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Histopathological Evaluation of Calcium Hydroxide Mixed with Gingerols Versus Metapexas Obturating Material in Treatment of Infected Pulp in Primary Teeth.: Experimental Study

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ABSTRACT

Use of Gingerolsin pulp treatment may lead to successful pulp treatment by reducing the body's exposure to chemicals and their potential toxicity. This experimental study was performed to compare the effectiveness of Gingerols versus Metapexas obturating material in the treatment of infected pulp in primary premolar teeth of puppies. Gingerols were extracted and mixed with calcium hydroxide to produce Ginge-Cal. 48 primary premolars of 4male mongrel puppies aged 4-8 weeks old were selected and divided into four main groups according to the applied root canal filling material. The groups were BL group (Baseline group normal teeth without intervention), INF (pathological group still without treatment), Ginge-Calexperimental group (root canal filling by calcium hydroxide mixed with Gingerols), and control group Metapex (root canal filling by Metapex). Both groups Ginge-Cal and Metapex divided into two subgroups according to treatment intervals of 4 weeks and 8 weeks (Ginge-Cal4, Ginge-Cal8, Metapex4 andMetapex8). Two puppies were sacrificed after 4 weeks and the other two after 8 weeks. Histological evaluation was done for inflammatory reaction at the apical area for each group. The histopathological results evaluated inflammatory reaction, bacterial colonies, bone destruction, bone remodeling, and periodontal ligament (PDL) thickness. The statistical results showed that the two materials had a positive effect in treating infected pulp teeth ($p<0.001$), with superiority of Ginge-Cal, especially at the end of the second period at 8 weeks in a number of comparison points ($p<0.001$). Depending on histopathological finding Ginge-Cal have the best result than Metapex. This makes it a promising material in the treatment of infected pulp.

INTRODUCTION

Most of the necrotic or treated teeth with pulp therapy have a high percentage level of inflammation recurrence. This is due to the large microbial diversity in children found in the pulp and tissues surrounding the apex of the root and furcation area. For this reason, additional chemicals and mechanical approaches are used to decrease the effect of the inflammation to preserve the primary teeth to perform their functions. Where until now, a number of root canal filling materials were developed such as Zinc oxide eugenol(ZOE), Calcium Hydroxide (CH), Iodoform, Walkoff paste, Guedes pinto paste, Maisto® paste, Endodlas®, Endoflas-chlorophenol-free, Ozone, Calen® paste, lesion sterilization, and tissue repair, Rifocort®, antibiotic mix, Vitapex®, and Metapex. [1]Among the most common of these materials is the CH mixture with iodoform as Metapex. But the problem remained because of the limited influence of substances on the microorganism. [2,3]Therefore, the idea of using some herbals started in many medical or dental treatments to seek better results than the use of chemicals. [4,5] In endodontic therapy, there are several herbals used as direct or indirect pulp dressing, pulpotomy filling, and pulp medicament as Allium Sativum (Garlic), Turmeric, Pomegranate, Green Tea, Neem, Cloves, and Aloe Vera. [6,7]. Ginger (*Zingiber officinale Roscoe, Rhizoma zingiberis*) is one of the earliest known oriental spices. It has been used as a spice as well as folk medicine in both forms fresh or dried since ancient times in China and India. It was also known in Europe from the 9th century and in England from the 10th century for its medicinal properties. This was confirmed by a United States Food and Drug Administration (FDA), Where it was considered ginger (and its ingredients) a natural source showing no toxicity and considered as "Generally Recognized As Safe" GRAS (a designation that a chemical or substance added to food is considered safe by experts and so is exempted from the usual Federal Food, Drug, and Cosmetic Act (FFDCA) food additive tolerance requirements). [8,9]Gingerols are the major powerful phenolic compounds present in ginger and are renowned for their role in human health and nutrition. A study of Gingerols found that different types of Gingerol are homologous and differ in the length of their unbranched alkyl side chain (6)-Gingerol, (8)-Gingerol, (10)-Gingerol (12)-Gingerol and (14)-Gingerol. [10] Most important properties of Gingerols it has anti-inflammatory by its inhibitory effects on prostaglandins and leukotrienes synthesis in RBL-1 cells and by mimicking dual-acting nonsteroidal anti-inflammatory drugs (NSAIDs) in intact human leukocytes. [11] As well as antimicrobial it has anti-fungal activity by a decrease or preventing the growth of fungi. [12]Based on the above, and the limited or lack of valuable studies to evaluate Gingerols in dentistry, especially in pulp

treatment; the present study focused on Gingerols extract from fresh ginger and the possibility of using it in treating primary teeth with induced infected pulp and compare it with Metapex that is used widely in dentistry, to avoid the recurrence of pulp inflammatiom and elemnate most of the microorganism.

MATERIALS AND METHODS

A study was conducted after obtaining the approval of the Ethics Committee of the Faculty of Dentistry Mansoura University and registered with the code number (06030718). All international and institutional guidelines for the use and care of animals were followed based on the ethical guidelines of Russell and Burch's. [13]According to the type of pulp obturation material, the teeth are divided into main 4 main groups (BL, INF, Ginge-Cal, and Metapex). Groups Ginge-Cal and Metapex each divided into two subgroups (Ginge-Cal_4, Ginge-Cal_8, $Metapex_4$, $Metapex_8$) depending on the period from root filling until sacrificed as flowing:

- **Baseline group (BL):** Eight primary premolars without any intervention for the same period as other main groups.

- **Infected group(INF):** Eight primary premolar teeth with contaminated root canals remain without canal treatment for the same period as other main groups.

- **Ginge-Cal group (Study group):** Sixteen primary premolar teeth with contaminated root canals were obturated with a **Ginge-Cal** which was divided into (8 teeth for each subgroup):

  - **Ginge-Cal_4**: Four weeks from obturation until obtaining the histopathological sample.

  - **Ginge-Cal_8**: Eight weeks from obturation until obtaining the histopathological sample

- **Metapex group (Control group):** Sixteen primary premolar teeth with contaminated root canals were obturated with a ready-made Metapex(Meta Biomed Co., Ltd.Korea), which divided into (8 teeth for each subgroup):

  - **$Metapex_4$**: Four weeks from obturation until obtaining the histopathological sample.

  - **$Metapex_8$**: Eight weeks from obturation until obtaining the histopathological sample.

Gingerols extract and mixture:

The Gingerols were extracted in the laboratories of the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University. [14]The semi-oily aqueous liquid extract of Gingerols, CH powder, and Barium sulfate powder were mixed on a glass slab, with a mixing ratio of semi-oily was 3:2:1 and abbreviated by Ginge-Cal. The mixture is packed into a sterile syringe similar to a Metapex syringe to facilitate its use in the filling of the pulp canal.

Experimental study procedures:

Puppies housing, preparation, and general anesthesia that was performed under the supervision of the veterinarians at the Medical Experimental Research Center (MERC) in the Faculty of Medicine, Mansoura University. In each dental procedure, the puppies were anesthetized by intravenous injection of Thiopental sodium 1gm (Egyptian International)with a dosage (10 to 25 mg/kg). [15]

Induction of dental pulp infection:

The selected 48 teeth of 4 male mongrel puppies aged 4-8 (wight 2200-2500g) were examined by dental x-ray to determine if the teeth are suitable for the study. Infection was induced for 40 selected teeth in two phases. The **first phase** started with the occlusal cavity opening by low or highspeed contra and medium round bur to carefully remove the pulp tissues with a Hedstrom size # 20 to # 40. The root canals were exposed to the oral cavity environment for 5-7 days. In the **second phase,** the opened tooth is sealed with ZOE without root canal treatment and covered with GIC-II material for 10-15 days under analgesic cover by Voltaren 75 mg (Novartis, Egypt) injectable ampules 1ml once daily.

Pulpectomy and restoration:

After confirming the presence of periapical lesions developed by clinical examination, that supported by dental X-ray. The root canal was initially widened and irrigated with 3% Sodium Hypochlorite and dried with a paper point. The root canal working length is measured by an electronic apex locator then start cleaning and shaping the root canal about three sizes larger than the initial file size. The root canal was filled according to the main groups (Ginge-Cal or Metapex). Then the access cavity and tooth were restored with glass ionomer restoration, with antibiotic cover Cefotaxime 250 mg (Cefotax Egyptian International) twice daily for 3 days.

Histopathological slide preparation and evaluation

In the fourth week after coronal restorations, two puppies were sacrificed by overdosage of I.V Thiopental sodium (150mg/kg). [16] While the other two puppies were sacrificed after 8 weeks by the same method. Specimens from the alveolar bone were collected by cutting the jaw that contained selected teeth and fixed in 20% buffered neutral formalin solution for 48 hours, decalcification by HCl 10% and Formic acid 10% for 3 weeks, dehydrated in gradual ascending ethanol (from 70 to 100%), cleared in xylene, and embedded in paraffin. An appropriate paraffin block section (five-micron thick) was sliced using a microtome (Leica RM 2155, England). The sections were longitudinally in the mesiodistal direction from the preapical area and the area of the related unerupted permanent tooth. Then each slide was stained with Hematoxylin and Eosin (H&E) for histopathology evaluation. [17] All slides of all groups were evaluated and analyzed by two different histopathologists for the following parameters: inflammatory reaction, bacterial colonies, bone destruction, bone remodeling, and periodontal ligament (PDL) thickness. The inflammatory reaction was evaluated depending on severity of inflammatory reaction (No alterations (0%), Mild (1-25%), Moderate (26-50%), Sever (51-75%), Intense (76-100%)). The inflammatory reaction was measured by calculating the surface area of inflammatory cells using a computer program (Image J- image processing and analysis in Java 1.8.0-112). Other parameters bacterial colonies, bone destruction, remodeling, and PDL thickening change were evaluated by the presence or absence in all analyzed slides. Then all data were tabulated and statistically analyzed.

RESULTS

a. Descriptive results:

The untreated infected pulp tooth after one month from inflammation induction and contact with the oral cavity environment, sections from the infected pulp of puppies' lower primary premolars were examined under a light microscope. The inflammatory events with abscess formation revealed the severity affected by the prominent periapical abscess formation which is characterized by the intense aggregation of life and dead neutrophils, which mixed with homogenous eosinophilic content (pus) besides to scattered bacterial colonies and multinucleated giant cells were common. In some sections, some calcified centers showed a result of the difference in pH level and calcium deposition (Figure No. 1).

The infected pulp tooth treated by Metapex after four weeks revealed intense infiltration of the inflammatory cells with the new formation of blood vessels in the periapical area zone angiogenesis. The inflammatory cell found in some areas contains several types of inflammatory cell aggregations mainly neutrophils and macrophages that extend to form zones of marked periapical abscess formation that contain in sometimes debris, bacterial colonies, and pus. Moreover, mild to moderate osteoporosis/degeneration of some alveolar areas with Extravaseted erythrocytes (hemorrhages) besides infiltration by inflammatory cells and distributed osteoclast cells (Figure No. 2). After eight weeks revealed many limited ameliorated effects ranging from mild to moderate with the still presence of some inflammatory reactions and cementoblasts cells especially near the apex. In addition to the presence of some wave bone formation (new bone formation). Mild focally inflammatory cells aggregations and edema were recorded in a few sections nearest the root apex of permanent teeth. The alveolar bone revealed many healing features as a wavy bone formation with marked thickening of the trabeculae surrounded by mild fibroblastic dysplasia (Figure No. 3).

Teeth treated by Ginge-Cal showed nearly normal histomorphological structures without pathological alterations after four weeks revealed treated by Gingerols. In some sections, abscesses and/or inflammatory aggregations disappear or still a varying degree of congested blood vessels and perivascular edema. Some sections showed thickening of periodontal ligaments due to edema, more collagen deposits, and hyper-cellularity with a new formation and congested blood vessels. Also, several sections showed thickening of the majority of alveolar trabeculae and blood cavities without fibroblastic dysplasia and active endosteum. Furthermore, some sections showed nearly normal preapical areas around an unerupted tooth area with slightly inflammatory cell aggregations (Figure No. 4). After eight weeks showed nearly remodeling to the normal status of the all-normal periapical and around unerupted tooth tissue structures with a still slightly thickening periodontal ligament without edema, congested blood vessels, and wave boon formation as well as any inflammatory reaction (Figure No. 5).

b. Statistical results:

Data were fed to the computer and analyzed using IBM SPSS software package version 27.0. (Armonk, NY: IBM Corp). Qualitative data were described using numbers and percentages. The significance of the obtained results was judged at the 5% level. The statistical results showed the severity of inflammatory reaction that present in all groups due to induction of inflammation except BL group. The represented percentage of the severity of inflammatory

reaction in the INF group ranged from moderate to intense (48.00 – 78.00%). However, the two subgroups that were treated with Ginge-Cal showed the severity of inflammatory reaction ranged from mild to moderate in Ginge-Cal$_4$ and mild reaction in Ginge-Cal$_8$ (10.13 – 20.01%). While other two subgroups that were treated with Metapex presented moderate severity of inflammatory reaction in Metapex$_4$ (29.39 – 41.09%) and mild reaction in Metapex$_8$ (15.40 – 23.12%). Table No. 1 showed the comparison between the two materials in the two different periods, depending on the inflammatory reaction. The bacterial colonies presented in different proportions in all groups except BL and Ginge-Cal$_8$ group that did not show any presence of bacterial colonies in all samples (100%). While INF, Ginge-Cal$_4$, Metapex$_4$ and Metapex$_8$ groups showed different proportions (0%), (75.0%), (62.5%) and (87.5%) respectively. Table No. 2 showed the comparison between the two materials in the two different periods, depending on the bacterial colonies presented. The BL group does not show any presence of bone distraction in all samples (0.00%). However, the INF group showed the presence of bone distraction in all samples (100%). The percentages of bone distraction differed for the Ginge-Cal$_4$, Ginge-Cal$_8$, Metapex$_4$, and Metapex$_8$ groups, and the percentages (50.0%), (25.0%), (50.0%) and (37.5%) respectively, with low percentages in Ginge-Cal$_8$group.Table No.3 showed the comparison between the two materials in the two different periods, depending on the presence of bone distraction. The INF group doesn't show any bone remodeling action in all samples (0.00%). While the percentages of bone remodeling for groups Ginge-Cal$_4$, Ginge-Cal$_8$, Metapex$_4$, and Metapex$_8$ presented differed percentages (62.5%),(87.5%), (62.5%) and (75.0%) respectively. Table No.4 showed the comparison between the two materials in the two different periods, depending on the presence of bone remodeling. The PDL thickening score was presented in all groups except the BL group. The represented score of PDL thickening in the INF group ranged from moderate (25.00%) to severe (75.00%). However, the two subgroups that were treated with Ginge-Cal showed the PDL thickening score ranged from normal to moderate [normal (37.5%), mild (37.5%) and moderate (25.0%)] in Ginge-Cal$_4$ group and (normal (37.5%), mild (50%) and moderate (12.5%)) in Ginge-Cal$_8$ group. Other two subgroup that treated with Metapex$_4$ group presented normal to sever PDL thickening score [normal (12.5%), mild (25.0%), moderate (50.0%) and sever (12.5%)] and Metapex$_8$ group presented normal to moderate (normal (37.5%), mild (37.5%) and moderate (25.0%)).Table No.5 showed the comparison between the two materials in the two different periods, depending on the presence of the PDL thickening score.

DISCUSSION:

Based on our knowledge, there is no previous study that studied Gingerolor the Ginge-Cal mixture that was prepared for the first time in this study for the treatment of the infected pulp of primary teeth. Also, for the lack of sufficient studies regarding Metapex. The aim of this topic was selected for study the Ginge-Cal in the treatment of infected primary pulp, to find a material capable of reducing or eliminating microorganisms and reducing the rate of inflammation recurrence. Metapex was chosen in this study for comparison because of its widespread use and its advantages in treating primary teeth with infected pulp. It is containing CH, which is a common material between it and Ginge-Cal. The CH has been used as the primary ingredient in Metapex and Ginge-Cal because of its safety, aiding in the healing and repair of hard tissues, it has an initial bactericidal effect and stops or prevents the occurrence of internal resorption. [18] While iodoform has been replaced due to doubts about its antimicrobial efficacy, relying on the results and recommendations of some studies such as Navit et al[19] and Ibrahim et al[20]. They confirmed that iodoform has no or very little antimicrobial effect. Also, the ability of gingerol extract to form a paste when mixed with CH, which is a suitable alternative to silicone oil because it is an oily-aqueous liquid. Gingerols have been selected because of their anti-inflammatory and antimicrobial action. [21,22] Also, for other therapeutic properties such as antioxidants and pain reduction. [23,24] As well as the lack of studies that evaluated gingerols in dental pulp or root canal treatment in adults or children.This study was conducted on animals to observe changes in the hard tissues within the alveolar bone or surrounding soft tissues. The effect of gingerols on the functions of cells extracted and implanted from bone, such as osteoclasts and osteoblasts, was evaluated, but the effect of the material on bone was not directly known. Regardless of the fact that the material is natural and considered safe. The use of new materials on humans directly or their introduction into living tissues is not in line with the ethics of scientific research and dealing with humans as a test field. It should be noted that the puppies were not only used for this study but some organs such as the liver, intestines, pancreas, heart, and some glands were extracted for further medical studies after reaching the stage of sacrifice. The puppies were selected for relative ease in dealing with their teeth in pulpectomy treatment, compared to other experimental animals. The age of puppies selected for this study was 4–8 weeks old, this is because of two causes first, the primary premolars of puppies complete their eruption into the oral cavity at 4-12 weeks; the second cause, primary teeth have grown out, and will begin to be replaced by the permanent ones, after roughly 4 months of age. [25]The result of the histopathological section revealed the

different percentages of inflammatory infiltration in all groups, however, there was a static significance difference when compared between the different studied groups with the INF group. That also supported when compared Ginge-Cal and Metapex subgroups in different intervals. This is consistent with that the two materials have an anti-inflammatory effect, which was proven by El-Ashry et al[26] Zhang et al[27]and there are no previous studies that contradict this result. On the other hand, no statistically significant difference was observed when comparing the two materials in the same period with the superior main value of Ginge-Cal. As for the resistance of the Ginge-Cal and Metapex groups in different intervals to bacterial colonies, a statistically significant difference was observed when compared with the INF group. This is in agreement with Riaz et al[28]and Zhang et al[27] in addition to Harini et al[29] with regard to Metapex. When comparing the two materials between them or the two materials in different intervals, it was noted that there is no statistically significant difference with the superiority of the main value for gingerols. This result is in line with Navit et al[9], which concluded that Metapex has an anti-bacterial effect, but is weak. When talking about bone distraction A high main value of the anti- bone distraction effect was observed in Ginge-Cal and Metapex subgroups, with a statistical significance difference in Ginge-Cal8 and Metapex8 group when compared with the INF group. This is due to the presence of CH in the composition of the two materials, and this result is consistent with Do Nascimento et al[30] and De Souza et al[31], who emphasized the role of CH as an anti- bone distraction material, also it is possible that adding gingerols to the treated material increased this effect. On the other hand, the Ginge-Cal and Metapex group showed their ability to remodel bone with statistical significance difference in different intervals when compared with the INF group, and this is also due to the presence of CH. But when comparing the two materials between them or the two materials in different intervals the result does not show any statistically significant difference. As for the slight increase in the main value of group Ginge-Cal4 and Ginge-Cal8 it may be due to the presence of antioxidants, which play an important role in bone remodeling, and these results and explanation are consistent with Domazetovic et al[32], who concluded that anti-oxidants that help in the bone healing process. Also, the result of gingerols coordinate with Fan et al[33] who suggested that 6-gingerol may have beneficial effects on bone formation as a therapeutic agent for treating bone disorders. The result of the histopathological section revealed the different percentages of PDL thickening in all groups, however, there was a static significance difference when compared between the different studied groups with the INF group. That also supported when compared Ginge-Cal and Metapex subgroups in different intervals but with no statically significance difference. This is consistent with that the two

materials have an anti-inflammatory effect, which affects PDL thickening, and the role of anti-inflammatory materials in a decrease of PDL thickening was proven by Mortazaviand Baharvand[34]and Siddiqui et al.[35]The low main value and low severity score ofPDL thickening observed in Ginge-Cal8 group with no statistically significant difference when compared with other subgroups Ginge-Cal4, Metapex4, and Metapex8. Perhaps this is due to the anti-oxidant effect noted in a study of Saad[36] or Kimet al[37] who confirm the anti-osteoclastogenic effect of ginger and suggest the possibility of application as an anti-resorptive strategy in periodontitis. In addition, due to the increase in blood nutrition in the PDL area that was noted in histopathological sections. With the limitations on this study due to the short period of experience on the primary teeth of puppies, we recommend studying some of the physical and biological properties of Ginge-Cal, as the material is considered applicable to the primary teeth of children with infected pulp.

CONCLUSIONS:

From this study, we can conclude that Ginge-Cal is a promising material that can be used in several dental pulp treatments, especially when dental treatments relate to fighting microorganisms within the pulp canal.

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Table No. 1: Comparison between the different studied groups according to infection reaction severity.

Infection reaction%	BL (n = 8)	INF (n = 8)	Ginge-Cal		Metapex		F (p)
			Ginge-Cal$_4$ (n = 8)	**Ginge-Cal$_8$ (n = 8)**	**Metapex$_4$ (n = 8)**	**Metapex$_8$ (n = 8)**	
Min. – Max.	0.0 – 0.0	48.0 – 78.0	22.76 – 38.82	10.13 – 20.01	29.39 – 41.09	15.40 – 23.12	112.599*
Mean ± SD.	0.0 ± 0.0	61.82 ± 10.77	29.77 ± 5.94	15.23 ± 3.73	34.70 ± 4.03	19.08 ± 2.64	<0.001*
P_0	-	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	
P_1	-	-	<0.001*	<0.001*	<0.001*	<0.001*	
Sig.	-	-	P_2<0.001*, P_3<0.001*, P_4=0.502, P_5=0.742				

F: **F for ANOVA test**, Pairwise comparison bet. every 2 groups were done using Post Hoc Test (Tukey)

P: p-value for comparing between the different studied groups

P_0: p-value for comparing between BL and each other groups

P_1: p-value for comparing between INF group and each other groups

P_2: p-value for comparing between Ginge-Cal in different interval

P_3: p-value for comparing between Metapex in different interval

P_4: p-value for comparing between Ginge-Cal_4 and $Metapex_4$

P_5: p-value for comparing between Ginge-Cal_8 and $Metapex_8$

*****: Statistically significant at $p \leq 0.05$

Table No. 2: Comparison between the different studied groups and subgroups according to bacterial colonies presence or absence.

Bacterial colonies	BL (n = 8)	BL (n = 8)	Ginge-Cal		Metapex	
			$Ginge\text{-}Cal_4$ (n = 8)	**$Ginge\text{-}Cal_8$ (n = 8)**	**$Metapex_4$ (n = 8)**	**$Metapex_8$ (n = 8)**
Absent	8 (100%)	0 (0.0%)	6 (75.0%)	8 (100%)	5 (62.5%)	7 (87.5%)
Present	0 (0.0%)	8 (100%)	2 (25.0%)	0 (0.0%)	3 (37.5%)	1 (12.5%)
P_0	-	$^{FE}<0.001^*$	^{FE} 0.467	–	^{FE} 0.200	^{FE} 1.000
P_1	-	-	^{FE} 0.007*	$^{FE}<0.001^*$	^{FE} 0.026*	^{FE} 0.001*
Sig.	-	-	$^{FE}P_2=0.467$, $^{FE}P_3=0.569$, $^{FE}P_4=1.000$, $^{FE}P_5=1.000$			

FE**: Fisher Exact**

P_0: p-value for **Chi-square test** for comparing between **BL** and each other groups

P_1: p-value for **Chi-square test** for comparing between **INF** and each other groups

P_2: p-value for **Chi-square test** for comparing between **Ginge-Cal** in different interval

P_3: p-value for **Chi-square test** for comparing between **Metapex**in different interval

P_4: p-value for **Chi-square test** for comparing between **Ginge-Cal** and**Metapex**at 4w

P_5: p-value for **Chi-square test** for comparing between **Ginge-Cal** and**Metapex**at 8w

*: Statistically significant at $p \leq 0.05$

Table No.3: Comparison between the different studied groups according to bone distraction

Bone distraction	BL (n = 8)	INF (n = 8)	Ginge-Cal		Metapex	
			Ginge-Cal$_4$ (n = 8)	**Ginge-Cal$_8$ (n = 8)**	**Metapex$_4$ (n = 8)**	**Metapex$_8$ (n = 8)**
Absent	8 (100%)	0 (0.0%)	4 (50.0%)	6 (75.0%)	4 (50.0%)	5 (62.5%)
Present	0 (0.0%)	8 (100%)	4 (50.0%)	2 (25.0%)	4 (50.0%)	3 (37.5%)
p_0	-	^{FE}<0.001*	^{FE} 0.077	^{FE} 0.467	^{FE} 0.077	^{FE} 0.200
p_1	-	-	^{FE} 0.077	**^{FE} 0.007***	^{FE} 0.077	**^{FE} 0.026***
Sig.	-	-	$^{FE}P_2$= 0.608, $^{FE}P_3$=1.000, $^{FE}P_4$=1.000, $^{FE}P_5$=1.000			

FE**: Fisher Exact**

P_0: p-value for **Chi-square test** for comparing between **BL** and each other groups

P_1: p-value for **Chi-square test** for comparing between **INF** and each other groups

P_2: p-value for **Chi-square test** for comparing between **Ginge-Cal** in different interval

P_3: p-value for **Chi-square test** for comparing between **Metapex**in different interval

P_4: p-value for **Chi-square test** for comparing between **Ginge-Cal4 and Metapex4**

P_5: p-value for **Chi-square test** for comparing between **Ginge-Cal8 and Metapex$_8$**

*: Statistically significant at $p \leq 0.05$

Table No.4: Comparison between the different studied groups according to bone remodeling

Bone remodeling	BL (n = 8)	INF (n = 8)	Ginge-Cal		Metapex	
			Ginge-Cal$_4$ (n = 8)	**Ginge-Cal$_8$ (n = 8)**	**Metapex$_4$ (n = 8)**	**Metapex$_8$ (n = 8)**
Absent	8 (100%)	8 (100%)	3 (37.5%)	1 (12.5%)	3 (37.5%)	2 (25.0%)
Present	0 (0.0%)	0 (0.0%)	5 (62.5%)	7 (87.5%)	5 (62.5%)	6 (75.0%)
P_0	-	-	^{FE} 0.026*	^{FE} 0.001*	^{FE} 0.026*	^{FE} 0.007*
P_1	-	-	^{FE} 0.026*	^{FE} 0.001*	^{FE} 0.026*	^{FE} 0.007*
Sig.	-	-	$^{FE}P_2$=0.569, $^{FE}P_3$=1.000, $^{FE}P_4$=1.000, $^{FE}P_5$=1.000			

FE**: Fisher Exact**

P_0: p-value for **Chi-square test** for comparing between **BL** and each other groups

P_1: p-value for **Chi-square test** for comparing between **INF** and each other groups

P_2: p-value for **Chi-square test** for comparing between **Ginge-Cal** in different interval

P_3: p-value for **Chi-square test** for comparing between **Metapex**in different interval

P_4: p-value for **Chi-square test** for comparing between **Ginge-Cal$_4$ and Metapex$_4$**

P_5: p-value for **Chi-square test** for comparing between **Ginge-Cal$_8$ and Metapex$_8$**

*: Statistically significant at $p \leq 0.05$

Table No.5: Comparison between the different studied groups according to PDL thickening

PDL thickening	BL (n = 8)	INF (n = 8)	Ginge-Cal		Metapex		F (p)
			Ginge-Cal$_4$ (n = 8)	**Ginge-Cal$_8$ (n = 8)**	**Metapex$_4$ (n = 8)**	**Metapex$_8$ (n = 8)**	
Min. –Max.	1.0 – 1.0	3.0 – 4.0	1.0 – 3.0	1.0 – 3.0	1.0 – 4.0	1.0 – 3.0	14.399* <0.001*
Mean ± SD.	1.0 ± 0.0	3.75 ± 0.46	1.88 ± 0.83	1.75 ± 0.71	2.63 ± 0.92	1.88 ± 0.83	
P_0	-	<0.001*	0.148	0.287	<0.001*	0.148	
P_1	-	-	<0.001*	<0.001*	0.029*	<0.001*	
Sig.	-	-	P_2=0.999, P_3=0.287, P_4=0.287, P_5=0.999				

F: **F for ANOVA test**, Pairwise comparison bet. every 2 groups were done using **Post Hoc Test (Tukey)**

P: p-value for comparing between the different studied groups

P_0: p-value for comparing between **BL** and each other groups

P_1: p-value for comparing between **INF group** and each other groups

P_2: p-value for comparing between **Ginge-Cal** in different interval

P_3: p-value for comparing between **Metapex**in different interval

P_4: p-value for comparing between **Ginge-Cal_4 and $Metapex_4$**

P_5: p-value for comparing between **Ginge-Cal_8 and $Metapex_8$**

*: Statistically significant at $p \leq 0.05$

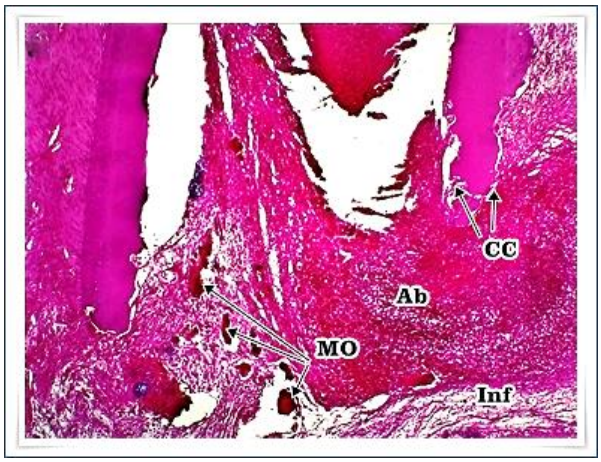

Figure No.1: Photomicrograph of the preapical area of puppy lower primary premolars after inflammation induction showing marked periapical abscess formation (**Ab**) Cementoclast (**CC**), Microorganism colonies (**MO**). Infiltration of inflammatory cells(**Inf**) [H&E stain with X40. X100 magnifications].

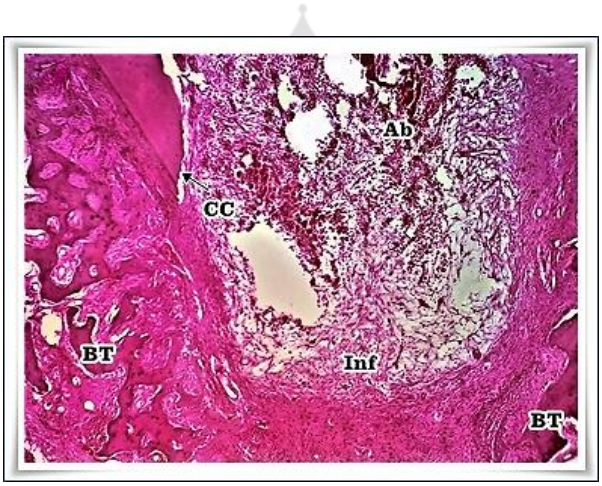

Figure No.2: Photomicrograph of the preapical area of puppy lower primary premolars after four weeks form is treated by Metapex showing abscess formation (**Ab**), infiltration of inflammatory cells (**Inf**), cementoblasts cells (**CC**), alveolar bone trabeculae (**BT**), and cementoblasts cells. [H&E stain with X40. magnifications].

Figure No.3: Photomicrograph of the preapical area of puppy lower primary premolars after eight weeks form is treated by Metapex showing inflammatory cell aggregations (**Inf**), edema (**Ed**), cementoblasts cells (**CC**), Blood vessels (**BV**), and wave bone formation (**WB**). [H&E stain with X40. magnifications].

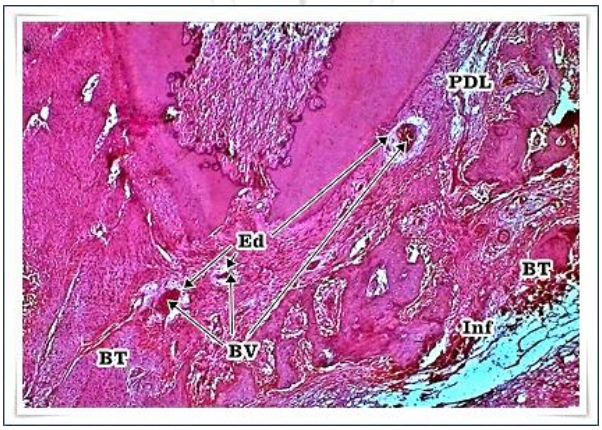

Figure No. 4: Photomicrograph of the preapical area of puppy lower primary premolars after four weeks form is treated by Gingerols showing prominent congested blood vessels(**BV**), perivascular edema (**Ed**), inflammatory cell aggregations (**Inf**), alveolar bone trabeculae (**BT**), and Periodontal ligaments(**PDL**).[H&E stain with X40 magnifications].

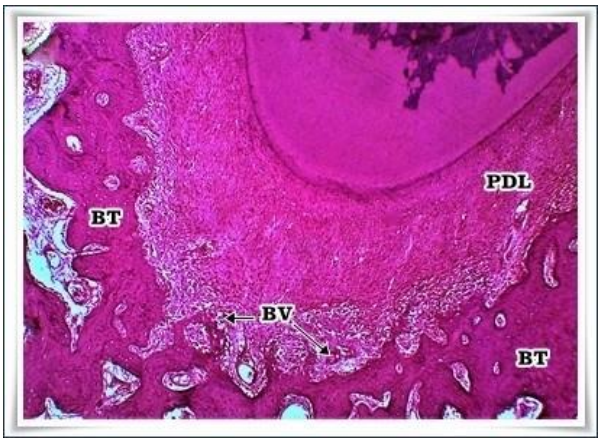

Figure No. 5: Photomicrograph of the preapical area of puppy lower primary premolars after eight weeks form is treated by Gingerols showing congested blood vessels(**BV**), Periodontal ligaments (**PDL**), alveolar bone trabeculae (**BT**) H&E stain with X40 magnifications].

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