

Antimicrobial Effect of German Chamomile Extract as Root Canal Irrigant (*in vitro* Study)

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Abstract:-

Aims: To evaluate the antimicrobial effect of German chamomile extract as a root canal irrigant *in vitro*, and comparing its effect with chlorhexidine 0.2% irrigant solution.

Materials and Methods: Twenty adult patients with age range 18–40 years attending dental school with symptomatic uniradicular teeth with necrotic pulp diagnosed by radiograph with no fistula or sinus tract were chosen; 10 subjects for each irrigant solution (the test and the control groups). Microbiological samples were obtained from the root canal at the beginning of the first appointment, and then moistened paper point is placed in a screw capped vial containing 5 ml Thioglycolate broth for anaerobic bacteria and the other containing Brain Heart Infusion broth for aerobic bacteria, which is incubated directly for 18 hours at 37 °C. Turbidity test was used to compare antimicrobial effect of German chamomile extract with chlorhexidine 0.2%.

Results: Mean reduction in bacterial count (0.36 ± 0.09 for aerobic and 0.19 ± 0.02 for anaerobic) for German chamomile and chlorhexidine 0.2% (0.084 ± 0.03 and 0.052 ± 0.016 respectively) were determined by using one sample t-test, which was much lower for German chamomile than for chlorhexidine 0.2% with significant difference. For comparison between two materials for aerobic and anaerobic, unpaired t-test was used. Results were considered significant at $p \leq 0.05$.

Conclusion: German chamomile extract is effective antimicrobial irrigant solution especially on anaerobic bacteria but much lower than chlorhexidine 0.2% irrigant solution.

تأثير مضاد الجراثيم للمستخلص المائي لنبات البابونج عند استعماله كغاسل لقناة الجذر

المستخلص:-

استهدفت الدراسة الحالية إجراء التقييم المختبري للتأثير المضاد للجراثيم للمستخلص المائي لنبات البابونج عند استعماله كغاسل لقناة الجذر، والقيام بمقارنته مع غسول الكلور هيكسيدين 0.2% المتداول حالياً. تم في الدراسة اختيار 20 مريضاً بالغاً تتراوح أعمارهم بين 18–40 سنة مقسمين إلى مجموعتين: المجموعة الأولى تستخدم المستخلص المائي لنبات البابونج، والثانية كمجموعة ضابطة تستخدم غسول الكلور هيكسيدين 0.2%. تم الحصول على عينات جرثومية من قناة الجذر في بداية الزيارة الأولى وفي بداية ونهاية الزيارة الثانية، وفي بداية الزيارة الثالثة باستخدام الأقماغ الورقية الرطبة المعقمة. وضعت إحدى الأقماغ الورقية في قنينة محكمة تحتوي على 5 مللتر من سائل الثايوكلايكوليت وتم وضع قمع ورقي آخر في قنينة أخرى تحتوي على 5 مللتر من سائل نقيع الدماغ والقلب كسانلين ناقلين، وتم فرشهما على وسطين زرعيين من أغار الدم الأساسي بدرجة 37 °م لمدة 18 ساعة، وتم عد الجراثيم النامية على الأوساط الجرثومية. أوضحت النتائج أن مستخلص نبات البابونج قابلية فعالة لقتل الجراثيم عندما يستخدم "مختبرياً" كغاسل لقناة الجذر لكن بدرجة أقل عن غسول الكلور هيكسيدين 0.2% وبفرق معنوي أقل ضمن 0.05. وكان تأثير مستخلص نبات البابونج فعال أكثر على البكتريا اللاهوائية من البكتريا الهوائية وبفرق معنوي أقل من 0.05 أي أن غسول الكلور هيكسيدين 0.2% كان فعالاً بفرق معنوي على البكتريا الهوائية واللاهوائية بدرجة معنوية 0.001 أكثر من مستخلص نبات البابونج؛ فتبين أن مستخلص البابونج يمكن استعماله كغاسل لقناة الجذر لكن بدرجة أقل من الكلور هيكسيدين 0.2%.

Key Words: German chamomile, chlorhexidine, aerobic, anaerobic, irrigant solution.

Introduction:-

Irrigating solutions used in endodontic treatment aid in cleaning the pulp chamber,⁽¹⁾ root canal, lubricate the files, flush out debris, having an antimicrobial effect and tissue dissolution properties.⁽²⁾

The ideal irrigant should have a strong antimicrobial action, dissolve the organic tissue but not toxic to the peri apical tissue if extruded through the apex.^(3,4)

Antimicrobial agent must suppress or destroy microbial growth, thus susceptibility of the microorganisms, penetration of the antimicrobial agent to the infected site, adequate concentration of the agent, low toxicity of the host cells and lack of microbial resistance to the agent are necessary.^(5,6)

Chlorhexidine gluconate (CHX) has a wide spectrum of antimicrobial activity and it is relatively non-toxic. However, it has no capacity to dissolve the vital tissues.⁽⁷⁾

Recently, there has been an increased interest in antimicrobial agents from medicinal plants which have been used in folk medicine.⁽⁸⁾ German chamomile (GC) is one of the oldest favorites amongst garden herbs and its reputation as a medicinal plant shows little signs of abatement. It is especially suited to teething children and those who have been in a highly emotional state over a long period of time.⁽⁹⁾ The herb kills certain bacteria and can be used as mouth wash for dental abscesses and tonsillitis; it is excellent in treating any type of inflammation whether internal or external.⁽¹⁰⁾

Thus, the objectives of this study were to evaluate antimicrobial activity of GC extract as endodontic irrigant solution since no animal or human studies verify its anti-infective activity.

Materials and methods:-

Patient Selection: A sample of twenty patients of both sexes was selected from those attending the dental school of Mosul. Their ages range from 18-40 years, and have no history of systemic diseases. Twenty asymptomatic uniradicular teeth with necrotic pulps were chosen. Pulp necrosis was determined by radiographic presence of apical rarefaction and lack of response to pulp vitality test by using electric pulp tester. There should be no fistula or

sinus tract at the time of sampling and the patients who were included in this study had not been treated by systemic antibiotic in the last three months.⁽¹¹⁾

Sample Collection: Microbiological samples were obtained from the root canals at the beginning of the first appointment. Following rubber dam isolation, the tooth, its surrounding and the clamp were disinfected with 70% ethanol for one minute. An access opening was made with a sterile round bur no. 2 at high speed. Canal length was determined by placing # 10 or # 15 K type files inside the canal. A radiograph was taken and the file length was adjusted within 1 mm of the radiographic apex. After that, a moistened paper point was placed in the canal (within the working length of the canal) and remained inside the canal for 1 minute and then removed and placed in a screw-capped vial containing 5 ml Thioglycolate broth (Appendix I). Another paper point was placed in the canal for 1 minute and then removed and placed in another vial containing Brain Heart Infusion broth (Appendix II). The samples were incubated directly in an incubator for 18 hours at 37 °C.⁽¹²⁾

Preparation of Irrigating Solutions: The CHX gluconate at concentration of 0.2%.⁽¹³⁻¹⁵⁾ A liquid extract of concentrated liquid preparations usually made by soaking chopped or mashed plant parts by steam distillation of 250 ml water liquid, and then the solid particles are filtered out. After separation of active ingredients such as apignine I + azulene III. A common dose of chamomile extract is 1 ml to 4 ml in water is used.^(16, 17) **Sterilization:** The culture media were sterilized by using an autoclave at 15 pound/inch² at 121 °C for 15 minutes, while petridishes and screw capped vials were sterilized by hot air oven at 180 °C for 1 hour.⁽¹⁸⁾

Microbiological Study: The microbiological study was carried out in the Microbiology Laboratory, Department of Dental Basic Sciences, College of Dentistry, University of Mosul. Turbidity method was used to determine the antimicrobial effect of GC extract and CHX gluconate 0.2%.

The method depends on measuring the light lost from the beam by scattering. Light absorption is assumed to be absent when transmitted through a tube of clear solution and it loses its clarity and becomes turbid as microbes grow in it. The measurement of light absorption was performed by using spectrophotometer at 590 nm.^(19, 20) This method was achieved using a series of screw capped vials containing equal amounts (4 ml) of sterilized Thioglycolate broth to support the growth of anaerobic microorganisms. Those have been divided according to examined solutions 0.2% CHX gluconate and 0.25 GC extract. So, as for each irrigating solution one vial contained 4 ml broth and 0.1 ml of examined solution and other three vials contained 4 ml broth and 0.1 ml of irrigating solution and 0.1 ml of 18 hours bacterial growth suspensions by using micropipette. Other three vials contain only 0.1 ml of bacterial suspension and 4 ml broth. Then those vials were incubated at 37 °C for 24 hours. The absorbance of each vial was measured using a spectrophotometer at 590 nm.⁽²¹⁻²³⁾ In order to determine the antibacterial effect of each examined solution, the turbidity of the examined solution itself must be excluded.⁽²⁴⁾

Data Analysis:

Standard statistical methods were used: Mean and standard error, one sample t-test was used to find the effect of the materials (CHX and GC) on the bacteria (aerobic and anaerobic).

The comparison between the two materials and between aerobic and anaerobic bacteria was analyzed using un-paired t-test. Results were considered significant at $p \leq 0.05$.

Results:-

The CHX 0.2% cause significant reduction on aerobic and anaerobic bacteria by using one sample t-test at $p \leq 0.001$ as it is shown in Table (1). While for GC extract there was significant reduction on aerobic bacteria at $p = 0.001$, but for anaerobic bacteria at $p \leq 0.001$ as it is shown in Table (2).

A comparison between CHX 0.2% and GC shows that CHX 0.2% was more effective in decreasing bacteria than GC at $p = 0.001$.

For the effect of CHX 0.2% (B + M) on aerobic bacteria shows better effect (0.084) than for GC

(0.36) at $p = 0.001$. for anaerobic also CHX 0.2% had better effect (0.05) than for GC (0.19) as it is shown in Table (3).

For the comparison of CHX 0.2% effect between aerobic and anaerobic bacteria, Table (3) shows no significant difference, while for GC extract Table (3) shows significant difference between aerobic and anaerobic bacteria. The difference was more significant for anaerobic bacteria than for aerobic with significant difference at $p \leq 0.05$.

Discussion:-

An infusion of the flowers is taken internally as an anodyne, anti-inflammatory, antiseptic, antispasmodic.^(2, 25) Evidence from laboratory studies shows that chamomile has antibacterial and antiviral properties. However, no animal or human studies verify its anti-infective activity; i.e., the potential antimicrobial effects as a root canal irrigant is not yet currently known. For this reason this study was carried out.

In vitro, the antimicrobial activity of GC extract as a root canal irrigant was evaluated using broth microdilution method. This method gives more better results in comparison with disk diffusion method, which has a low credibility for samples of plants that are difficult to diffuse in the media and also there is no relationship between diffusion power and antimicrobial activity and, therefore, may not express its full effective potential.⁽²⁶⁾

Accordingly, dilution method is more suitable than the agar diffusion method to determine the susceptibility of microorganisms.⁽²⁷⁾ Results revealed that CHX 0.2% cause significant reduction on aerobic and anaerobic bacteria, with no significant difference, which is much greater than GC extract. The antibacterial activity of CHX *in vitro* is not outstanding but the spectrum of activity is broad. Gram positive bacteria are more susceptible than are Gram negative bacteria; *Streptococcus mutans* seems to be particularly sensitive.⁽²⁸⁾ Whereas *Streptococcus sanguis*, for example, exhibits a great variation in susceptibility between and among various strains. Its antimicrobial activity is of membrane active type which damages the inner cytoplasmic membrane. Bacterial cell wall is

characteristically negatively charged.⁽²⁹⁾

While for GC extract, the antimicrobial effects are primarily the result of an active components α -bisabolol, azulenes which have anti-inflammatory activity.⁽¹⁷⁾ This activity has been demonstrated, not only by long empirical use, but by a number of different laboratory model as well.⁽³⁰⁾ But the total anti-inflammatory effect of whole chamomile depend upon the presence of flavonoids such as apigine and luteoline.⁽³¹⁾ Several of flavonoids are believed to help strengthen capillaries and other connective tissue.⁽³²⁾

The effect of GC extract was found to be greater on anaerobic microorganisms than aerobic microorganisms. The supposed resistance of the aerobic microorganisms may be related to several factors, such as: Cell wall structure, metabolic product secreted and resistance to irrigants. The true nature of such factors need further clarification.⁽³³⁾

Various methodologies can be used to assess the antimicrobial activity of endodontic irrigants. Indeed, the methodology could be a possible explanation for the differences in the results between different studies. Some methodologies allowed direct contact of the substances with the microorganisms, whereas other organisms located inside the dentinal tubules did not necessarily have direct contact with the antimicrobial agent.⁽³⁴⁾

In this study, the effect of CHX 0.2% on aerobic bacteria (0.084 ± 0.03) has lower antimicrobial effect than the study of Al-Bazaz⁽³⁵⁾ (0.07 ± 0.05), while for anaerobic bacteria it was 0.052 ± 0.016 , but of Al-Bazaz it was 0.06 ± 0.05 .

When comparing CHX with different concentrations of aqueous extract of *Nigella sativa*,⁽³⁵⁾ the differences in the findings of different studies in which the same methods were used are explained by the fact that the result depends on the microbial strains, type and concentration of irrigating solution,⁽³⁴⁾ but it was in contrast with that of Al-Shaekh Ali, who reported equal antibacterial effect on aerobic and anaerobic bacteria respectively (98.94%, 98.62%).⁽¹³⁾

The irrigant of choice should be one that exerts its antimicrobial activity quickly against

the majority of microorganisms found in the root canal and dentinal tubules.

Conclusion:-

- 1) The GC extract is natural product, cheap, safe and well-tolerated, showed an effective antimicrobial action *in vitro* when it is used as an irrigant during biomechanical instrumentation of root canal.
- 2) The GC antimicrobial effect is much lower than that of CHX 0.2% irrigating solution.
- 3) The GC exhibit more effect on anaerobic bacteria than for aerobic with significant difference.

Suggestions:-

- 1) More researches are needed for all of the potential oral uses of chamomile.
- 2) An *in vivo* study is suggested to compare the antibacterial activity of GC extracts with CHX 0.2%.
- 3) A study to assess the use of GC extract as a medicament between root canal appointments.

Appendix (I): Thioglycolate broth (oxid)

Formula	g/l
Beef Extract	1.0
Yeast Extract	2.0
Balanced Peptone No.1	5.0
Methylene Blue	0.002
Sodium Thioglycolate	1.1
Agar No.1	1.0
pH 7.1-0.2	

Appendix (II): Brain Heart Infusion broth (oxid)

Formula	g/l
Calf Brain, Infusion Form	200
Beef Heart, Infusion Form	250
Proteose Peptone	10
Dextrose	2
Sodium Chloride	5
Disodium Phosphate	2.5

Table (1): Mean and standard error for chlorhexidine 0.2% reduction on aerobic and anaerobic bacteria

Bacteria	B + CHX	B	t-value	p-value
Aerobic	0.084 ± 0.03	0.90	54.4	<0.001
Anaerobic	0.052 ± 0.016	0.68	78.0	<0.001

B: Bacteria, CHX: Chlorhexidine.

Table (2): Mean and standard error for German chamomile extract on aerobic and anaerobic bacteria

Bacteria	B + GC	B	t-value	p-value
Aerobic	0.36 ± 0.09	0.90	11.34	0.001
Anaerobic	0.19 ± 0.02	0.68	53.5	<0.001

B: Bacteria, GC: German chamomile.

Table (3): Comparison between B + chlorhexidine and B + German chamomile on aerobic and anaerobic bacteria

Bacteria	B + CHX	B + GC	p-value
Aerobic	0.084 ± 0.03	0.36 ± 0.09	0.001
Anaerobic	0.052 ± 0.016	0.19 ± 0.02*	<0.001

B: Bacteria, CHX: Chlorhexidine, GC: German chamomile.

* Significant difference between aerobic and anaerobic at $p < 0.05$ for B + GC

No significant difference between aerobic and anaerobic bacteria for B + CHX.

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