

Effectiveness of *Azadirachta indica* (Neem tree) leave extract mouth rinse in preventing presenting complaints of pregnant females

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Abstract:

Background: *Azadirachta indica*, commonly known as the Neem tree, has various reported therapeutic effects including its antimicrobial effects. Multiple researchers have studied the effects of Neem extract gels against gingival and periodontal bacteria and reported a significant antibacterial effect and minimum or no adverse effects as to chlorhexidine. Neem tree extracts induced-decrease in gingival inflammation, dental plaque, and bacterial count have also been reported when compared with standard chlorhexidine. Thus, the rationale of our study is to evaluate the effectiveness of Neem tree (*Azadirachta indica*) extract against presenting complaints of pregnant females.

Methodology: This experimental study was performed at tertiary care hospital of Karachi. The sample size was calculated to be as $n = 46$. The participants were recruited using a sequential sampling strategy. The research was conducted on pregnant women who agreed to participate and reported to OPD in 1st trimester. For group randomization, the participants were handed an envelope, and randomization was done by single-blind technique. Before sampling, participants were told not to clean their teeth. Each group comprised 23 pregnant females and was separated into two groups (Group 1, positive control (who used commercially available mouth rinse), and Group 2, Neem tree extract rinse group). For rinses, the experimental group received diluted neem tree extract, and the positive controls received a typical commercially available mouth rinse. The participants were instructed to use the rinses twice a day after breakfast and before sleeping. The gingival pocket depth, bleeding tendency, halitosis, and gingival swelling were evaluated before and after 3 months to compare the results, and participants were asked about the taste and any post-use discomfort by the rinse.

Results: The mean age of females was 27 ± 8 , and most of the females 32 (69.5%) visited the OPD at the 11th -14th week of gestation. The most common complaint was halitosis 36 (82.6%) and gingival bleeding 28 (60.8%). The mean pocket depth observed pre-experimentally was 1.9 ± 0.4 . After the 3 months of the experiment when patients were asked about their previous presenting complaints their responses were similar in both the groups. Chi's square analysis didn't show any

significant difference between commercially available mouth rinse and neem tree extract rinse. When examined intraorally we also had the nonsignificant findings.

Conclusion: Neem tree extract rinse and commercially available mouth rinse showed comparable results in reducing presenting complaints, gingival bleeding, and pocket depth.

Key words: *Azadirachta indica*, Mouth rinse, Pregnancy, Presenting Complaints

Introduction:

Various hormonal and immune variations occur in almost all systems of a pregnant women's body (1). These multiple alterations in body systems, including the endocrine, inflammatory, immune, and stress responses, increase the chances of various infections in the body (2). This also increases the risk of periodontal infections, especially gingivitis and periodontitis, in pregnant women facing hormonal alterations (3). The gingival swelling in pregnant women could be marginal or generalized (4). Although, the initiating factor in most cases of gingivitis or gingival swelling is plaque accumulation that contains certain bacteria leading to enlargement of the gingiva (5). But the hormonal disturbances in pregnant women can predispose them to gingivitis and increases the chances of gingival hyperplasia (6). Apart from pregnancy, certain other systemic conditions may cause gingival hyperplasia such as vitamin C deficiency, puberty, leukemia, and certain tumors (6). These hormonal alterations cause an increase in the estrogen and progesterone levels in pregnant females which as a result increases the vascular permeability (7). This surge in permeation of vessels also causes a significant inflammatory response, by the immune system, even to an unremarkable amount of accumulated plaque (8). An increase in subgingival microbiota has also been reported such as *Fusobacterium nucleatum* and *Prevotella intermedia*, causing gingival edema in pregnant females (9).

The primary cause of gingivitis is subgingival and supragingival plaque that contains multiple bacteria (10). So, the recommended treatment or prophylaxis for gingival inflammation is chlorhexidine-containing mouthwashes, which have certain bacteriostatic and bactericidal properties (11). Chlorhexidine has been prescribed and used widely around the world for having remarkable antibacterial effects, especially against oral pathogens, causing gingivitis or periodontitis (12). Although, chlorhexidine has no reported complications or adverse effects in pregnant mothers and fetuses (13). But still, there are various general adverse effects related to

chlorhexidine that cannot be put aside. These adverse include irritation of oral mucosa, dry mouth, staining of teeth or oral soft tissues, unpleasant taste, and decreased taste sensation (14). Even though chlorhexidine is a primary and gold standard for treating oral infections but patient compliance is not up to the mark due to various adverse effects, mentioned previously (15). That is why, in the last decade many studies have been carried out to find a newer herbal alternative and substitute to chlorhexidine that may have equal or better antimicrobial properties against oral pathogens with the least complications and adverse effects on oral tissues (16).

New treatment modalities, such as extracts of various herbs and plants having therapeutic properties, are now replacing the standard chemotherapeutics to avoid various adverse effects and drug resistance (17). *Azadirachta indica*, commonly known as the Neem tree, has various reported therapeutic effects including its antimicrobial effects (18). In India and Pakistan region, this traditionally used medicinal plant is considered a "village dispensary" (19). Multiple researchers have studied the effects of Neem extract gels against gingival and periodontal bacteria and reported a significant antibacterial effect and minimum or no adverse effects as to chlorhexidine (20). Neem tree extracts induced-decrease in gingival inflammation, dental plaque, and bacterial count have also been reported when compared with standard chlorhexidine (21).

Thus, the rationale of our study is to evaluate the effectiveness of Neem tree (*Azadirachta indica*) extract against presenting complaints of pregnant females.

Methodology:

From February to April 2022, an experimental study was done at tertiary care hospital of Karachi. The sample size was calculated to be as $n = 46$. The participants were recruited using a sequential sampling strategy. The research was conducted on pregnant women who agreed to participate and reported to OPD in 1st trimester. For group randomization, the participants were handed an envelope, and randomization was done by single-blind technique. Neem tree leaves (1000 g) were obtained from a local Karachi market and given an authentication number, Specimen voucher 1081. The leaves were washed and dried before being ground into powder. The leaves were steeped for 15 days in 2500mL of 70% ethanol with intermittent shaking. The filtrate was filtered with Whatman filter paper (number 1) after 15 days and then treated in a water bath at 60°C. On a rotary evaporator, the mixture was dried at 50°C until a well-concentrated extract was obtained. The extract was kept in an airtight bottle and refrigerated until it was used. The extract was diluted in distilled water to a concentration of 1:4 (Extract: Distilled water). Before sampling, participants were told not to clean their teeth. Each group comprised 23 pregnant females and was separated into two groups (Group 1, positive control (who used commercially available mouth rinse), and Group 2, Neem tree extract rinse group). For rinses, the experimental group received diluted neem tree extract, and the positive controls received a typical commercially available mouth rinse. The participants were instructed to use the rinses twice a day after breakfast and before sleeping. The diluted extract and commercially available mouth rinse were provided by the principal investigator on monthly bases and this was the strategy to monitor the followup of the experiment. The gingival pocket depth, bleeding tendency, halitosis, and gingival swelling were evaluated before and after 3 months to compare the results, and participants were asked about the taste and any post-use discomfort by the rinse. Student t-test was applied to identify the intergroup comparison, chi-square test was used to check the association of the categorical variable. <0.05 p-value was considered significant at 95% confidence interval.

Results:

The mean age of females was 27 ± 8 , and most of the females 32 (69.5%) visited the OPD at the 11th -14th week of gestation. The most common complaint was halitosis 36 (82.6%) and gingival bleeding 28 (60.8%). The mean pocket depth observed pre-experimentally was 1.9 ± 0.4 . Table 1 represents the demographic data of study participants. After the group allocation proforma of the participants were separated and pre-experimental data was aligned (Table 2). After the 3 months of the experiment when patients were asked about their previous presenting complaints their responses were similar in both the groups. Chi's square analysis didn't show any significant difference between commercially available mouth rinse and neem tree extract rinse. When examined intraorally we also had the nonsignificant findings (table 3). The t-test analysis after the experiment revealed an insignificant finding ($p=0.071$, Pocket depth $< 1.2\text{mm}$ in both the groups) as both the treatments reduced the pocket depth equally. Furthermore, after the completion of the experiment participants were asked about the experience of using the mouth rinse table 4 represents the post-experimental experience of the study participants.

Education status	Frequency (percentage)
Matric	18 (39.13%)
Intermediate	15 (32.6%)
Graduation	13 (28.2%)
Presenting Complaints	
Pain in gingiva	29 (63%)
Gingival bleeding	28 (60.8%)
Bad breath	36 (82.6%)
Pain in tooth	18 (39.13%)
Mobile tooth	2 (4.3%)
Hot cold sensation	30 (65.2%)
Intraoral examination	
Carious teeth	19 (41.3%)
Mobile teeth (grade 3 mobility)	3 (6.5%)
Probing depth (mean \pm SD) $> 1.5\text{mm}$	33 (71.7%)
Bleeding on probing	35 (76.08%)
Oral ulcers	12 (26.08%)

Table 2. Pre-experimental data after group distribution

Presenting complaint	Total (N=46)	Group 1 (n=23)	Group 2 (n=23)
Pain in gingiva	29 (63%)	13 (56.52%)	16 (69.5%)
Gingival bleeding	28 (60.8%)	15 (65.2%)	13 (56.52%)
Bad breath	36 (82.6%)	9 (39.1%)	14 (60.8%)
Pain in tooth	18 (39.13%)	11 (47.8%)	8 (34.8)
Mobile tooth	2 (4.3%)	2 (8.6%)	1 (4.3%)
Hot cold sensation	30 (65.2%)	13 (56.5%)	10 (43.4%)
Intraoral examination			
Cariou teeth	19 (41.3%)	11 (47.8%)	8 (34.8)
Mobile teeth (grade 3 mobility)	3 (6.5%)	2 (8.6%)	1 (4.3%)
Probing depth (mean \pm SD) > 1.5mm	33 (71.7%)	13 (56.5%)	20 (86.9%)
Bleeding on probing	35 (76.08%)	17 (73.9%)	18 (78.2%)
Oral ulcers	12 (26.08%)	7 (30.4%)	5 (21.7%)

Table 3: Chi-Square analysis after experimental followup

Presenting complaint		Group 1	Group 2	p-value
Pain in gingiva	Yes	3 (23.07%)	2	0.317
	No	10 (76.9%)	14	
Gingival bleeding	Yes	4 (26.6%)	1	0.061
	No	11 (73.3%)	12	
Bad breath	Yes	1 (11.1%)	1	1.000
	No	8 (99.9%)	13	
Pain in tooth	Yes	8 (72%)	6	0.152
	No	3 (27.3%)	2	
Mobile tooth	Yes	2 (8.6%)	1 (4.3%)	-*
	No	0	0	
Hot cold sensation	Yes	3 (23.07%)	1 (10%)	0.051
	No	10 (76.9%)	9 (90%)	
Intraoral examination				
Cariou teeth	Yes	11 (47.8%)	8 (34.8)	-*
	No	0	0	
Mobile teeth (grade 3 mobility)	Yes	1 (8.6%)	0	-*
	No	0	0	
Probing depth (mean \pm SD) > 1.5mm	Yes	2 (56.5%)	4 (86.9%)	0.014
	No	11	16	
Bleeding on probing	Yes	3 (73.9%)	2 (78.2%)	0.162
	No	14	16	
Oral ulcers	Yes	0 (30.4%)	0 (21.7%)	-*
	No	7	7	

*p-value not generated because of "0" (zero) in two by two table

Table 4. Post experimental experience of participants

	Group 1 (n=23)	Group 2 (n=23)
Bitter test	4 (17.39%)	3 (13.04)
Alter in taste	2 (8.69%)	5 (21.7)
Nausea	4 (17.39%)	3 (13.04)
Vomiting	1 (4.34%)	0
Dry mouth	0	2 (8.69)

Discussion:

This study was conducted on pregnant women who reported the dental OPD with various complaints like gingival pain, gingival bleeding, halitosis, tooth pain, mobility of teeth, and hot and cold sensation. The participants inducted into the study were having last weeks of their 1st Trimester. The demographic data reported that all of the participants were educated and literate. These participants were divided into two equal groups (23 each). Group 1 was treated with commercially available mouthwash containing chlorhexidine while the 2nd group was treated with Neem leaves extract rinse.

After the 3 months use of both the rinses, chlorhexidine, and Neem tree extract, different parameters related to the oral health of both groups were evaluated. Gingival pain was relieved in 10 participants out of 13 who used standard chlorhexidine mouthwash. Almost the same but slightly better results were observed in patients who used Neem tree extract rinses where 14 out of 16 participants got relief from gingival pain. Same as our study, a study done by Nikita Dandekar reported equal effects of Neem tree extract and chlorhexidine against gingival pain and gingivitis (22). Regarding gingival bleeding, both chlorhexidine treated group and the Neem tree extract-treated group showed equal effects in curing. In group 1, 11 out of 15 participants got cured of gingival bleeding while 12 participants out of 13 were cured in group 2 who received Neem tree extract. In India, results of a randomized controlled trial reported equally effective and significant reduction of gingival bleeding and plaque indices in both chlorhexidine and Neem tree mouthwash groups. In our study, post-experimental results showed that halitosis was treated remarkably in both groups. In the chlorhexidine mouth rinse group, 8 out of 9 participants were treated for halitosis while in Neem tree extract mouthwash, 13 out of 14 participants of the study were treated. Another study done by Ranjan Malhotra compared chlorhexidine with herbal preparation containing various herbs including Neem tree extract. This study reported that the herbal

preparation was found to be a potent plaque inhibitor and decreased halitosis. The participants of this study preferred the herbal mouth rinse for its taste, convenience of use, and taste after rinse which are frequent complaints by most chlorhexidine mouth wash users (23).

The results of our study did not report any significant effects of both chlorhexidine and Neem tree extracts in patients with complaints of mobile teeth and pain in any tooth. Only 3 out of 8 patients got relieved from toothache in group 1 while in group 2, only 2 out of 6 participants were relieved from toothache. This also shows equal effects of both mouth rinses. But the pre and post-experimental evaluation of hot and cold sensations showed remarkable improvement in both, group 1 and 2 in our study. Post experiment evaluation reported that 10 patients out of 13 were not sensitive to hot and cold in group 1. While group 2 participants also reported improved results same as the chlorhexidine group, where 9 out of 10 participants were not feeling hot and cold sensations. Many other studies have reported a reduction in sensitivity and gingival bleeding with the use of Neem tree extract as a mouth wash (24).

Pre and post intraoral examinations of participants reported that there was no effect on the teeth that were affected with dental caries, in both chlorhexidine and Neem tree extract groups. Only one participant had grade 3 mobility and was treated with the chlorhexidine group. The results reported no effect on the tooth mobility with the use of commercial mouthwash. Probing depth was significantly reduced after the experimental period in both group 1 and group 2. In group 1, treated with chlorhexidine, 11 out of 13 participants reported a reduction in probing depth less than 1.5mm. While 16 out of 20 in group 2, treated with Neem tree extract, reported reduced probing depth of less than 1.5mm. As a validation of our results, another study has reported that Neem and Turmeric extracts had reduced the periodontal depth (25).

Regarding bleeding on probing, the results of our study were significant. In the chlorhexidine group, 14 out of 17 patients showed no bleeding on probing. Same as this, the 2nd group, Neem tree extract, also showed no bleeding on probing in 16 out of 18 patients. University of Dental Medicine, Yangon conducted a research to evaluate the effects of Neem tree extract in chronic periodontitis. It reported improved gingival inflammation and bleeding on probing (20). Regarding oral ulcers, no effect was produced by both chlorhexidine and Neem tree extract rinses.

Few adverse effects were also compared between both groups whose results were not much remarkable. Only 4 participants from group 1 reported a bitter taste while 3 from group 2.

Alteration of taste was reported in only 2 participants from group 1 and 5 from group 2. Nausea, vomiting, and dry mouth were also not significantly found in both groups, 1 and 2. But various studies have reported different adverse effects related to the use of chlorhexidine mouthwash including taste alteration, staining of oral tissues, and dry mouth (26).

Conclusion:

Neem tree extract rinse and commercially available mouth rinse showed comparable results in reducing presenting complaints, gingival bleeding, and pocket depth. Using herbal rinses in patients who do not want to use medicated mouth rinses may prevent them from the burden of oral diseases. Furthermore, none of the groups showed bad experiences with using either commercial or herbal mouth rinse.

Conflict of interest: None

Ethical Approval: The study was approved by ERC of the university.

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