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RESEARCH ARTICLE

ANTIFUNGAL EFFICACY OF SODIUM HYPOCHLORITE AND CHLORHEXIDINE WITH AND WITHOUT AN ANTIFUNGAL HERBAL IRRIGANT: AN INVITRO STUDY

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Abstract

Aim: The aim of this in vitro study was to evaluate the efficacy of 5.25% sodium hypochlorite (NaOCl) & 2% chlorhexidine gluconate (CHX) as a final irrigant with and without the inclusion of an antifungal herbal agent (Neem extract) on Candida albicans.

Materials & Methods: Thirtyfive single-rooted premolars were instrumented and inoculated with a suspension of C. albicans. The experimental specimens were divided into two groups. The irrigant group was divided into 2 subgroups and irrigated with 5.25% NaOCl& 2% CHX respectively. The irrigant with antifungal group was divided into two subgroups and irrigated with 5.25% NaOCl& 2% CHX respectively followed by neem extract. The control group was irrigated with normal saline. Aliquots from the experimental teeth were plated on 4% Sabouraud agar, and the colony-forming units (CFUs) were evaluated under light microscopy (400x).

Results: 2% CHX exhibited superior antifungal efficacy compared with 5.25% NaOCl with no significant difference. On inclusion of Neem extract, there was a significant decrease in the CFU; 5.25% NaOCl and 2% CHX with Neem extract showed significantly greater antifungal properties.

Conclusion: Neem extract proved to be effective against C. albicans when used along with root canal irrigants as a final rinse.

Introduction:-

Microorganisms are linked to almost all diseases affecting the pulp and periradicular tissues. The microbial environment in infected root canals may comprise various bacteria, including spirochetes, and fungi.[1]

Fungi play a crucial role in the failure of root canal treatment within the microbiota of root canals. These fungi are prevalent opportunistic pathogens found in the oral cavity. Interestingly, approximately one-third of healthy individuals harbor fungi as part of their normal flora.[2]

Candida albicans stands out as the most frequently isolated yeast from the oral cavity, found in both healthy individuals and those with immunocompromised health conditions.[3]

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In a study by Najzar-Fleger et al. (1992) that investigated the prevalence of the Candida genus in various sites of the oral cavity, it was confirmed that 55% of root canals contained these microorganisms.[4]

Candida is regarded as common inhabitants in the oral cavity; however, it can lead to disease when certain local or systemic factors make the individual more susceptible to infection.[1]

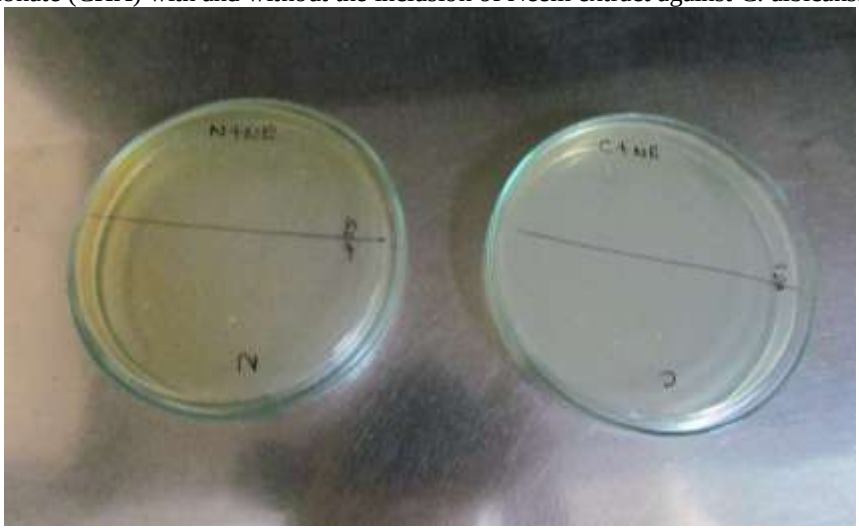
The effective use of irrigants in meticulously cleansing root canal systems during instrumentation is essential for the success of endodontic treatment. Irrigation complements the process of instrumentation, aiding in the elimination of pulp tissues and/or microorganisms. Beyond its mechanical flushing action, an irrigant should possess microbicidal effects without causing harm to periradicular tissues.[5]

Sodium hypochlorite (NaOCl) is presently the most widely employed substance in endodontic procedures due to its various properties, including the ability to dissolve organic matter, provide lubrication, and neutralize toxic content.[6]

Developed in the late 1940s in the United Kingdom[7], chlorhexidine (CHX) is a cationic bisbiguanide disinfectant that has found extensive use as a final irrigation solution in endodontics. It demonstrates antimicrobial efficacy, established substantivity, and the ability to inhibit the adherence of specific microbes to dentin. At a concentration of 2%, it exhibits a broad spectrum of antimicrobial activity.[8]

Azadirachta indica, commonly known as Neem, stands as the most frequently utilized traditional medicinal plant in India. Research has shown that Neem leaf and its components possess a range of beneficial properties, including immunomodulatory, anti-inflammatory, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic effects.[9]

The aim of the present study was to evaluate the antifungal efficacy of 5.25% sodium hypochlorite (NaOCl) and 2% chlorhexidine gluconate (CHX) with and without the inclusion of Neem extract against C. albicans.



Materials &Methods:-

Thirty five permanent single-rooted, premolar teeth were selected for this study. Each tooth was digitally radiographed in both the mesiodistal and buccolingual directions to confirm the presence of a single canal. The teeth were immersed in 3% sodium hypochlorite for 15 minutes to remove debris and organic tissue and subsequently cleaned using an ultrasonic scaler to render them free from calculus and tissue tags. They were stored in 0.2% sodium azide until use.

The teeth were decoronated at the cementoenamel junction, and pulpal remnants were extirpated using barbed broaches. A 25-mm, size 15 K-File was then inserted into the root canal until it was seen at the apical foramen. One millimeter was subtracted from this length and was taken as the working length. Gates Glidden drills 1 to 4 were used for the coronal flaring, and apical preparation was done until ISO size 50. Three milliliters of 5.25% NaOClwas

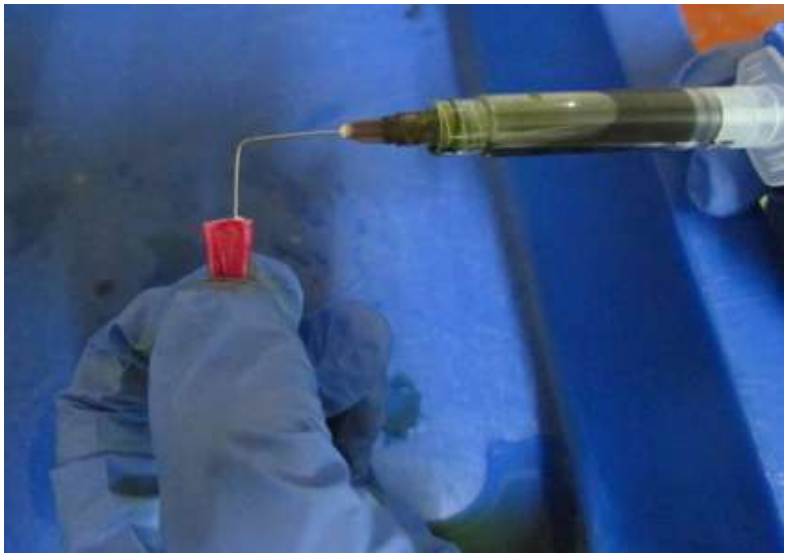
used between files. After instrumentation, the removal of the smear layer was accomplished with a final rinse of 1 mL of 17% EDTA for 1 minute followed by 3 mL of 5.25% NaOCl.

Finally, the canals were flushed with 5 mL of distilled water to remove any debris and residual irrigants. The roots were coated with two coats of nail varnish and apical foramen sealed with type II GIC. Subsequently, the roots were sterilized in an autoclave for 15 minutes at 121^{o}C and 15 lb of pressure. A suspension of C. albicans was adjusted to 0.5 turbidity on the McFarland scale (1.5 108 bacteria/mL). The canals of the experimental teeth were cautiously inoculated with 0.5 mL of the freshly prepared suspension and were stored in a glass test tube. The samples were stored incubated at 37^{o}C and 91% humidity for 96 hours. Every 24 hours, the vials containing the experimental teeth were replenished with freshly prepared suspension of C. albicans. At 48 hours, aliquots were taken from each tooth using a syringe and plated on 4% Sabouraud dextrose agar plate to verify the growth of C. albicans.

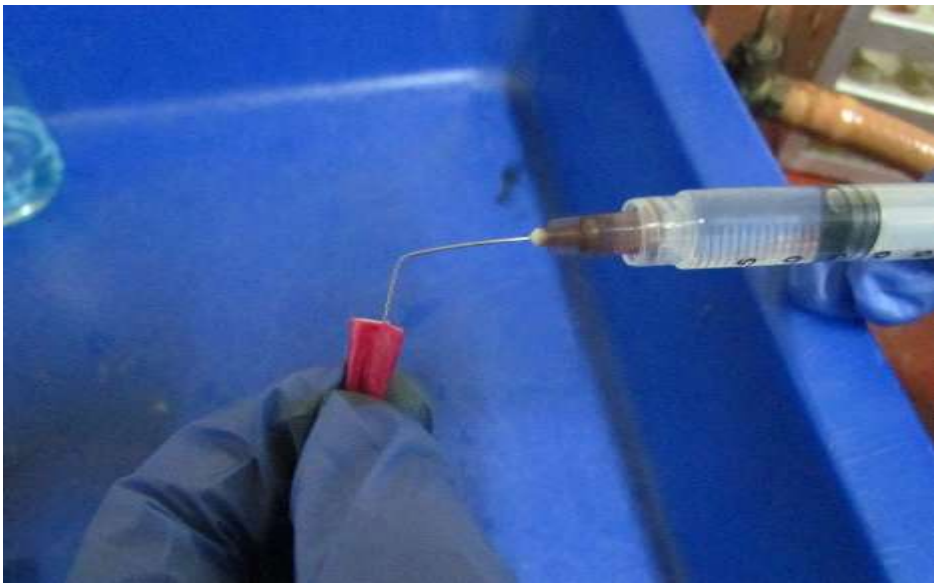
At the end of 96 hours, teeth were removed from the glass test-tube vials and excess fluid in the canal was removed with sterile paper points and then subjected to the following experimental groups.

The teeth were randomly divided into two experimental groups each comprised of 16 teeth. Samples in the irrigant group (group 1) were further divided into two subgroups consisting of 8 teeth each. The subgroups were rinsed as follows: 1A: 2 mL of 5.25% NaOCl, 1B: 2 mL of 2% CHX. The time of contact of each irrigant was 1 minute, and a final flush of 5 mL of distilled water was performed to terminate the action of the irrigant.

The samples in the irrigant with the antifungal group (group 2) were divided into three subgroups (2A & 2B) each containing 8 teeth. The samples in group 2A were irrigated with 2 mL of 5.25% NaOCl (Dentapro); samples in group 2B were irrigated with 2 mL of 2% CHX. The time of contact of each irrigant was 1 minute. A flush with 5 mL of distilled water was done to terminate the action of irrigant. Subsequently, all samples in group 2 were irrigated with 2 mL of alcoholic neem extract. The antifungal agent was injected into the root canal by using a 26-gauge needle.



The time of contact was 1 minute; after which, the samples were flushed with 5 mL of distilled water to prevent the carryover of irrigants. The control group contained 3 samples, and Saline was used for irrigation.



All experimental teeth were then flushed with 15 mL of sterile saline, and canals were dried with sterile absorbent paper points. A small amount of saline solution was introduced into the canal, and an endodontic hand file was used in a filing motion to a level approximately 1 mm short of the root apex. A 1-mm inoculation loop was used to remove aliquots from the fluid and was plated on Sabouraud 4% dextrose agar. The plates were incubated at 36C and 91% humidity for 48 hours. After the incubation period, the growth of C. albicans was assessed with light microscopy at 400x. The number of colonyforming units (CFUs) of Candida served as a measure of the antifungal activity.

Data were analyzed statistically using SPSS software, and one-way analysis of variance, the Tukey honestly significant differences test, and the Student t test were performed.

Results:-

GROUPS	CFU	P VALUE
NaOCl	8.75 ±1.48	0.000000552
NaOCl+ Neem Extract	2.75 ±1.28	
CHX	7.25 ±1.03	0.0000089
CHX + Neem extract	2.50 ±1.69	
Control group	>50	

Among the irrigants evaluated in the irrigant group (group 1), 2% CHX was the most effective and statistically significantly superior when compared with 5.25% NaOCl.

In the irrigant with antifungal group (group 2), CHX with Neem extract showed better Antifungal properties when compared with 5.25% NaOCl& Neem extract.

Discussion:-

Candida albicans is the predominant fungus observed in root canals, occurring in 21% of primary infections and 18% of retreatment cases.[10] This microorganism demonstrates remarkable adaptability across a broad spectrum of pH levels. It displays pleomorphism, manifesting in various morphological forms such as germ tubes, blastospores, pseudohyphae, true hyphae, and chlamydospores. Candida exhibits the capacity to grow on dentinal surfaces even in the absence of oral tissue fluids. Additionally, it can penetrate dentinal tubules through diverse growth patterns, including hyphae and blastospores. These distinctive characteristics enable Candida to thrive in the challenging ecological conditions within the root canal.[11]

Sodium hypochlorite (NaOCl) stands out as the preferred irrigant utilized during instrumentation. Numerous in vitro and in vivo studies have consistently demonstrated its effectiveness in eradicating Candida albicans.[5]

With both tissue-dissolving and antimicrobial properties, sodium hypochlorite has been proven to be effective. Studies have shown that Candida albicans is highly susceptible to its effects at a concentration of 0.5% within a brief contact time of 10 seconds.[13]

Sen et al. evaluated the antifungal properties of 1% NaOCl, and 5% NaOCl and 0.12% CHX against Candida albicans using cylindrical dentine tubes. When smear layer was absent, NaOCl started to display antifungal activity after 30 minutes.[12]

Chlorhexidine digluconate serves as a broad-spectrum antiseptic commonly employed as an endodontic irrigant. Its effectiveness extends across a diverse range of organisms, encompassing Candida albicans.[13]

Chlorhexidine (CHX) possesses a distinctive characteristic wherein dentine treated with it gains antimicrobial substantivity. The positively charged molecules of CHX can adhere to dentine, creating a barrier that hinders microbial colonization on the dentine surface for a duration extending beyond the initial medication period.[15]

In the study conducted by Ballal et al., the antimicrobial efficacy of CH paste, 2% CHX gel, and their combination was evaluated against E. faecalis and Candida albicans. The findings indicated that, after 72 hours, 2% CHX gel exhibited greater effectiveness compared to CH alone or the combination of both medicaments.[16]

Neem (Azadirachta indica), with a pH of 6.8, possesses antimicrobial properties attributed to the presence of various compounds, including alkaloids, glycosides, saponins, flavonoids, steroids, anthraquinone, and tannic acid.[9]

The antiviral, antifungal, antibacterial, and anticarcinogenic properties of Neem make it a promising agent for root canal irrigation. Additionally, Neem leaf extract is employed in the treatment of dental plaque and gingivitis. Being a bio-compatible anti oxidant, use of neem is advantageous as it is not likely to cause the severe harms to patients that might occur through sodium hypochlorite accidents.[17]

Bohora et al. have concluded that neem leaf extract exhibits a notable antimicrobial effect against Candida albicans. These findings align with the results of our study as well.[18]

In the field of dentistry, Azadirachta indica (Neem) has been used for its antimicrobial potential against oral microorganisms. Additionally, it demonstrates anti-adherence activity by modifying bacterial adhesion and impeding the organism's ability to colonize.[19]

Hegde and Kesaria reported significant effectiveness of Neem extract against Candida albicans, corroborating the findings of the present study.[20]

India is renowned for its abundant heritage of traditional medicinal systems. Over the past decade, there has been a notable shift toward the utilization of phytochemicals in the field of Endodontics, aligning with the growing popularity of herbal remedies and holistic medicine among the public. Herbal endodontic irrigants offer various advantages, including high antimicrobial activity, anti-inflammatory, antiseptic, and antioxidant properties. They are also known for their biocompatibility, easy availability, cost-effectiveness, low toxicity, and the absence or minimal staining of teeth compared to synthetic irrigating solutions. Additionally, the use of herbal irrigants is associated with reduced microbial resistance against various microorganisms.[21]

Conclusion:-

Under the limitations of the present study, the inclusion of Neem extract along with the experimental irrigants further enhanced the antifungal efficacy. Hence, the use of alcoholic Neem extract can be recommended as an adjunct in the final irrigation protocol.

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