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Effectiveness and safety of aqueous neem leaf extract versus chlorhexidine mouthwash in post-operative care of dis-impacted third molars: a randomised controlled clinical trial

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ABSTRACT

Background: Chlorhexidine is usually used as a mouth disinfectant after third molar surgery however; it lacks anti-inflammatory and analgesic properties. There is a possibility that neem leaf extracts, with analgesic, anti-inflammatory properties and can serve as a disinfectant, will be a better alternative. We aimed to evaluate the effectiveness and safety of neem leaf extract in controlling postoperative complications after third molar surgeries in comparison to chlorhexidine mouthwash.
Methods: This was a randomised clinical trial that enrolled patients who had impacted mandibular third molars and were randomly assigned to the control (chlorhexidine) or experimental (neem leaf extract) groups using simple randomisation method. We collected data on patients' clinical demographic, type of mouthwash, pain, facial swelling, trismus, wound healing, acute alveolar infection, localised alveolitis and adverse effects. Both descriptive and inferential statistics were performed and p-values < 0.05 were considered significant.
Results: 48 patients were randomly divided into control and neem groups (n = 24 each). There was no significant difference in clinical demographic characteristics between the two groups. Post-operative pain was significantly lower in the neem group at post-operative day (POD) 3 (p = 0.001) and POD 7(p = 0.001). Facial swelling reduced significantly at POD 7 in the neem group (p=0.02). There was significant decrease in trismus at POD 3(p = 0.01) and POD 7 (p = 0.004) in the neem group. Wound healing was better among those who rinsed with neem (p=0.03). Neem mouthwash did not reduce incidence of localised alveolitis (p = 0.595), acute alveolar infection (p = 0.346) and adverse effects (p = 0.257) than chlorhexidine mouthwash.
Conclusion: Neem mouthwash was more effective in the control of pain, facial swelling, trismus and poor wound healing compared to chlorhexidine mouthwash but has same effect as chlorhexidine mouthwash in the control of localised alveolitis, acute alveolar infection and adverse effects.

1. Introduction

Impacted third molar extraction is one of the most common surgical procedures performed by the oral and maxillofacial surgeon globally[1]. Often, the surgical removal of impacted lower third molars involves trauma to the soft and hard tissues from retraction of the mucoperiosteal flap and the removal of bone. Clinical conditions precipitated include pain, swelling, and trismus[2]. In the postoperative phase, swelling and pain are the main complaints[3] Complications such as alveolar osteitis, hematomas, and damage to adjacent teeth or nerves have also been reported[4] Various studies in the literature have reported several therapeutic protocols to improve the postoperative complications after extraction of the lower

third molars by integrating or modifying different aspects of the treatment and these include use of preoperative antibiotics[5], antimicrobial irrigants [6], different flap designs [7],and osteotomies using high- or low-speed instruments[8]. Other reported treatment protocols are healing by either primary or secondary intention[9] use of ice during the postoperative period [10], and use of corticosteroids (both via parenteral and oral administration)[11-13].

Conventionally, chlorhexidine mouthwash is prescribed following third molar surgeries for its antibacterial properties globally[14]. It should be noted that the main aim of treatment in the acute phase is to reduce inflammation[15,16] and chlorhexidine lack this role . Moreover, chlorhexidine is a luxury in some health facilities in developing countries[17]. Hence, the search for an effective and safe alternative to chlorhexidine mouthwash has led to the introduction of various herbal products in dentistry that are without any major side effects besides being cheap, readily, and locally available[18]. Neem (*Azadirachta indica)* is a popular medicinal plant in Africa and Asian continents [19]. Numerous studies [20-25]have reported that neem is rich in compounds such as azadiractin that possess various properties, including antioxidant, anti-diabetic, anti-mutagenic, antiviral, antibacterial, and anti-inflammatory. Also, it has been reported that neem contain limonoids, which show anti-inflammatory activity[23]. Neem is reported to remarkably reduce the release of monocyte chemotactic protein-1 (MCP-1)[22] . Extract of neem leaves shows a reduction in the release of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β. On the other hand, it enhances the release of anti-inflammatory cytokines such as IL-10[24]. Despite the well documented[22-24] anti-inflammatory and analgesic role of neem, it appears this is yet to be reported following third molar surgeries. Though several treatment protocols are reported[6-13] in the control of postoperative sequelae and complication after third molar surgeries, none has been reported to be optimum. Therefore, the aim of this study is to evaluate the effectiveness and safety of neem leaf extract in controlling postoperative complications after third molar surgeries in comparison to chlorhexidine mouthwash. We hypothesize that neem mouthwash will be effective and safe in controlling postoperative complications after third molar surgeries compared to chlorhexidine mouthwash.

Materials and methods

Study design and participants: To answer the study objective, a parallel, two-arm, randomised, double-blind controlled clinical trial was designed and implemented. The clinical trial (PACTR202301888753718) result was reported according to the Consolidated Standards of Reporting Trials (CONSORT) statement. Written informed consent was taken from the consecutive patients. Ethical approval (ADM/22/A/VOL. VII/14831289) for this study was obtained from the Ethics and Research Committee of the study centre. Patients who had lower third molar surgery were recruited and they were screened for inclusion and exclusion criteria (Table 1).

Table 1: Inclusion and exclusion criteria

Inclusion criteria

- Age-> 18 years
- American Society of Anaesthesiologists physical status class 1 Patients (A normal healthy patient without systemic disease).
- Patients who did not take any antibiotic or anti-inflammatory agent seven days before surgery.
- Those who understood the procedure and agreed to sign the informed consent form.
- Those with mesioangular, distoangular, vertical and horizontal mandibular third molars impaction with moderate difficulty index according to Pederson's criteria[4].
- Presence of recurrent pericoronitis, unrestorable carious lesions, fractured teeth, and non-treatable pulpal pathology.
- Surgical site free from active infection

Exclusion criteria

- Patients with systemic conditions like uncontrolled diabetes, uncontrolled hypertension, and blood dyscrasias.
- Those non-consenting
- Duration of surgery more than 45 minutes.
- Individuals with dentition lack natural opposing central incisors or prosthetic equivalent which would not allow standardization of the measurement of the maximum inter-incisal opening.

- Those on medications that might influence the surgical procedure or affect wound healing, e.g., anticoagulants and corticosteroids.
- Smokers and alcoholics
- Those with Pederson severe difficulty Index.
- Patients who had a history of head and neck cancer or radiotherapy.
- Those pregnant or breast feeding.

The minimum sample size with attrition was calculated to be 24 for each group using the formula for comparison study in randomized clinical equivalence trials[26]: N= (r+1) (Zα/2 + Z1-β)2 Ϭ2 / rd2 based on a previous study[27] where N = Minimum sample size for the study; Zα/2 = Is the standard normal deviate at 95% level of significance (this represents the chance for making a type I error) = 1.96.; Zβ = Is the standard normal deviate at (1- β%) 80% power with 20% chance of making a type 2 error = 0.84; Ϭ = Pooled standard deviation in previous study [27] = 1.13; r = Ratio of the sample size for the two groups = 1 (as both groups will have equal sample size); d= Difference in mean of the outcome variable of the 2 groups in previous study[27] = 0.98.

Enrolment, randomisation and intervention: Participants were enrolled into the study by the investigators. An independent statistician performed sequence generation with the aid of a computer and concealed them in opaque sealed envelopes, which were assigned into test (neem) and control (chlorhexidine) groups using blocked randomisation. All participants and investigators were unaware of the contents of each bottle. Treatment assignments were blinded to the research assistant responsible for recording the outcome measures. The mouthwashes were dispensed in two similar 500ml opaque plastic bottles that masked the contents of the two bottles. The test group received 5% neem mouthwash, while the control group received 0.2% chlorhexidine mouthwash. Each group was instructed to rinse with 20 ml 12 hourly for 7 days. The chlorhexidine gluconate (Hexicol®, Biofield Pharma., PVT LTD,Panchkula, India) is commercially available and was obtained from the pharmacy of the University of Benin Teaching Hospital, Benin-City, Edo State.

Neem mouthwash preparation: Identification of the neem plant was carried out under the expert supervision of the Herbarium Unit of the Department of Plant Biology and Biotechnology, University of Benin, Benin-City with a voucher number of UBH-A286. Neem mouthwash preparation was performed in the Department of Pharmacognosy, University of Benin, Benin-City. The neem leaves were picked, washed, and air dried at room temperature for 7 days and subsequently oven-dried at 60 degrees centigrade using the plant oven (Gullian®, Germany) and then pulverized into a coarse powder using a milling machine (Miller®, UK). The grounded leaves were subsequently weighed (500 g) and stored in containers at room temperature. Later, the well-soaked (24 hours in water) neem powder was transferred to a distillation apparatus along with ten parts of water. The mixture was continuously heated until 60% of the distillate was collected after the water maceration. The mixture left in the flask was cooled at a low temperature and subsequently freeze-dried. The freeze-dried sample weighed 28 g after cooling. The preparation of neem mouthwash described in this study was previously described[28]. To prepare the neem mouthwash at 5% concentration, 5 g of the extract was dissolved in 100 ml distilled water.

Surgical procedure: All surgical procedure was done by a single-blinded oral and maxillofacial surgeon using the same surgical protocol for all patients. A pre-procedural mouth rinsing with the two mouthwashes selected for this study was administered to the participants according to their study protocol. The local anaesthetic technique employed was the standard inferior alveolar nerve block and long buccal nerve infiltration using 1.8 mls of 2% lidocaine with 1:100,000 adrenaline. This was administered to all subjects using a dental syringe and a 3.5 cm long 27-gauge dental needle through an intraoral approach to anaesthetise the inferior alveolar nerve, lingual nerve and long buccal nerve. An anterior relieving incision was created from the vestibule upward and at an angle toward the midpoint of the marginal gingiva of the second mandibular molar to achieve access. The posterior relieving incision was then made, and the incision was continued along the buccal gingival sulcus to the external oblique ridge. A full-thickness mucoperiosteal flap was reflected using Howarth's periosteal elevator and retracted using a Lagenbeck retractor. Under continuous irrigation with 0.9% sterile normal saline solution, buccal and distal bone

was removed with a round bur on a straight handpiece, and guttering was completed slightly beyond the furcation. When necessary, the crown was sectioned. After the extraction, the socket was inspected and irrigated, and the flap was sutured with a 3-0 polyglactin suture (Vicryl®, Williams Medical, London, UK). One suture was placed just distal to the lower second molar and another on the distal aspect of the extraction socket. All subjects received verbal and written post-operative instructions and were given either to rinse with neem or chlorhexidine mouthwash. They were instructed to commence the mouth rinsing twice a day (morning and evening) the day after surgery by pouring 20 ml of the solution and rinsing with it for 60 seconds. All patients were placed on oral codeine/paracetamol (co-codamol®) 8/500 mg every 12 hours for three. The cocodamol was given since it lacks anti-inflammatory effect. Antibiotics was not administered since none of the participant had acute infection in the operation site and the procedure was done in aseptic condition

Pain assessment: Pain was measured and recorded pre-operatively and postoperatively using visual analogue scale (VAS). The VAS is in the form of a horizontal 10cm line without demarcations, with the number 0 cm (no pain) on the left edge and the number 10cm on the right edge. Patients were instructed to mark, with a vertical trace, the point of the scale that best defined their degree of pain sensation after the surgical procedure, which was later measured with a ruler. The pre-operative assessment was done before the surgical procedure. The POD was 1, 3 and 7.

Facial swelling assessment: Facial swelling (oedema) was determined by measurement with a tape measure, according to Gabka and Matsumura[29]. Three measurements were performed between the five reference points: tragus, pogonion (soft tissue), lateral corner of the eye, angle of the mandible, and external corner of the mouth. The measurements were obtained preoperatively (baseline) and POD 1, 3, and 7.

Trismus assessment: Maximum mouth opening was used to assess the level of trismus. The distance between the upper and lower incisors was measured with a digital calliper in millimetres. The measurement was determined in the pre-operative period (baseline) and on day 1, 3 and 7 after surgery.

Alveolar osteitis assessment: Alveolar osteitis was defined as post-operative pain in and around the extraction site accompanied by a partially or totally disintegrated blood clot within the alveolar socket[30]. This was recorded as "absent" or "present" and was used to estimate the prevalence of alveolar osteitis. The POD was 3.

Acute alveolar infection: Acute socket infection was defined as the presence of pus in the socket[30.] This was recorded as "present" or "absent" and was used to estimate the prevalence of acute socket infection. The POD was 3.

Wound healing assessment: This was evaluated with a modified Inflammatory Proliferative Remodeling (IPR) wound healing scale[31] at day 7 postoperatively. The modified IPR scale has eight parameters: bleeding, granulation tissue, hematoma, tissue colour, incision margins, suppuration, oedema, and pain (Table 2). The parameters were scored as 1 or 0, and the total range score was 0-8. The score was categorised as poor wound healing if the score is from 0-2, acceptable healing if the score is from 3-5, and excellent wound healing if the score is from 6-8. The scores 0-2, 3-5, and 6-8 were categorised as poor, acceptable, and excellent wound healing, respectively

Table 2: The modified IPR wound healing scale

SN	Parameter	Score 0	Score 1
1	Bleeding spontaneously or on palpation	Yes	No
2	Haematoma	Yes	No
3	Tissue colour	Redder or whiter than opposite tissue	Like the opposite side tissue
4	Granulation tissue	Yes	No
5	Incision margin	Incomplete flap closure/fibrin clot/ partial necrosis/complete necrosis	Complete flap closure/fine fibrin line
6	Suppuration	Yes	No
7	Oedema VAS (1-10)	VAS 6 – 10	VAS 1 – 5
8	Pain VAS (1-10)	VAS 6 – 10	VAS 1 – 5

Adverse effect: The presence or absence of any adverse effect following the rinsing of any of the test mouth rinses was recorded and used to estimate the prevalence of adverse effects. The assessed adverse effects were teeth discolouration, burning sensation, altered taste, epithelial desquamation, nausea, altered tongue sensitivity, and dry mouth. Patients were instructed to report any adverse effect beginning from POD 1-7.

Data analysis: The predictor variable was the type of mouthwash (neem or chlorhexidine) used in each group. The outcome variables were self-reported pain, facial swelling, trismus , wound healing, acute wound infection, and alveolar osteitis. The other study variables were demographic variables, including age and sex. Both descriptive and inferential statistics were performed. In the descriptive analysis, the mean and standard deviation of the numerical data were estimated, while the frequency and percentage of the categorical data were estimated. In the inferential statistics, normality of continuous data between the two groups was tested with the Shapiro-Wilk test; an independent t-test was used to compare mean values of pain, swelling, trismus, and wound healing scores between the two groups, and Chi-square test was used for categorical variables. Data were analysed on per-protocol basis as data of drop-outs were not analysed. Data were analysed using Statistical Package for the Social Science (SPSS) software version 20 (SPSS Inc., Chicago, USA). P <0.05 was considered significant.

Results

An illustration of the study participants' recruitment, randomisation, allocation, and analysis is represented in the CONSORT flow diagram below (Fig.1). A total of 60 participants were assessed for eligibility to participate in the study, but only 43 participants (neem, n = 22; chlorhexidine, n = 21) completed the study.

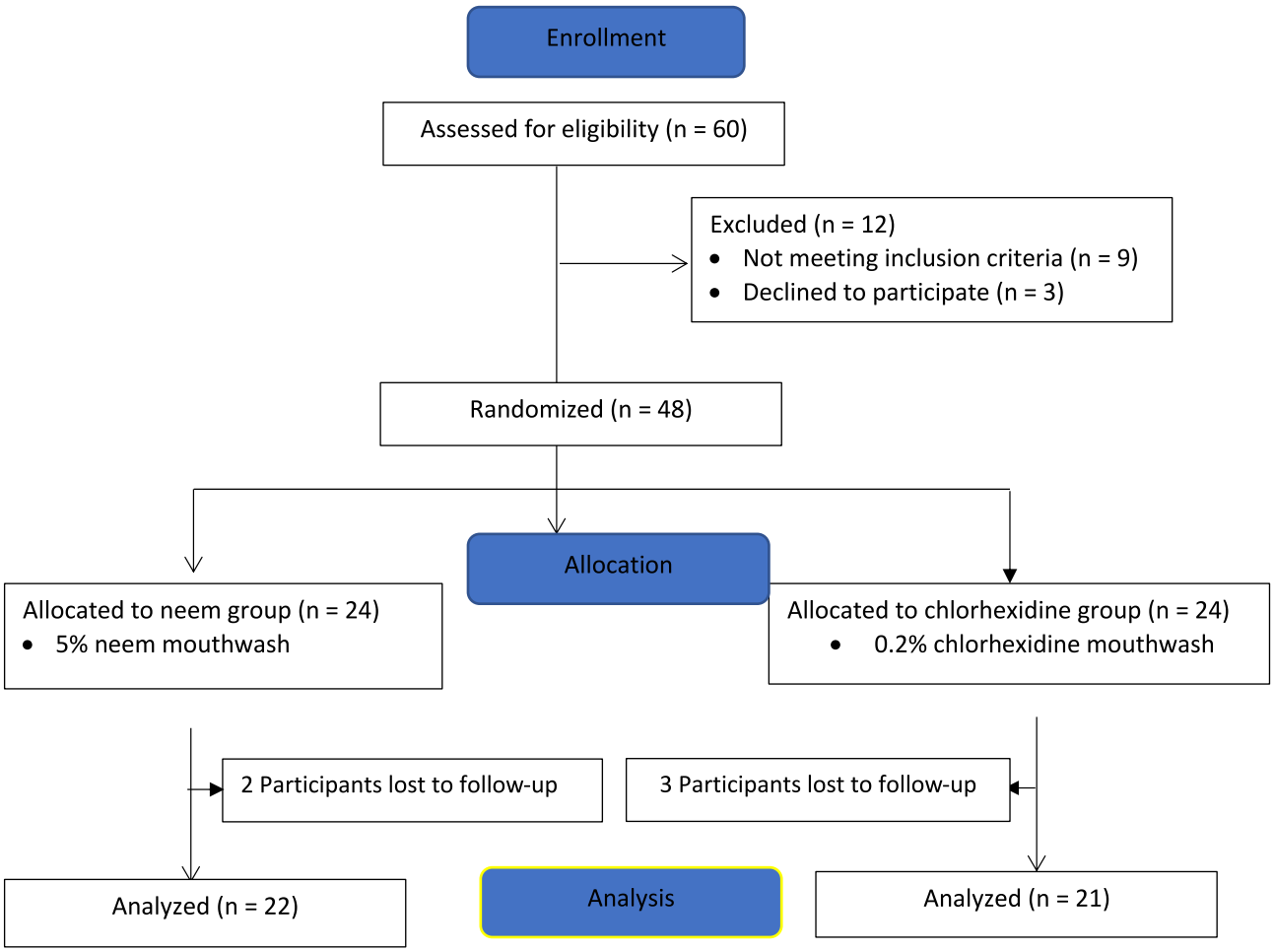

Figure 1: The CONSORT flow diagram

The clinical demographic characteristics of the participants are shown in Table 3. The participants' age range and mean age were 20-43 years and 30.10 ± 6.42 years respectively. Overall, a female preponderance was observed with a male: female ratio of 1:1.2. The neem and chlorhexidine groups' clinicodemographic variables was not statistically significantly (p = 0.759). The most (39.5%) extracted impacted lower molar were mesioangular, while the least (9.3%) was distoangular impaction. Recurrent pericoronitis (46.5%) was the commonest indication for disimpaction while orthodontic reasons were the least (2.3%). There was no statistical difference in the type of impaction (p = 0.974) extraction site (p = 0.443) and the indication for extraction (p = 0.324). No systemic complications were noted in both groups studied. χ

Table 3: Clinical demographic characteristics of the study participants

Variables	Neem group (n = 22)	Chlorhexidine group(n=21)	Total(n=43)	x	p- value
Mean Age ± SD*	29.95 ± 6.34	30.10 ± 6.53		0.154	0.697
Gender					
Male	11 (25.6)	8 (18.6)	19 (44.2)	0.617	0.432
Female	11 (25.6)	13 (30.2)	24 (55.8)		
Type of impaction					
Mesioangular	9 (20.9)	8 (18.6)	17 (39.5)	0.224	0.974
Distoangular	2 (3.7)	2 (4.7)	4 (9.3)		
Vertical	7 (16.3)	6 (14.0)	13 (30.2)		
Horizontal	4 (9.3)	5 (11.6)	9 (20.9)		
Indication of extraction					
Recurrent pericoronitis	9 (20.9)	11 (25.6)	20 (46.5)	6.969	0.324
Abscess	4 (9.4)	3 (6.9)	7 (16.3)		
Dental caries	3 (6.9)	2 (4.7)	5 (11.6)		
Periodontal disease	5 (11.6)	3 (6.9)	8 (18.6)		
Periapical pathology	1 (2.3)	1 (2.3)	2 (4.7)		
Orthodontic reasons	1 (2.3)	0 (0.0)	1 (2.3)		

SD = Standard deviation; χ = Chi Square of independence test

The effect of chlorhexidine and neem mouthwashes on pain, facial swelling and mouth opening is shown in Table 4. Pain score was higher among the chlorhexidine group compared to the neem group on POD 1 (p = 0.04). On the 3rd day after surgery, pain score was lower in the neem group compared to the chlorhexidine group (p = 0.001). Similarly, pain score was lower in the neem group compared to the chlorhexidine group on the 7th day post-operative (p=0.001). There was a reduction in facial swelling in the neem group compared to the chlorhexidine group, and this finding was observed only on day 7 after surgery (p=0.02). On day 3 postoperative, the mouth opening was better in the neem group compared to the chlorhexidine group (p=0.01). Also, on the 7th day after surgery, there was a better mouth opening among the neem group as compared to the chlorhexidine group (p = 0.004).

Of the total 43 samples studied, 6 (14.0) patients presented with acute alveolar infections of which the neem group had 2 (4.7%) compared to 4 (9.3%) of the chlorhexidine group. However, the observed differences were not statistically significant (relative risk (RR) = 2.095; 95% confidence interval (CI) = 0.428 to 10.26; p = 0.346). A total number of 5 (11.6%) patients had post-operative alveolar osteitis of which the neem group has 2 (4.65%) compared to 3 (6.97%) of the chlorhexidine group. However, the observed differences did not get to the statistical significant level (RR = 1.571; 95% CI = 0.291 to 8.48; p = 0.595). A total number of 9 (20.9%) patients developed postoperative poor wound healing, of which the neem group has 2 (4.6%) compared to 7 (16.3%) of the chlorhexidine group, and the observed difference was statistically significant (RR = 4.19; 95% CI = 0.509 to 34.5; p = 0.03). Table 5 shows the prevalence of adverse effects between the two groups. Of the total 43 samples studied, 16 (37.20%) patients presented with postoperative adverse effects, of which the neem group had 4 (9.30%) compared to 12 (27.90%) of the chlorhexidine group. However, the observed differences did not reach statistical significant level (p = 0.257).

Table 4: Effect of chlorhexidine versus neem on pain, swelling, and trismus

Variables	Neem group (n=21)	Chlorhexidine group (n=22)	MD(95%C.I)	T	p - value
Pain(Mean ± SD) (cm)					
Day 0(preoperative)	0.50 ± 0.01	0.53 ± 0.01	-0.03(-0.19-0.07)	2.24	0.16
Day 1	3.77 ± 0. 01	3.88 ± 0.02	-0.11(-0.75-0.18)	5.82	0.04
Day 3	1.63 ± 0.02	3.47 ± 0.021	-1.84(-2.66-2.02)	41.2	0.001
Day 7	0.50 ± 0.01	1.98 ± 0.01	-1.48(-1.85-1.51)	209.3	0.001
Facial swelling (Mean ± SD)(cm)					
Day 0(preoperative)	19.2 ± 0.14	19.4 ± 0.04	-0.20(-0.37-0.62)	1.70	0.23
Day 1	27.4 ± 0.78	28.6 ± 0.71	-1.20(-2.95-4.45)	1.70	0.24
Day 3	23.9 ± 0.35	23.7 ± 0.57	0.2(-10.2- 6.12)	17.3	0.08
Day 7	19.4 ± 0.35	22.1 ± 0.35	-2.7(-3.18-4.22)	7.64	0.02
Trismus (Mean ± SD)(cm)					
Day 0(preoperative)	5.46 ± 0.06	5.51 ± 0.03	-0.05(-0.12-0.23)	1.36	0.31
Day 1	3.41 ± 0.06	3.06 ± 0.04	0.35(-2.15-1.26)	-1.12	0.38
Day 3	4.41 ± 0.03	3.47 ± 0.03	0.94(-12.2-3.6)	-3.10	0.01
Day 7	5.60 ± 0.03	4.89 ± 0.06	0.71(-0.90- 5.2)	-15.9	0.004

SD = standard deviation; MD = mean difference; C.I= confidence interval; t = t-test;

Table 5: Effect of chlorhexidine versus neem on adverse effect

Adverse events (n (%))	Neem group (n=22)	Chlorhexidine group (n=21)	Relative risk (95%C.I)	χ	p– value
Burning sensation					
Mild	2(9.52)	2(9.52)			
Moderate	0(0.0)	1(4.76)	1.57 (0.29-8.49)	2.82	0.595
Severe	0(0.0)	0(0.0)			
Altered taste sensation					
Mild	1(4.76)	4(19.0)			
Moderate	0(0.0)	0(0.0)	4.19 (0.51-34.5)	2.99	0.138
Severe	0(0.0)	0(0.0)			
Epithelium desquamation					
Mild	0(0.0)	1(4.76)			
Moderate	0(0.0)	0(0.0)	0.95 (0.87-1.05)	1.07	0.300
Severe	0(0.0)	0(0.0)			
Nausea					
Mild	0(0.0)	1(4.76)			
Moderate	0(0.0)	1(4.76)	0.91 (0.79-1.04)	2.20	0.138
Severe	0(0.0)	0(0.0)			
Altered saliva color					
Mild	0(0.0)	1(4.76)			
Moderate	0(0.0)	0(0.0)	0.95 (0.87-1.05)	1.07	0.300
Severe	0(0.0)	0(0.0)			
Dry mouth					
Mild	0(0.0)	1(4.76)			
Moderate	1(4.76)	0(0.0)	1.05 (0.07-15.6)	0.001	0.973
Severe	0(0.0)	0(0.0)			

n= frequency; % = percent; C.I = Confidence interval; χ = Chi Square of independence test

Discussion

The age at which an individual undergoes personal and professional growth is when the third molars usually erupt, and a series of functional and structural alterations are expected after their extraction, which is mostly expressed as pain, swelling, and trismus[4]. Other complications that can arise are acute alveolar infection, alveolar osteitis, and poor wound healing [3]. In the current era in which quality of life is a prime concern, no one can happily accept these discomforts even for 1-3 days. So, a remedy that can lessen these postoperative sequelae and complications should be used regularly. Neem remedy represents such a class of therapy. They possess anti-inflammatory, antimicrobial, and healing properties, as reported in both animal and human studies [23,24.] To the best of our knowledge, it appears these properties have not been studied following third molar surgery.

Pain is subjective and is influenced by many factors, such as age of patients, cultural background, educational level, previous experience, pain threshold, and tolerance, and consequently, its assessment may be challenged[33]. The results of this study showed that pain was significantly lower in patients who used neem mouthwash than those who used chlorhexidine mouthwash at all times of assessment. This could be due to the anti-inflammatory action of neem, which is lacking in chlorhexidine. Anti-inflammatory and analgesic effects of neem demonstrated in this study corroborate those reported in previous studies[20,23]. The anti-inflammatory role of neem as mouthwash during periodontal procedures has been demonstrated in the literature [33], but this is unclear as regards third molar surgery. Aside from the anti-inflammatory role of neem, it is readily available and easy to prepare. Warm saline mouthwash may be unsuitable in patients with salt-retention conditions such as heart and renal failure[34], and chlorhexidine may not be readily available in some settings. However, phytochemical studies [35,36]on neem in animals showed that a major constituent of neem extract called azadiractin can markedly reduce TNF-a and IL-1b levels, thus inhibiting tissue infiltration by neutrophils and other inflammatory cells and relieving inflammatory pain.

Facial oedema following surgery is challenging to assess precisely because it necessitates a three-dimensional measurement of an uneven and convex surface and might appear internally and externally. Oedema has been measured objectively using a variety of methods over the years. Standardised stereo-radiography or photography measurements, computed tomography, linear measurement, the use of Vernier calipers to measure the cheek circumference, modified face-holding devices, facial plethysmographs, or various direct face-taking techniques are some of the measurement techniques mentioned in the literature [29]. The comparative superiority or accuracy of any of the techniques used in oedema analysis is yet to be proven. This study used the flexible tape rule, a practical, affordable, and trustworthy tool for assessing swelling of the face. In the present study, the results indicated that postoperative oedema increased less and was limited better in the groups receiving neem-based mouthwash compared to chlorhexidine mouthwash. This finding was profound on day 7, indicating prolonged action of the anti-inflammatory role of azadiractin[24]. Studies have shown that post-operative oedema usually subsides after I week of surgery [37, 38]. Though there were no previous studies to compare with this finding, the role of neem mouthwash in the reduction of facial swelling is evident in this study, thereby encouraging further studies on this topic.

Trismus, or limited mouth opening, is another undesirable effect commonly reported after oral surgeries. Trismus prevents eating and talking and impairs patients' quality of life; thus, decreased trismus translates to patients' reduced discomfort and increased quality of life[39]. The results showed significant differences in the two mouth rinse groups; there was more mouth opening after surgeries in participants using neem mouth rinse. This could be related to the anti-inflammatory role [23] of neem, which is absent in chlorhexidine gluconate.

Though more patients who use chlorhexidine mouthwash presented with a higher prevalence of alveolar infection, the observed difference in the prevalence was insignificant, and this is an indication that both mouthwashes are potent antimicrobial agents[14]. Nimbidin is reported to be the anti-microbial component of neem [23]. In this study, both neem and chlorhexidine have same potential in mitigating alveolar osteitis following third molar surgeries. This could be due to the fact that both agents have antimicrobial functions[14]. No clinical trials have been published to date, similar to the present study, in which neem was used in third molar surgery. The present study carried out the first comparison of this compound with chlorhexidine use as the gold standard [14]in third molar surgery. A clinical study of six weeks was made to check the efficacy of neem extract dental gel with chlorhexidine gluconate (0.2% w/v)

mouthwash as a positive control, and results of the study showed that the dental gel containing neem extract significantly reduced the plaque index and bacterial count compared to that of the control group[40].

Alveolar osteitis is generally characterised by delayed healing associated with degradation of clot and is usually accompanied by persistent, radiating pain postoperatively in and around the extraction site that is not easily relieved by analgesics. It can be a burden for both patients and surgeons [2]. Though the role of neem and chlorhexidine in the relief of periodontal diseases [41-42] and alveolar osteitis [43,] respectively, are well documented in the literature, neem efficacy in alveolar osteitis seems not yet documented. In this study, fewer patients who use neem mouthwash had less alveolar osteitis following third molar surgery, but this finding was statistically insignificant. This finding could be related to the fact both neem and chlorhexidine have antimicrobial properties.

The results of this study showed better soft tissue healing at one week post-operatively on sites that were rinsed with neem aqueous extract when compared to chlorhexidine gluconate solution. The prevalence of poor wound healing recorded in this study was 11.7%, more in patients who use chlorhexidine mouthwash following third molar surgery. However, it appears that no previously published studies compared wound healing between neem and chlorhexidine mouthwashes. However, some investigators [44, 45] have reported excellent wound healing with the use of neem. A previous study conducted to evaluate the wound healing activity of the extracts of leaves of neem using excision and incision wound models in Sprague Dawley rats revealed that extract of neem plants significantly promoted the wound healing activity in both excision and incision wound models [46]. Furthermore, in incision wounds, the tensile strength of the plant-treated group's healing tissue was significantly higher than in the control group[46]. Other results showed that leaf extracts of neem promote wound healing, increased inflammatory response and neovascularisation[19].

The most common adverse event reported was a mild burning sensation, with more incidences in the chlorhexidine group, followed by altered taste, which was also reported by the participants in the chlorhexidine group. Altered taste, which participants in both groups reported was mild and transient. In a similar randomised control trial, similar and contrary findings were noted as they reported burning sensation as the most common adverse event, but this was 38% and 14% for the neem and chlorhexidine groups, respectively[46]. The neem compound was demonstrated to be relatively safe. Adverse events were common but less frequent in the neem group, but these were mild in all cases and resolved without therapy. However, long-term adverse events are unknown and must be addressed by appropriately designed studies.

This study has some limitations. First, pain assessment was done using linear measurement instead of three-dimensional measurement. Second, the sample size would have been relatively small. Secondly, other pharmacological activities of neem were not evaluated. Lastly, although GOT, GPT, BUN and creatinine were not estimated in this study, neem is reported to be safe locally and systemically.

Conclusion: The Neem aqueous mouthwash was more effective in the control of pain, facial swelling, trismus and poor wound healing compared to chlorhexidine mouthwash but has same effect as chlorhexidine mouthwash in the control of localised alveolitis, acute alveolar infection and adverse effects.

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Data availability: The data that support the findings of this study are available in the department of the corresponding author, but restrictions apply to the availability of this data, which were used under license for the current study and are not publicly available. Data are, however, available from the authors upon reasonable request.

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References

1. Carter K, and Worthington S (2016) Predictors of third molar impaction: a systematic review and meta-analysis. *Journal of Dental Research*, 95: 267-276.
2. Chaparro-Avendaño AV, Pérez-García S, Valmaseda-Castellón E, Berini-Aytés L, and Gay-Escoda C (2005) Morbidity of third molar extraction in patients between 12 and 18 years of age. *Medicina Oral, Patologia Oral, Cirugia Bucal*, 10:422-431.
3. Bouloux GF, Steed MB and PerciaccanteVJ (2007) Complications of third molar surgery. *Oral and Maxillofacial Surgery Clinic of Northern America*, 19: 117-128.
4. Bui CH, Seldin EB and Dodson TB (2003) Types, frequencies, and risk factors for complications

after third molar extraction. *Journal of Oral MaxillofacialSurgery*, 61: 1379-1389.

5. Gopee P and Rikhotso E (2017) Impacted mandibular third molars: the efficacy of prophylactic antibiotics and chlorhexidine mouthwash in preventing postoperative infections. *South African Dental Journal*, 72:213-218.
6. Jadhao VA , Rao A, Hande P, Mahajani M, Raktade PP and Gedam R (2018) The efficiency of three irrigating solutions after surgical removal of impacted mandibular third molars: A cross-sectional study. *Journal of Contemporary Dental Practice,* 19: 1147-1151.
7. Korkmaz YT, Mollaoglu N and Ozmeric N (2015) Does laterally rotated flap design influence the short-term periodontal status of second molars and postoperative discomfort after partially impacted third molar surgery?. *Journal of Oral and Maxillofacial Surgery*, 73: 1031-1041.
8. Sortino F, Pedullà E and Masoli V (2008) The piezoelectric and rotatory osteotomy technique in impacted third molar surgery: comparison of postoperative recovery. *Journal of Oral and Maxillofacial Surgery*, 66(2008) 2444-2448.
9. Bello SA, Olaitan AA and Ladeinde AL (2011) A randomised comparison of the effect of partial and total wound closure techniques on postoperative morbidity after mandibular third molar surgery. *Journal of Oral and Maxillofacial Surgery*, 69: 24-30.
10. Hill CM and Walker RV (2006) Conservative, non-surgical management of patients presenting with impacted lower third molars: A 5-year study. *British Journal of Oraland Maxillofacial Surgery*, 44: 347-350.
11. Ngeow WC, Lim D (2016) Do corticosteroids still has a role in the management of third molar surgery? . Advance Therapy, 33: 1105-1139.
12. Moraschini V, Hidalgo R and Porto E (2016) Effect of submucosal injection of dexamethasone after third molar surgery: a meta-analysis of randomised controlled trials. *International Journal of Oral and Maxillofacial Surgery*, 45: 232-240.
13. Darawade DA, Kumar S, Mehta R, Sharma AR and Reddy GS(2014) In search of a better option: Dexamethasone versus methylprednisolone in third molar impaction surgery. *Journal of International Oral Health*, 6(6):14-17.
14. Zhou J, Hu B, Liu Y, Yang Z and Song J (2016) The efficacy of intraalveolar 0.2% chlorhexidine gel on alveolar osteitis: a meta-analysis. *Oral Diseases*, 3**:** 38-41.
15. Wade WG and Addy M (1989) In vitro activity of a chlorhexidine-containing mouthwash against subgingival bacteria. *Journal of Periodontology*, 60: 521–525.
16. Southern E N (2006) The comparative effects of 0.12% chlorhexidine and herbal oral rinse on dental plaque-induced gingivitis. *Journal of Dental Hygiene*, 80: 12-16
17. Haffajee AD, Yaskell T and Socransky S (2008) Antimicrobial effectiveness of a herbal mouth rinse compared with essential oil and a chlorhexidine mouth rinse. *Journal Of American Dental Association*, 139: 606-611.
18. Shahakbari R (2014) Effectiveness of green tea mouthwash in comparison to chlorhexidine mouthwash in patients with acute pericoronitis: a randomised clinical trial. *International Journal of Oral and Maxillofacaial Surgery*, 43: 1394–1398.
19. National Research Council(1992) Neem: A tree for solving global problems. Washington, DC: The National Academies Press. https://doi.org/(1992)10.17226/1924.
20. Vanka S (2001) The effect of indigenous Neem (*Azadirachta indica*) mouthwash on Streptococcus mutans and lactobacilli growth. *Microbiosynthesis*, 105: 183-189.
21. Khosla P (2000) A study of hypoglycaemic effects of *Azadirachta indica* (Neem) in normal and alloxan diabetic rabbits. *Indian Journal of Physiology and Pharmacology*, 44: 69-74.
22. Emran TB (2015) Phytochemical, Antimicrobial, Cytotoxic, Analgesic and Anti-Inflammatory Properties of *Azadirachta indica*: A Therapeutic Study. *Journal of Bioanalysis and Biomedicine*, 12: 007. doi:10.4172/1948-593X.S12-007
23. Srinivasa K, Jagadeesh K and Revankar S (2014) Analgesic effect of *Azadirachta indica* (Neem) in albino rats. *Journal of Medical and Health Sciences*, 3: 93-96.
24. Oktavia SN, Ifora I, Fauziah F(2022) Anti-inflammatory, Analgesic, and Antipyretic Potential of *Azadirachta indica*: A Review. *Journal Drug Delivery and Therapy*, 12(3): 245-254

25. Kagbo HD and Ejebe D (2010) Phytochemistry and Preliminary Toxicity Studies of the Methanol Extract of the Stem Bark of *Garcinia kola* (Heckel). *International Journal of Toxicology*, 7: 2-4.
26. Krejcie RVand Morgan DW (1970) Determining Sample Size for Research Activities. *Educational and Psychological Measurements*, 30: 607-610.
27. Jesudasan JS, Wahab PU and Sekhar MR (2015) Effectiveness of 0.2% chlorhexidine gel and a eugenol-based paste on postoperative alveolar osteitis in patients having third molars extracted: a randomised controlled clinical trial. *British Journal of Oral and Maxillofacial Surgery*, 53(9):826-30
28. Wolinsky LE, Mania S, Nachnani S and Ling S(1996) The inhibiting effect of aqueous *Azadirachta indica* (Neem) extract upon bacterial properties influencing *in vitro* plaque formation. *Journal of Dental Research*, 75: 816-22.
29. Gabka J and Matsumura T (1971). Measuring techniques and clinical testing of an anti-inflammatory agent (tantum). *Munch Med Wochenschr*, 113: 198-203
30. Eshghpour M and Nejat AH (2013) Dry socket following surgical removal of impacted third molar in an Iranian population: incidence and risk factors. *Nigeria Journal of Clinical Practice*, 16: 496–500
31. Hamzani Y and Chaush G (2018) Evaluation of early wound healing scales/indexes in oral surgery: A literature review. *Clinical Implant and Dental Related Research*, 20:1-6
32. HuskissonEC (1974) Measurement of pain. *Lancet*, 304: 1127-1131.
33. Botelho MA (2008) Efficacy of a mouth rinse based on leaves of the neem plant (*Azadirachta indica*) in the treatment of patients with chronic gingivitis: a double-blind, randomised controlled trial. *Journal of Medical Plants Research*, 2: 341-46.
34. Osunde OD, Anyanechi CE and Bassey GO (2017) Prevention of alveolar osteitis after third molar surgery: Comparative study of the effect of warm saline and chlorhexidine mouth rinses. *Nigeria Journal of Clinical Practice*, 20: 470–473.
35. Madhu M, Sailaja V, Satyadev S, and Satyanarayana MV (2016). Quantitative phytochemical analysis of selected medicinal plant species by using various organic Solvents. *Journal of Pharmacology and Photochemistry*, 5(2): 25-29.
36. Khanal S (2021) Qualitative and Quantitative Phytochemical Screening of *Azadirachta indica* Juss. Plant Parts. *International Journal of Applied Science and Biotechnology*, 9(2): 122-127.
37. White RP, Jr., Shugars DA, Shafer DM, Laskin DM, Buckley MJ and Phillips C (2003). Recovery after third molar surgery: clinical and health-related quality of life outcomes. *Journal of Oral and Maxillofacial Surgery*, 61**:**535-44.
38. Obimakinde OS, Fasola AO, Arotiba JT, Okoje VN and Obiechina AE (2010) Comparative effect of tube drain on post-operative inflammatory complications of impacted mandibular third molar surgery College Hospital, Ibadan, Nigeria. *Nigerian Postgraduate Medical Journal*, 17:194-199.
39. Aimuamwosa OD, Edetanlen EB, and Obuekwe ON (2020) Transdermal patch and oral routes of administration of diclofenac for the control of postoperative sequelae after third molar surgery: a single-blinded, randomised clinical trial. *Journal Dental and Oral Disorders,* 6(4): 1-6.
40. Pai MR., Acharya LD and Udupa N (2004). Evaluation of antiplaque activity of Azadirachta indica leaf extract gel - a 6-week clinical study. *Journal of Ethnopharmacology*, 90 (1) :99-103**.**
41. Kankariya AR, Patel AR and Kunte SS (2016) The effect of different concentrations of water-soluble azadirachtin (neem metabolite) on Streptococcus mutans compared with chlorhexidine. *Journal of Indian Society of Pedodontics and Preventive Dentistry*, 34(2): 105.
42. Dandekar NV and Winnier JJ (2021) Assessment and evaluation of the effect of neem and mango mouth rinse on *S. mutans* count *in vitro* and in children. *Journal of Herbal Medicine,* 29: 100469. doi:10.1016/j.hermed.2021.100469
43. Delilbasi C, Saracoglu U and Keskin A (2002) Effects of 0.2% chlorhexidine gluconate and amoxicillin plus clavulanic acid on the prevention of alveolar osteitis following mandibular third molar extractions. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontic*, 94: 301-304.
44. Barua CC(2010) Evaluation of the wound healing

activity of methanolic extract of *Azadirachta indica* (Neem) and *Tinospora cordifolia* (Guduchi) in rats, Pharmacologyonline, 1: 70–77.

45. Osunwoke EA, Emamoke OJ, Theodore AA and Julius OC (2013) The wound healing effects of aqueous leave extracts of *Azadirachta indica* on Wistar rats. *Journal of Natural Science Research*, 3(6):181-186

46. Sharma PT, omar L, Bachwani M and Bansal V (2011) Review on Neem (*Azadirechta indica*): Thousand Problem One Solution. *International Research and Journal of Pharmacology*, 2: 97-100