, China K, Kaushik A. Salivary enzymes as diagnostic markers for detection of gingival/periodontal disease and their correlation with the severity of the disease. *J Indian Soc Periodontol*. 2012;16(3):358-64.

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