Antimicrobial effect of Tropolone containing versus Tropolone free mouthwash - Randomized clinical trail

التأثير المضاد للميكروبات لغسول الفم المحتوي على تروبولون مقابل التي لا تحتوي على تروبولون : دراسة سريرية عشوائية

Protocol

Submitted to the Faculty of Oral and Dental Medicine, Cairo University for partial fulfillment of the Requirements of Master Degree in Conservative Dentistry

By

Mohamed Awad Mohamed Abdelkadir

B.D.S (Misr University for science & technology) 2011

Under supervision of

Prof. Dr. Mohamed Adel Ezzat

Prof. of Conservative Dentistry, Cairo University

Associated prof. Dr. Eman Ali Abouauf

Conservative Dentistry Department, Cairo University

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Administrative information:

1. **Trial registration:**

   www.clinicaltrials.gov (NCT03384537)

2. **Protocol version:**

   August - 2017, First version. (will be finalized after registration)

3. **Funding:**

   The trial will be Self-funding by the main researcher.

4. **Roles and responsibilities:**

4.1. Mohammed Awad Mohamed Abdulkadir

- ✓ Role: main researcher, assessor data enterer and writing the research.
- ✓ Affiliation: Master degree candidate in Conservative Dentistry Department, Faculty of dentistry, Cairo University Egypt.

4.2. Prof. Dr. Mohamed Adel Ezzat

- ✓ Role: Main supervisor, assessor, data monitoring, auditing.
- ✓ Affiliation: Professor Conservative Dentistry Department, Faculty of dentistry, Cairo University, Egypt.

4.3. Associated prof. Dr. Eman Ali Abuauf

- ✓ Role: Co-supervisor, assessor, data entry and auditing.
- ✓ Affiliation: Conservative Dentistry Department, Faculty of dentistry, Cairo University, Egypt.

4.4. Eman Desouky

- ✓ Role: Sample size calculation.
- ✓ AFFILIATION; Statistician, Faculty of dentistry, Cairo University, Egypt.

4.5. Research Ethics Committee (CREC)

- ✓ Role: Protocol reviewer of the clinical trial in order to protect the right, safety, dignity and well-being of the participants.
- ✓ Affiliation: Faculty of dentistry, Cairo University, Egypt.
- ✓ Approval No: 18 2 12

Scientific Background:

Removal of dental bio-film is important as it may become acidic causing demineralization of the teeth (dental caries) or harden into dental calculus (tartar).

Calculus cannot be removed through mouthwash, tooth-brushing or with inter-dental aids and can only be removed through professional cleaning.

Therefore, removal of the dental bio-film will prevent the development of dental caries and gum diseases. **(Haider et al, (2013).**

Statement of the problem:

Oral health has great impact on for prevention of oral diseases. Therefore, people have used different methods to promote their oral health, among which mechanical techniques such as flossing and brushing, are the most well-known ones.

However, these methods are not able to eliminate all disease causing factors alone, especially in interproximal areas.

On the other hand, many people with specific physical and psychological conditions are required to use an antiseptic in order to do away with many deficiencies of eliminating the mechanical plaque.

Thus, using chemical materials (such as mouth rinses) are taken more into account.

As an aid along with mechanical plaque control, mouth rinses play a pivotal role in supra-gingival plaque control and gingivitis control.

An appropriate mouthwash, in addition to antimicrobial spectrum, should have low pharmaceutical interaction and cause less damage to the normal micro-flora of oral cavity. **E Marchetti et al., 2017**

Rationale:

Dental Caries disease prevented through the antibacterial mouthwash.

The prevalence of dental caries disease continues to be a challenge for oral health care professionals to this day.

In fact an alarming 92% of US adults (aged 20 to 64 years) have a history of dental caries.

While strides have been made since the early 1970s the problem persists.

From the mid1990s until 2004 according to the National Health and Nutrition examination survey there was a small but significant increase in primary decay.

This trend was even more severe in younger.

Dental Caries disease are not being treated can eventually turn into more serious issues.

Fluoride has long been known as one of the key components to good oral health and the prevention of dental caries disease.

It is a naturally occurring mineral that makes tooth enamel more resistant to acid producing bacteria that cause dental caries disease while also repairing teeth in the very early microscopic stages.

While the use of fluoride in toothpaste has been responsible for a drop in dental caries disease since 1960 this significant problem still persists.

Yet, many people do not realize that also using a mouth rinse can result in 50% stronger teeth than brushing with fluoride toothpaste alone.

The American Dental Association (ADA) recommends the use of mouth rinses with fluoride to help resist tooth decay.

However, while it may appear so on the surface, not all fluoride mouth rinses are created the same.

At the Listerine brand, our commitment to innovation led to the creation of unique anti-cavity mouth rinses powered by breakthrough science called rapid fusion technology a unique fluoride delivery system which binds calcium ions with fluoride to create fluoride reservoirs that attach to tooth enamel and are then released over time for a greater fluoride uptake and greater enamel content of fluoride to the tooth surface.

Rapid fusion technology provides fluoride in a safe acidic environment that rapidly liberates calcium and phosphate ions.

These ions combine to create millions of tiny fluoride reservoirs on tooth enamel and in saliva increasing the amount of fluoride on the tooth surface.

Throughout the day as enamel is exposed to acids from dietary sugars, the reservoirs dissolve and release fluoride to re-mineralize the teeth.

The fluoride then binds to areas of weak demineralized enamel and attracts calcium and phosphate from saliva.

These ions penetrate the enamel and combine with fluoride to create a new stronger and more acid resistant mineral surface**.**

In this way Rapid fusion technology enhances re-mineralization and inhibits demineralization of tooth enamel to provide.

Stronger teeth than brushing with fluoride toothpaste alone and greater re-mineralization of enamel **May 11, 2016. Johnson & Johnson Consumer Inc.**

Benefits for patient/clinician:

- ✓ **For the Patient:** The antimicrobial mouthwash is proved to prevent the development of dental plaque.
- ✓ The use of antimicrobial agents will lead to the avoidance of side effects complained by patients associated with the use of chlorhexidine.
- ✓ And proving their potency against mutans streptococci will dramatically improve the oral health of patients as it is the major cause of dental plaque.

- ✓ **For the clinician:** Dental plaque is reported to be a major health problem in public.
- ✓ Therefore, reducing the incidence of caries disease through the use of antimicrobial agents will decrease the number of visits of patients complaining of plaque and associated health problems.
- ✓ Also, complaints of patients regarding side effects of will be of no concern to dentists.

Objectives:

The objective of this study is to compare the effect of Tropolone containing mouthwash versus CHX 0.2% mouthwash in reducing intraoral microorganism. Randomized clinical trial study

Hypotheses:

The null hypotheses tested is that there is no different between using alcohol-free essential oils containing mouth rinse and other mouth rinse regarding anti-plaque and anti-gingivitis microbial agent.

Trial design:

The study design in this investigation will be an in vivo diagnostic study.

Trial type:

This study will be a parallel study design.

Allocation ratio:

In this study, the allocation ratio will be 1:1.

Review of Literature:

Search strategy:

- ✓ **Source:** Database used in searched are pub-med, Cochrane library database and Google Scholar.

Index terms	Mesh terms
Antimicrobial dental plaque	• **Index, Dental Plaque** • **Dental Plaque Indexes** • **Indexes, Dental Plaque** • **Dental Plaque Indices** • **Indices, Dental Plaque**
Anti-Infective agents (Tropolone containing)	**Supplementary Concept**
Anti-Infective agents (Chlorhexidine)	• **Chlorhexidine Hydrochloride** • **Hydrochloride, Chlorhexidine** • **Tubulicid** • **Novalsan** • **Sebidin A** • **Chlorhexidine Acetate** • **Acetate, Chlorhexidine**
Bacterial count	• **Bacterial Loads** • **Load, Bacterial** • **Loads, Bacterial** • **Bacterial Count** • **Count, Bacterial** • **Counts, Bacterial** • **Bacterial Count**

Inclusion and exclusion criteria:

Inclusion	Exclusion
In vivo study	**Patient with systemic disease that affect study result**
Articles published in English language	**Study for ages below 18 and above 54 years**
Article published after 2010	**Intervention study other than Listerine total care mouthwash**

Antimicrobial Tropolone containing mouthwash (Total care zero)	**Study that include product other than mouthwash or rinsing**
Chlorhexidine mouthwash	**Study didn't measurement dental plaque (bacterial count)**

- ✓ **<u>Antimicrobial effect of Tropolone containing mouthwash :</u>**

E Marchetti et al., 2017 The aim of this study was to evaluate the antiplaque effects of an alcohol-free essential oil (alcohol-free EO) mouthwash and an amine fluoride compared to a positive control of chlorhexidine they found there was less of an effect compared to the CHX group, with an overall plaque index of 1.41.

The differences of 0.96 between alcohol-free EO and CHX were all statistically significant ($P < 0.001$).

Conclusion alcohol-free (EO) mouthwash has the same effect of CHX control on an inhibiting plaque regrowth.

Mogharehabed et al. 2016 . The current study was aimed to compare the effectiveness and side effects of chlorhexidine mouth rinses with and without alcohol.

Chlorhexidine mouth rinses have widely been recognized for their contribution in maintaining plaque control. Most of them contain alcohol that makes them impractical for many patients.

They found both mouthwashes significantly reduced the mean scores of plaque ($P < 0.0001$) and gingival ($P < 0.032$).

The extent of stain was the comparable in both groups. While Epimax mouth rinse caused severe stains on the teeth, Hexidine mouth rinse caused burning mouth.

Conclusion Epimax mouthwash it was less suitable and caused more dental stain. Ethanol-free Hexidine mouthwash seems to be more proper for gingivitis, but its side effects are required to be taken into consideration.

C. Vlachojannis et al . 2015. The aim of this study was to get preliminary information about the antimicrobial activities of individual Listerine components and their mixtures against Streptococcus mutans.

They found thymol was the most effective against S. mutans and phenols and their concentrations increase their general effectiveness that do not induce harm.

Conclusion based on our experiments and considering the antimicrobial effects against the resident physiological micro-flora in the oral cavity, we suggest optimizing the phenolic composition of Listerine and the concentrations of those phenols that were found to have a beneficial effect within the combination.

BR Charugundla et al 2015. The objective of this study was to compare the effectiveness of fluoride essential oil (EO) and chlorhexidine (CHX) mouth rinses on dental plaque and gingivitis and to compare their relative efficacy in patients with and without dental caries.

They found significant reduction in plaque after use of mouth rinses ($P < 0.05$) and no significant differences were observed with respect to each other in reducing gingivitis ($P > 0.05$).

Further significant differences were found in reducing plaque and gingivitis in dental caries-free subjects in comparison to those with dental caries ($P < 0.05$).

Conclusion all the three mouth rinses significantly reduced plaque accumulation and gingivitis especially in dental caries-free subjects in

comparison to those with dental caries, and amongst the three fluoride and CHX proved to be more effective than EO mouth rinse.

Christian Vlachojannis et al . 2013. The studies support the claim that ListerineW shows benefit for oral health, but the concerns over its safety remain to be clarified.

They found until these have been addressed, high risk populations (children, alcohol addicts, patients with genetic deficiencies in ethanol metabolism) should use alcohol-free mouthwashes for the maintenance of oral health.

Conclusion the mouthwash ListerineW is not a medicinal plant product.

The mixture of four essential oil ingredients in an aqueous ethanol solution showed benefit for oral health, in terms of gingivitis and dental plaque reduction.

However, the concern over its safety should be clarified.

Since the use of ListerineW is not restricted, rigorous preclinical and human long-term pharmacological safety data are warranted to exclude a possible link with cancer.

Until the safety concerns have been addressed, high risk populations (children, alcohol addicts, patients with genetic deficiencies in ethanol metabolism, oral cancer or smokers) should use alcohol-free mouthwashes for the maintenance of oral health.

HANS RAGNAR PREUS et al 2013. The aim of the present study was to test the clinical effect of Listerine on plaque formation as primary and gingivitis as secondary end point, with or without mechanical oral hygiene in a modified experimental gingivitis model.

They found when comparing the gingival condition in the proximal sites only, there were no differences between the mouth rinses.

When tooth brushing, flossing and rinsing were performed in the same quadrant the plaque scores were very low and gingival scores showed no statistically significant differences between the mouth rinses.

Conclusion clinical efficacy of Listerine total care on plaque formation and gingivitis in this modified experimental gingivitis model, with 22% hydro-alcohol and 0.2% CHX solutions as controls, no statistically significant antibacterial effect of Listerine over its placebo vehicle was found.

Neither Listerine nor alcohol had any effect of clinical value to the user, since the amount of accumulated plaque after rinsing was still enough to cause any of the dental plaque related diseases.

Sharukh S. Khajotia et al .2013. The objective of this study was to report a methodology for quantification and comparison of the concurrent three dimensional distributions of three cellular and extracellular components of biofilms.

The method consists of distinct but interconnected steps involving biofilm growth, staining, Confocal laser scanning microscopy (CLSM) imaging of biofilms, biofilm structural analysis and visualization and statistical analysis of structural parameters.

The biofilm growth assay permits biofilm growth on relevant substrates and produces biofilm structures that are reproducible.

The combination of novel simultaneous staining of exopolysaccharides (EPS) proteins and nucleic acid components with the measurement of three dimensional biofilm structural parameters results in quantifiable distributions of components within biofilms.

Statistical analysis of the biofilm structural parameters facilitates evaluation of biofilms under specific experimental conditions

Van Strydonck DAC et al 2012. The aim of study to systematically evaluate the efficacy of chlorhexidine (CHX) mouth rinses on dental plaque, gingival inflammation and staining in gingivitis patients.

They found CHX molecule result in a broad bactericidal and bacteriostatic spectrum of action and a high substantively of up to 12 hour within the oral cavity.

Because CHX binds strongly to tissues, it is poorly absorbed from the gastrointestinal tract and therefore lacks systemic toxicity.

Conclusion to demonstrate the efficacy of the different CHX mouth rinse formulations in the inhibition of dental plaque, gingivitis and stain formation, multiple comparative clinical studies have been performed.

Anthony L. Neely et al. 2012. This systematic review studies had to be randomized clinical trials in healthy human subjects comparing the effects of essential-oil mouthwash (EOMW) with chlorhexidine on dental plaque accumulation, tooth staining and gingival inflammation.

Studies could be either short-term less than four weeks duration or long-term more than four weeks duration.

Studies were required to include a specific formulation of EOMW (Listerine).

They reportedly selected this standard formula of EOMW because it was representative of essential oil based mouthwashes and because it has the American Dental Association seal of approval.

Conversely, there were no restrictions on the concentration of CHX used in studies.

They found nineteen controlled clinical trials were included in this systematic review.

In five of the seven studies of plaque index, CHX was found to be significantly better than EOMW at reducing plaque accumulation.

Stain development was assessed in five long-term brushing trials. CHX was significantly associated with more staining than EOMW in the systematic review.

The calculus index was significantly greater among CHX users versus EOMW users.

Gingivitis levels four of five studies provided statistical data that could be used in the systematic review.

Two of these investigations showed significantly lower gingival inflammation with CHX. Bleeding indices were assessed in five short-term and four long-term studies.

Only one of the short-term studies showed a significant difference, whereas three of four long-term studies showed no difference between CHX and EOMW.

Meta-analyses were included for plaque index, gingival index, and tooth staining index. In two of three meta-analyses of plaque index trials, CHX was shown to be significantly more effective than EOMW at reducing plaque.

One of the significant meta-analyses involved plaque regrowth in less than four weeks of no oral hygiene measures.

The overall weighted mean difference (WMD) for the plaque index was 0.46 (95% (CI) = 0.09, 0.84)) confidence interval.

Other meta-analysis was a comparison of more than four weeks in which either daily EOMW or CHX supplemented normal oral hygiene measures.

This long-term study of plaque accumulation showed significantly less plaque with CHX than with EOMW.

The WMD for the long-term plaque control studies was smaller than for the short-term studies (0.19; 95% CI = 0.08, 0.30).

No significant differences were found in meta-analyses for gingival inflammation (gingival index17) or stain accumulation (stain index12).

Significant heterogeneity was identified in one of two of the meta-analyses for both gingival index and stain index.

Conclusion CHX was significantly better at reducing plaque accumulation than EOMW in short-and long-term studies.

Staining and calculus accumulation were greater among CHX users compared to EOMW.

CHX and EOMW were not different with respect to long-term control of gingival inflammation.

They concluded that EOMW might be a reliable alternative to CHX for controlling gingival inflammation in cases where a dental professional deems that anti-inflammatory oral care is beneficial.

Enrico Marchetti et al. 2011. The study was held to evaluate the antiplaque effect of a new alcohol free essential oil (zero alcohol) mouthwash with respect to a control of an essential oil with alcohol mouthwash.

They found the amounts of mouthwashes used indicated good compliance with the instructions.

No adverse events or side effects were reported or observed.

With regard to the subjects rating of the rinsing time the results demonstrated a difference between the test and control groups (6.50 and 4.23 respectively, $p < 0.005$).

This suggest than the rinsing time of EO with alcohol seemed longer than EO without alcohol, probably due to the typical burning effect of EO with alcohol.

They also considered EO with alcohol (visual analogue scale, VAS 6.07) as more effective in reducing plaque in the mouth compared to the EO without alcohol (visual analogue scale, VAS 4.97; $p < 0.05$).

However, duration of taste, alteration in taste perception and convenience, the statistically significant differences were not noted between the groups.

Conclusion Epidemiological studies however are often inconsistent and many reviews conclude there are no data demonstrating the direct correlation between alcohol containing mouthwashes and oral cancer.

Recently, Werner and Seymour have reviewed the two most recent revisions on the role of alcohol in the onset of oral cancer stating that there is evidence showing the existence of this association, but these are still weak and inconclusive and randomized clinical trials would be needed on a large sample to verify this hypothesis.

These authors concluded that the benefit of alcohol in mouthwashes is negligible and it may carry a risk of oral cancer which is difficult to quantify and so it is preferable not to prescribe or recommend them.

This three day plaque regrowth study showed that the EO containing mouthwash without alcohol was a less potent plaque inhibitor than the traditional alcohol containing EO mouthwash.

It appears that the subjects appreciated the effect on plaque reduction of the traditional mouthwash better.

Aim of study:

The study will be conducted to evaluate the antimicrobial effect of recent rapid fusion technology mouthwash in reducing intra-oral cariogenic microorganism.

PICOTS:

P: intra-oral cariogenic microorganism.

I: (Recent rapid fusion technology mouthwash) Tropolone containing mouthwash.

C: Chlorhexidine Mouthwash (0.2%).

O: bacterial count.

Type Outcome	Outcome name	Measuring device	Measuring unit
Primary	Viability counts	Counts	Colony-forming unit per milliliter (CFU/ml)

T= T_0 = base line

T_1= immediate after using mouthwash.

T_2= one week after regular use of mouthwash.

T_3 = two weeks after regular use of mouthwash.

S= Randomized Clinical Trial

Research question:

Will a Tropolone containing mouthwash decrease the cariogenic microorganism than Chlorhexidine mouthwash (0.2%)?

1. **Material:**

Steps:

1.1. Tropolone containing mouthwash (Listerine total care zero).

1.2. Chlorhexidine mouthwash as a positive control (Hexitol).

2. **Methods**

2.1. Study setting

The study will be conducted in outpatient clinic of the Conservative department of the Faculty of Dentistry, Cairo University. The bacterial count test will be conducted in the Microbiology department, Faculty of Medicine, Cairo University Egypt.

The researcher will bear ultimate responsibility for all activities associated with the conduct of a research project including recruitment of patients, explaining and performing the procedures to them.

2.2 Eligibility criteria

➢ Inclusion Criteria:

- ❖ Patients should be between 18- 45 years of age.
- ❖ All the volunteers participated in this study will be healthy looking with free medical history.
- ❖ The volunteers will be asked to suspend their usual oral hygiene practice from two to four days before experiment studying.

➢ Exclusion criteria:

- ✓ Patients with a compromised medical condition.
- ✓ Volunteers that receive any antimicrobial agent during at least two weeks prior to study.
- ✓ Volunteer with fixed, removable prosthesis or orthodontics appliance.
- ✓ Volunteers with DMF above will be excluded.

2.3. Variables of the study:

A total of 30 Volunteers patients will be assigned in this study.

Thirty participants in this study will be randomly divided into two groups (n= 15) according to the application of the mouth rinse agent (M); the first group (M1) will use Tropolone containing mouthwash as a mouth rinse, the second group (M2) will use chlorhexidine 0.2% as a mouth rinse. Then each patient will be monitoring at the base line T0: before using any mouthwash, T1: immedially after using tested mouthwash, T2: after One week and finally, T3: after Two weeks of using the tested mouthwash.

Each patient will be the reference/counted for self as a record.

- **Table 1: Variables of the study**

Variable	Symbol	Refer to
Mouth rinsing agent	**M_1**	Tropolone containing mouthwash
	M_2	Chlorhexidine mouthwash
Time relation to rinsing	**T_0**	Base line
	T_1	Immediate time
	T_2	After one week
	T_3	After two weeks

Table 2 : Interaction of Variables of the study

Rinsing agent / **Time**	**M_1**	**M_2**	**Total**
T_0	**$M_1 T_0$**	**$M_2 T_0$**	**15**
T_1	**$M_1 T_1$**	**$M_2 T_1$**	**15**
T_2	**$M_1 T_2$**	**$M_2 T_2$**	**15**
T_3	**$M_1 T_3$**	**$M_2 T_3$**	**15**
Total	**30**	**30**	**60**
N=15 **(N represent a number of subjects in each groups)**			

2.4. Intervention/control assessment:

The participants in this study will be randomly divided into two groups according to the application of the mouth rinse agent (M); the first group M1) will use Tropolone containing as a mouth rinse, the second group (M2) will use chlorhexidine 0.2% as a mouth rinse.

Then each patient will be monitoring at the base line T0: before using any mouthwash, T1: immedially after using tested mouthwash, T2: after one week and finally, T3: after two weeks of using the tested mouthwash.

Each patient will be the reference/counted for self as a record.

Examination:

Patients will be selected and examined according to inclusion and exclusion criteria.

Criteria for discontinuing or modifying allocated interventions for a Participant:

At the given percentage of antimicrobial Tropolone containing mouthwash no complications should be anticipated.

Strategies that will be used to improve adherence to interventions protocol:

The antimicrobial Tropolone containing mouthwash will be kept sealed until use.

2.5. Outcome assessment:

Procedure of viability count measurement

Effects of antimicrobial Tropolone containing mouthwash versus Chlorhexidine mouthwash 0.2% on viability counts of Mutans Streptococci and lactobacilli:

Each volunteer will be given a piece of Arabic gum and asked to chew it for one minute only, then stimulated saliva was collected in sterilized screw capped bottles.

After one minute, each volunteer will be asked to rinse with 10 ml of test agent for one minute then expectorate.

Stimulated saliva will be recollected in the following points: after 30 minutes of rinsing, one hour, and, two hours.

During this time, the volunteers will be asked not to eat or drink anything except water.

Salivary samples will be dispersed for two minutes by vortex mixer, then 0.1 ml of saliva transferred to 0.9 ml of sterile phosphate buffer saline (pH 7.0), and tenfold dilutions will be performed.

From the dilution 10^{-3}, 0.1 ml will be taken and spread in duplicate on Mitis Salivarius Bacitracin agar plates, these plates will be incubated anaerobically for 48 hour at 37 ºC then aerobically for 24 hour at room temperature.

The number of colonies will be expressed as colony forming units multiplied by the dilution factor per milliliter of saliva (CFU/ml) and compared before and after rinsing.

2.6. Clinical relevance of the outcome:

Reduction in the number of the streptococcus mutans and lactobacilli after application of the intervention which is the main cause of dental

caries will mean that this material is an adjuvant to other mouthwash rinses and could be used in the clinical practice as its easily use with not reported side effects.

2.7. Sample Size calculation:

The aim of this study is to compare the antimicrobial effects of Tropolone containing mouthwash versus antimicrobial Chlorhexidine mouthwash 0.2% (Hexitol) on oral streptococcus mutans and lactobacilli with repeated measures bacterial count test of variance will be performed to study the effect of different solutions at different times.

Based on previous papers by Weli & Mohammed 2013 and Rashad 2008 a large effect size is expected (f=0.4).

A minimum total sample size of 30 patients (15 in each group) will be sufficient with the power of 85% and a significance level of 5%.

The sample size was calculated using G*Power program (University of Dusseldorf, Dusseldorf , Germany).

2.8. Recruitment:

Patient will be recruited from the outpatient clinic of the Conservative department of the Faculty of Dentistry, Cairo University Egypt.

After explaining the benefits from the application of the interventions to their oral hygiene which is alcohol free .

Unlike other mouth rinses that has complications such as staining, burning sensation and dry mouth from which eligible patients will be recruited to fulfill the eligibility criteria.

2.9. Randomization:

Sequence generation: Randomization will be computer generated using (www.randomization.com).

Allocation concealment: Allocation of rinsing agents to groups will be done through sealed black opaque envelopes to ensure complete concealment.

Implementation:

A third party will perform the allocation sequence and assign the participants to rinsing agent in sequentially numbered opaque envelop.

Blinding:

The allocation group will be blinded from those assessing the outcome, data collectors, and data analysts.

The microbiologist will be blinded by labeling the specimens with non-identifying terms.

2.10. Data collection methods:

Baseline data collection:

The main researcher will collect the baseline data through a chart which is composed of medical, dental history and clinical examination for every patient will be filled.

The report will be anonymous where patients identified by their serial numbers (the first letter of the first and last name and date of birth) only will be registered.

The main researcher will write the full detailed personal data of the patient in a separate sheet having the patient's serial number for further contact with patient.

This sheet can only be seen by main researcher and co-supervisor.

Outcome data collection:

A Microbiological technician will assess the viability counts of Mutans Streptococci and lactobacilli in patient's saliva at baseline, immediate time, after one week and after two weeks of rinsing.

Patient retention:

The main researcher should understand the participant the final result will be expected and how it will differ in his/her oral hygiene.

The main researcher will told the participant that the whole procedure will take a short time and it will be painless.

2.11. Data management:

Data entry will be carried out by the main researcher and revised by co-supervisor.

All data will be stored on computer and will be encrypted using a password.

This will be done to allow accurate data entry through revision and protect data from being incorrectly used.

Data will be packed up on another storage device to prevent it from being lost.

2.12. Statistical methods:

Data will be analyzed using IBM SPSS advanced statistics (Statistical Package for Social Sciences, version 21 (SPSS Inc. Chicago.IL).

Numerical data will be described as mean and standard deviation or median and range, while qualitative data will be described as number and percentage.

Two ways with repeated measures bacterial count test of variance (ANOVA) will be performed. A p-value less than or equal to 0.05 will be considered statistically significant. All tests will be two tailed.

2.13. Monitoring:

A. Data monitoring:

The main supervisor will monitor this study. His role is to monitor any risk of bias could be done from participants, operator or assessors, blinding of the assessors and patient safety, outstanding benefits or harms.

B. Harms:

The main researcher should inform participants about the possible harms, if present.

Participants are allowed to contact the operator at moment through telephone. The data will be reported to main researcher.

C. Auditing:

In the present trial, auditing will be done by the main and co-supervisors to assure quality of the research frequency procedures.

2.14. Ethics and dissemination

a. Research ethics approval:

Application forms for carrying out the clinical trial, checklist and informed consent of Research Ethics Committee (CREC) Faculty of Oral and Dental Medicine, Cairo University will be retrieved and filled, then will be delivered for (CREC) committee for approval, this is done to prevent any ethical problems during the study or any harm for any of the participants.

Research ethics approval: 18 2 12

b. Protocol amendments:

If a new protocol will be used a protocol amendment will be submitted; containing a new copy of the new protocol and brief explanation about the differences between it and the previous protocols.

If there is a change in the existing protocol that affects safety of subject investigation scope or scientific quality of the trial, an amendment containing a brief explanation about the change will be submitted.

If a new author will be added to accomplish the study, an amendment including the investigator's data and qualifications to conduct the investigation will be submitted to prevent ghost authorship.

c. Consent or assent:

The operator (Mohamed Awad Abdulkadir) is responsible for admitting and signing the written consents during the enrolment day.(Fig.1).

d. Confidentiality:

Name, personal data and pictures of the participants will not appear on the protocol form and will be maintained secured for ten years after the trial. This is done for protection of participant's privacy and civil rights.

e. Declaration of interests:

There is no conflict of interest, no funding or material supplying from any parties.

f. Access to data:

Access to final data will be allowed to the operator, the main and co-supervisors of the study who are involved in assessment of the outcome.

g. Ancillary and post-trial care:

Patients will not be followed up after intervention as there is no harm from our material we used.

h. Dissemination policy:

Full protocol will be published online in www.clinicaltrials.gov to avoid repetition and to keep the integrity of the research work. Thesis will be discussed in front of judgment committee.

The study will be published to report the results of this clinical trial.