

Effectiveness of Avrgam Herbal Mouthwash Against Plaque and Gingivitis: A Randomized Controlled Double Blind Trial

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ABSTRACT

A randomized, double blind, parallel and controlled clinical trial (RCT) was carried out to evaluate the safety and efficacy of a novel herbal mouthwash *Avrgam* formulation, using three of the highly valuable Pakistani herbs: *Aloe vera* (L.) Burm. f., *Achillea millefolium* L. and *Berbris aristata* DC. These herbs have scientifically proven as anti-inflammatory and antimicrobial potentials. In this research, phase I safety evaluation trial was conducted in which thirty six (36) subjects were enrolled and after successful completion phase II efficacy trial was then conducted. A total of fifty six (56) subjects of both the genders having at least 1.01 score of plaque index (PII) and gingival index (GI), respectively, were enrolled. Among them, twenty eight (28) patients received test rinse and the same number received control rinse. Chlorhexidine gluconate in 0.12% concentration was used as a positive control and a seven day twice a day dosing were managed for both the test and control groups. PII and GI were measured at day zero (0) and day seven (7) of the study. Both the rinses decreased significantly PII ($p < 0.025$). Although test rinse decreased the GI ($p < 0.01$) but chlorhexidine did not show significant reduction in GI. *Avrgam* herbal mouthwash was found more effective than the chlorhexidine gluconate ($p < 0.001$).

Keywords: Pakistani medicinal plants, RCT, Periodontal diseases, Mouthrinse, Plaque.

INTRODUCTION

Microbial plaque and its by-products are the primary cause of gingival inflammation and periodontal diseases. After few hours of plaque biofilm formation it can lead to secrete certain acids which start damaging gingival tissues which would be manifested as the clinical signs of gingivitis that may include changes in the color, size, shape, tenderness and tendency to bleed from gingival sulcus (Lindhe and Nyman, 1975; Lindhe et al, 1982 and Theilade E, 1996). In combating dental plaque and gingivitis nothing is better than to consult the nature and natural products (Lauten et al., 2005 and Van der Weijden et al., 1998).

Achillea millefolium L. (Asteraceae) is used as astringent, lithotriptic agent and antimicrobial and is an antiseptic to urinary infections. It is diaphoretic, antihypertensive, cholagogue, spasmolytic, antispasmodic and it is used topically for wounds, anti-inflammatory, while orally as antipyretic, common cold, digestive and carminative. Its use has been documented in varicose ulcer, cuts and injuries. It is also found very effective in leucorrhoea and all kinds of piles (Pullaiah 2006; WHO Monograph; Khare, 2007; Shinwari et al., 2006 and Amelio 1999). *Achillea* genera, famous for their essential oils, mostly contains

terpenes from mono to sesquiterpenes, lignanes, flavonoids (Si et al., 2006). In the aerial parts of *Achillea millefolium*, achilleine, achimillic acids A, B and C, azulene and chamazulene 1,8-cineole and flavonoids such as apigenin and luteolin have been isolated (Cavalcanti et al., 2007).

Aloe vera (L.) Burm. f. (Liliaceae), another famous and valuable Pakistani plant, traditionally, is being used extensively as emollient, anti-inflammatory and antimicrobial in skin ailments like burns and wounds. Anti hyperglycemic effects were proved in a clinical study. Moreover it's a tonic to intestinal movement in small doses and possess anti ulcer activities. It is cooling agent and is used in eye infections. Angiogenic effects were reported of β -sitosterol, a constituent of it, on damaged blood vessels. *Plasmodium falciparum* was also found to be inhibited by the aloe extract as mosquito repellent. Indian aloe is used in treating skin rashes, insect bites and nipple sores due to breast feeding. (Pullaiah 2006; WHO Monograph; Khare, 2007 and Shinwari et al., 2006). Moreover, aloe drug has strong laxative and cathartic action and can be given in mild constipation but it is contraindicated in intestinal obstructions. (Khare 2007). Aloe contains as its major and active principles hydroxyanthrone derivatives, mainly of the aloe-emodin-anthrone 10-C-glucoside type. The major constituent is known as barbaloin (aloin). Hydroxylaloin,

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gum, resins and uronic acids, flavonoids, aloeresin B, C and D and anthraquinone derivatives like emodin and chrysophanic acid were also present. (WHO monograph, volume 1; Rehman M, 2006). Aloesone and aloesin are found in plant while barbalion, chrysophanol glycoside and its aglycone aloe-emodin in leaves while, mucilage of leaves contains glucose, galactose, mannose and galacturonic acid as well as an aldopentose and a protein of 18 amino acids (Anonymous, 2003).

Berberis aristata DC. (Berberidaceae) is emmenagogue and it is used in the treatment of jaundice. Fresh berries are used in ophthalmics, especially in conjunctivitis. *Berberis aristata* contains berberine which have been studied extensively and successfully being tested for many activities like antimicrobial, antiinflammatory, antineoplastic, trypsin and chymotrypsin inhibitor, intestinal antiseptic, bitter stomachic, cardiotonic, antispasmodic, anti HIV and antiplatelets. It is also employed in the diagnosis test of malaria. In modern medicine berberine is used for the treatment of diarrhea. Its berries are antiscorbutic and laxative. Rot bark of the plant is recommended in digestive and hepatic ailments including diarrhea, cholera, dysentery, enlargement of spleen and metabolism disorders. *B. aristata* is also an anticoagulant, anti-inflammatory, hypoglycemic, hypotensive, antiamoebic and antibacterial (Pullaiah 2006 and Khare 2007). Major phytochemical agents present in it as berberine, berberine chloride, palmatine, berbamine, karachine, oxycanthine, oxyberberine, aromoline and oxycantine (Chakravarti et al., 1950; Dhiman 2006 and Pullaiah 2006).

In addition to such vast therapeutic activities, these plants have antimicrobial activity against oral pathogens. They are reported in the literature against periodontal diseases (Lee Sean S et al. 2004; Rhim et al. 2002; Villalobos et al. 2001; Soler, Blasco Ramon et al, 2010; Van der Weijden et al. 1998; Koo et al., 2003; Kumar et al., 2006; Singh et al. 2007; Yu Zhan-hai et al. 2008 and Shahid et al., 2009). Keeping in view these scientific facts, the aim of this study is to develop such natural origin formulation that can make a cure to the destructive effects of dental plaque and its' by products and the mild, moderate and severe gingivitis. As, the Herbal mouthwashes have been used since ancient times in folk medicine, involving both Eastern and Western medical traditions. Based on this, over the past decades, pharmaceutical companies have been interested in investigating plants as sources for new phytotherapeutic

agents with proven efficacy, safety and quality products for oral hygiene. The phytopharmaceutical preparation *Avrgam* herbal mouthrinse was done by using these above mentioned three renowned medicinal plants of Pakistan which are distributed frequently available in this region and we performed an randomized controlled trial on it.

MATERIALS AND METHODS

Achillea millefolium L., *Aloe vera* (L.) Burm. f. and *Berberis aristata* DC. were purchased from Hakim sons, street Darya gali, Boltan market, Karachi and these specimens were then identified by Prof. Dr. Ghazala H. Rizwani, Dept. of Pharmacognosy, Faculty of Pharmacy, University of Karachi and deposited in the Herbal museum of the Department of Pharmacognosy, University of Karachi with the voucher specimen number 088, 089 and 047, respectively.

Extraction: The dried herbs were reduced in size by grinder (West point automatic series TSK-333, France). Coarse herbal parts of *Achillea millefolium* L. and *Berberis aristata* DC. were macerated with the solvent (90%E/W) for 15-20 days in separate glass containers. After that percolate was concentrated at reduced pressure and controlled temperature (40°C) in the rotary vacuum evaporator (Rotary evaporator, Eyela, Japan). Brown, shiny and lumpy viscous liquid was obtained with very nice and flowery fragrance as *Achillea millefolium* L. extract (140 g) with the extract yield of 14 %. When followed the same procedure, around 50 g dark brown, smooth and odorless semi-solid paste like material was obtained as *Berberis aristata* DC. extract and the extract yield was around 5 %. *Aloe vera* L. was processed to be used in the composition and the extract yield was 36%.

Formulation design. The herbel mouthrinse *Avrgam* consisted of 0.62% (w/v) *Achillea millefoium* L., 16.67 % (v/v) *Aloe* juice, 0.84% (w/v) *Berberis aristata* DC. and 15.91 % ethanol in water base system. It was prepared at Faculty of Pharmacy, University of Karachi according to author's instruction.

Standard. Chlorhexidine gluconate, Gold standard for plaque and gingivitis control, was used as a positive control in the study at 0.2% (w/v) concentration. The organoleptic features of the positive control was made as close as possible to that of the designed test rinse.

Study population. All the study subjects were at first provided with a written informed consent form approved by ethical committee of ICCBS, University of Karachi (approval #). The participants (18-60 years) were procured having different socio-economic status and from different communities as the study was conducted at various dental clinics that were located in diverse regions of the Karachi city. Firstly, a phase I safety study was conducted recruiting 36 healthy individuals and then phase II efficacy trial was carried out. Each and every individual, meeting the given inclusion criteria, was briefed about the clinical trial design and mouth rinses, individually, and the agreed participants signed the informed consent forms. The demographics of the phase II recruited participants were shown in table 1. In phase II trial 56 patients took part in randomized controlled double blind parallel clinical trial.

Clinical design. Inclusion criteria for the trial was set as the participants should have PII >1 and GI > 1. Pregnant ladies or nursing mothers, a history of immunosuppressive diseases, diabetics, disease that would hinder the use of substance that would be used in the study (e.g. mental diseases), cancer patients, having disease of unknown etiology, subjects that were using antibiotics or had used any within a month of this study, subjects having less than 20 uncrowned teeth and that have any periodontal pocket depths >4mm were set as exclusion criteria. The study was kept double blind by dispensing the control and test in opaque sealed packs labeled A for control and B for test rinse. Each patient was randomized for test or control group by tossing a coin. The mouth rinse A was dispensed for every head and for every tail, B was dispensed. Subjects were instructed to take 5 ml mouthwash solution in the given dispensing cup and dilute it with water till the solution reached 10 ml before use then rinse with it twice a day, one dose after lunch and other after dinner for 30 seconds continued for 7 days. They were instructed further not to use any other commercial or medicinal rinse during the study, although, they are allowed to continue their usual dental cleaning habits and not to rinse with the given mouthwash just after their usual daily mouth hygiene practice. The compliance with the dosage protocol was monitored for every dose through a research assistant by calling them at prescribed timings and missed dose was considered as an indication of non-compliance.

Out of 56 recruited patients, 28 received the *Avrgam* mouthwash while the other 28 received the positive control

mouthwash. The initial clinical assessments included the plaque index scoring (Silness and Loe, 1964) and gingival index measures (loe and silness, 1963) on every selected tooth that included maxillary right first molar (16), maxillary right lateral incisor (12), maxillary left first bicuspid (24), mandibular left first molar (36), mandibular left lateral incisor (32), mandibular right first bicuspid (44). The final score was the cumulative mean. All the data was collected on carefully designed case report forms (CRFs). Each CRF included demographic data of the participants, pretreatment medical history and disease status, efficacy measurements and adverse drug reactions, if any.

Statistical analysis. The initial demographic data of the recruited individuals of phase II trial were expressed as percentage, mean $\pm$ standard deviation and median with interquartile range and shown in table 2. Whether or not the demographics of both the groups, test and control, were similar, were tested by applying *t-test*. Wilcoxon sign rank test and Mann-whitney *U* test were used to evaluate the significance difference in PII and GI of each mouthrinse including test and positive control and compare the effectiveness of both the mouth rinses, respectively. Additionally, *t-test* was also used for these analyses. Outcomes with $p < 0.05$ were considered significant, $p < 0.01$ highly significant, while, $p < 0.001$ as very highly significant. Analyses were performed with different Statistical software.

RESULTS

Demographics and compliance

In phase I safety study, among 36 recruitments, 19 were available for follow-up assessments after seven days of dosing. All the follow-ups were satisfactory advising for further trials.

In phase II efficacy round, out of 56 recruited individuals, 11 from test group while 14 from positive control group were available for follow up visits after the proper dosing. Remaining were either unable to meet the planned follow up appointments or were non-compliance towards the trial. As shown in table 2, the study recruited more female for the control group (39.28%) than that for the treatment group (32.14%), current or previous tobacco users were same in both the groups i.e. 35.71%, the range of age in the test group was 18-50 years while for the control group it was 18-45 years. In the test group more subjects were having education less than matriculate (28.57%) as

compared to the other group (25%). While, the number of teeth was almost same in both the groups. The demographics of both the groups, test and control were same, very highly significantly. Initial PII in median (IR) was

1.2705 (1.1-1.5) for the test group while 1.5 (1.25-1.937) for the control group though that of GI was 1.5 (1.16-1.6) in test and 1.46 (1.01-1.75) control group respectively (Table 1).

Table 1. Demographic and clinical data analysis of phase II trial.

	Test rinse	Control rinse	p
Female (%)	32.14%	39.28	-
Age (years)			$p < 0.001^*$
Mean $\pm$ SD	25.25 $\pm$ 5.75	28.07 $\pm$ 8.35	
Range	18-50	18-45	
Current or previous tobacco users (%)	35.371	35.71	-
Education			-
Illiterate (%)	28.57%	25%	
Income (Rs/year)			NS*
Mean $\pm$ SD	237804 $\pm$ 146600	217371 $\pm$ 130530	
Number of teeth			$p < 0.001^*$
Mean $\pm$ SD	30.14 $\pm$ 1.71	30 $\pm$ 2.01	
PII			$p < 0.001^{**}$
Median (IR)	1.27 (1.1-1.5)	1.5 (1.25-1.937)	
GI			$p < 0.001^{**}$
Median (IR)	1.5 (1.16-1.6)	1.46 (1.01-1.75)	

Table 2. Significance in decrease in indices after using mouthrinses.

PII control	$p < 0.025^{**}$	$p < 0.01^*$
PII Avrgam	$p < 0.025^{**}$	$p < 0.025^*$
GI control	NS**	NS*
GI Avrgam	$p < 0.01^{**}$	$p < 0.001^*$
PII Avrgam versus control	$p < 0.001^{***1}$	$p < 0.001^*$
GI Avrgam versus control	$p < 0.001^{***}$	$p < 0.001^*$

*t-test, **Wicoxon-sign rank test, ***Mann-whitney U test, ¹Decrease in PII after using both the mouthrinses were equal very highly significantly, NS: non-significant.

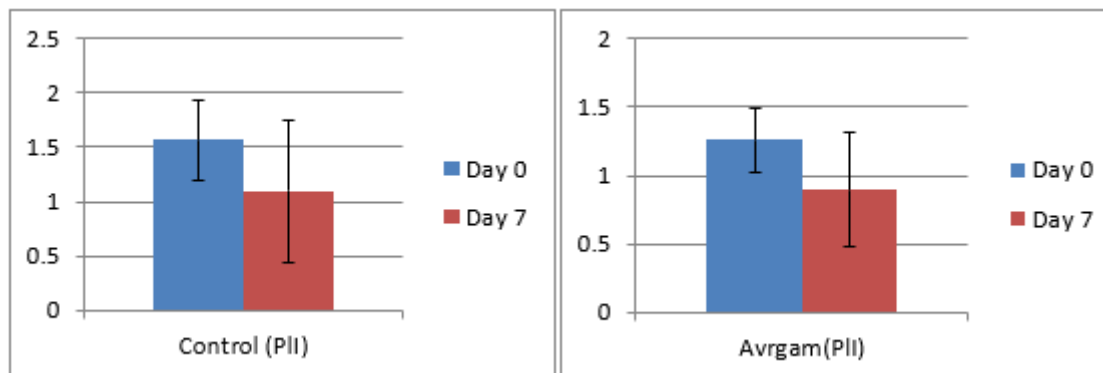


Figure 1. Comparison of decrease in the PII after using test versus control mouthrinse. Vertical bars represents SD of the mean.

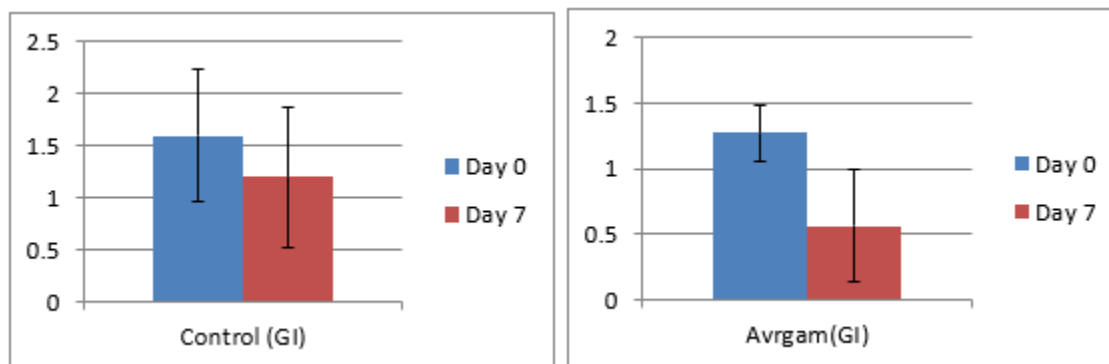


Figure 2. Comparison of decrease in the GI after using test versus control mouthrinse. Vertical bars represents SD of the mean.

Effectiveness of treatment

Statistical analysis showed that both the rinses decreased plaque index ($p < 0.025$) and test rinse decreased gingival index ($p < 0.01$), significantly. Decrease in PII by the control rinse were not significant. Test rinse showed more efficacy than the gold standard in reducing the PII and GI ($p < 0.001$), highly significant (Table 2). Decrease in PII and GI, after a week of dosing, for both the mouthwashes was also compared in the Graph 1 and 2, respectively.

DISCUSSION

Herbs for the formulation were taken from different plant families with the aim of adding a vast array of natural products and secondary metabolites in it including the entire major classes of natural products i.e. alkaloids, glycosides, phenolics, essential oils, terpenes, tanins etc. *Aloe vera* is known for healing purpose for centuries, yarrow is an analgesic drug and berberine has tremendous anti-inflammatory potential and was proved to be antimicrobial to some of the major periodontopathic and cariogenic microorganisms (Khare, 2007 and Hwang *et al.*, 2003); these pharmacological actions altogether are required for treating periodontal diseases.

Aloe vera in concentration of 50% showed a significant reduction of plaque and gingivitis after a 30-day use of mouth rinse containing *Aloe vera* associated to tooth brushing (Villalobos *et al.* 2001) but its use in toothpaste was incapable to show any efficacy (Oliveira *et al.*, 2008). In our study it, might be due to the additive effects and comparatively higher concentration, expressed its role in dentistry. *Aloe vera* nullified the burning sensation, if any, of the essential oils. Its soothing effects probably protected from any adverse effects to the oral cavity. We used the

plant extracts in the formulation, not individual component; essential oil or any one or few phytochemical; so that variety of the secondary metabolites in the final composition may cancelled out each other's' adverse effects, if any. Tooth powder containing *Berberis aristata* DC. for oral daily cleaning is available in the subcontinent (Agrawal *et al.*, 2005). The hydroalcoholic extracts of root and stem of the *Berberis aristata* DC. has proven antimicrobial activity against oral pathogens (Singh *et al.*, 2007). Further, *Berberis aristata* indication in periodontal diseases has been proved by the fact that Berberine hydrochloride, a major alkaloid of the plant, inhibited the expression of IL-1beta and TNF-alpha in periodontal tissues in rats periodontitis model and promoted the regeneration of the periodontal tissues. This study suggested that berberine hydrochloride may have potential clinical application. (Yu Zhan-hai *et al.*, 2008). In addition, Van der Weijden *et al.* (1998) worked on *Achillea millefolium* L. alone, as well as in combination for its antimicrobial activity against oral pathogens. Later, Koo and coworkers in 2003 studied on chemical constituents of *Achillea millefolium* (e.g. apigenin) and showed that its topical applications, twice daily (total of six applications), resulted in lower amounts of polysaccharides in the *Streptococcus mutans* biofilms. These three plants never being used together, earlier, as in this combination in any formulation in natural medicine. Although, *Achillea millefolium* L. has been used in a combination for treating dental problems but the combination of herbs in that trial was unable to produce significant effects (Van der Weijden *et al.* 1998). But in our research proved its high efficacy might because the use of *A. millefolium* in the different combination.

In this study, some patients with severe plaque and gingivitis were also get enrolled in the trial and there were also some tobacco users like *pan*, *gutka*, *niswar* and smokers. That might be the reason in not getting statistically significant results in reducing GI by the chlorhexidine gluconate rinse. Even after six months use of the *Avrgam* mouthwash neither of the participants reported any side effects of it although they were advised to report ill effects, if any, any time, any day but no complains received from them.

In testing the rinse on dental calculus patients by one of the dentists, it was observed that after 30 second rinsing just before the ultrasonic scaling, it takes comparatively less efforts in removing debris. Therefore, this advice might be use as another therapeutic significance in future. It was also observed that it has antibleeding and soothing effects on gums. Although, chlorhexidine gluconate is considered a standard for controlling dental plaque and periodontal diseases but its use may cause dental staining and this restricts its use for relatively longer duration. Herbs, used in this research exercise were considered safe from this aesthetic side effect and thus might be preferred over the synthetic chemicals having the same efficacy. The herbal preparation *Avrgam* was appreciated by the participants of the study due to its marvelous effects on various dental problems as compare to the use of synthetic mouth preparations. This not only showed the effectiveness of the natural dosage form but also reflects that the interest of people going towards nature more and more day by day.

CONCLUSION

This research study suggested that the *Avrgam* mouthwash (a combination of *A. millefolium*, *A. vera* and *B. aristata*) is highly effective in reducing plaque and gingivitis and is proved to be highly safe (Karim et al., 2015 and Karim et al., 2015) for human consumption. The present study prospects the long term clinical use recruiting large samples. Believing in the miracles of phytomedicine and the highly efficacy that was manifested in the research exercise recommended that the herbal rinse could be investigated for other oral pathologies, for instance in periodontitis and even in oral cancers with the blessings and benefits of nature.

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