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Histopathologic Evaluation of the Subcutaneous Tissue Response toThree Endodontic Sealers

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Abstract: *The materials used for obturation of root canal system may get extruded through apical foramen into the per apical tissue. Therefore, biocompatibility of these materials is very important. The purpose of this study was to evaluate the in vivo biocompatibility of three endodontic sealers: Sealapex, Diaket and Tubliseal after their subcutaneous implantation in rats. Each of the materials was injected subcutaneously in the dorsal connective tissue of 20 Wistar albino rats. Tissue biopsies were collected at first day, fifth day, tenth day and thirtieth day after the procedure. The specimens were processed and stained with hematoxylin and eosin and examined microscopically. According to this study, all of the sealers cause inflammatory reactions immediately after contact with tissue, but the intensity of these responses decrease with time. The acute inflammatory responses of initial days changed to chronic proliferative, phase and later to healing processes by the end of one month.*

Key words: *Endodontic Sealers, Tissue reaction (response), Subcutaneous tissue reaction.*

I. Introduction

The materials used for obturation of root canal system such as sealers, may get extruded through apical foramen into the periapical tissue. Therefore, the biocompatibility of these materials is very important. Currently, there are three recommended tests for the biological evaluation and acceptance of endodontic materials: a primary test or cell culture test which provides a general profile of toxicity for the material (level 1), a secondary test or material implantation test which evaluates its local toxicity in experimental animals (level 2) and usage test in which the material is used in the endodontic treatment of teeth in experimental animals (level 3).

In cell culture tests, several studies have been performed and all have found different degrees of cytotoxicity for various sealers [1, 2, 3]. Bouillaguet et al (2004)[1] evaluated cytotoxicity of several sealers at cell culture and reported that the cytotoxicity of sealers increases with time from 24hr to 1 week and most sealers are potentially, cytotoxic specially when they are mixed freshly . Huangetal [3] demonstrated that Diaket exhibit not only in vitro dose dependent cytotoxicity but also genotoxicity and that cytotoxicity of ZOE was detectable as early as 1hr after mixing and remained at a high level until 5 week. Diaket, however, induced early cytotoxic effects that lasted for 1week, followed by a substantial reduction in cytotoxicity [8]. In usage tests, Bernath and Szabo has found that after filling the root canals of monkeys, all sealers cause inflammatory response and reported that if root filling by Apexit and Grossman's sealers confine to the canal system, it would not cause inflammation. But similar situations with Diaket, cause mild lymphocytic- plasmocytic infiltration in some cases .The reports about biocompatibility of sealers are different. Thus in present study the biocompatibility of three conventional sealers such as Diaket, Sealapex, and Tubliseal are evaluated by subcutaneous injection (secondary test) in rats.

II. Materials and methods

Twenty mature male Albino rats, weighing from 250 to 500gms, were used in this study. . Animal care was carried out according to the Institutional Animal Care and Use Committee .Animals were divided into four group with 5 animals in each group to avoid bias .Every 5 animal in each group were sacrificed at the end of time interval of one day, five days ,ten days and thirty days. The animals were anesthetized with an intraperitoneal injection of 65 mg/ml sodium pentobarbital at a dose of 5.1 mg/100 g body weight.).

After disinfection of skin , the dorsum was shaved at 4 points, two points at anterior or cranial portion (right and left) and two points at posterior or caudal portion (right and left).The materials were freshly mixed in accordance with the manufacturer's instruction. All rats were injected with .05ml of each of the three sealers and distilled water at four predesignated sites which were specifically encircled. Once procedure was completed each group of animals were caged in separate cages. Post-operatively local examination of sites was done for detection of infection.

The study was conducted in four parts; the rats were sacrificed at different time intervals after injecting the test sealers, five rats after one day (Group A), five rats after 5 days (Group B), five rats after 10 days and another five after 30 days (Group D) by anesthetic over dosage. Biopsy (specimen and 2mm of surrounding

normal tissue) of the injection sites were taken. Macroscopically examined for inflammation or any other abnormalities and placed in 10% formalin. The tissue was processed to be embedded in paraffin after48 hrs of fixation. The blocks were cut to thickness of 6 micrometer . The sections were mounted on glass slides and were stained with haematoxylin and eosin. The histopathological evaluations of specimens were performed by pathologist under light microscope.

For each material, the sum and average of inflammatory cells (polymorphonuclear leukocytes (PMNs), plasma cells, lymphocytes, macrophages and giant cells) and fibroblasts were determined in ten separate areas at 400x magnification. The observer was blinded to the tissue source. Reactions in the tissue were scored as: 0, none or few inflammatory cells (no reaction); 1, less than 25 cells (mild reaction); 2, between 25 and 125 cells (moderate reaction); and 3, 125 or more cells (severe reaction) Results were statistically analyzed using the Kruskal-Wallis and Wilcoxin Signed rank test

III. Materials

3.1Sealapex

A severe reaction was observed on the 1st day .The tissue was disorganized and infiltrated with neutrophils, but there were no giant cells or areas of necrosis (Figures 1A). On the 5th day, the tissue was more organized and was characterized by the presence of chronic cells and absence of fibrous capsule formation and areas of necrosis (Figures 1B). The intensity of the reaction was milder on the 10th day (Figures1C) .At 30th day moderate amount of chronic inflammatory cells with mild amount of fibrosis was seen (Figure 1D)

3.2Tubliseal

A severe inflammatory reaction with some edematous tissue was seen on the 1stday. The tissue was infiltrated with neutrophils and few macrophages. There were no giant cells or areas of necrosis (Figures 2A). The intensity of the inflammatory reaction was milder on the 5th day and the tissue was more organized exhibiting the formation of connective fibers. The tissue was infiltrated with macrophages, plasma cells and lymphocytes. There were no giant cells or area of necrosis and organization of a fibrous capsule was observed in this period of time (Figures 2B). On the 10th day, granulation tissue with muscle necrosis was seen(Figure 2 C). There inflammatory reaction was more severe on the 30th day characterized by presence of chronic cells including giant cells (Figures 2D).

3.3 Diaket

Mild acute inflammation without areas of edema was observed on the 1stday. The inflammatory reaction was characterized by presence of neutrophil (Figures 3A). The intensity of the reaction was attenuated at the 5th day and chronic inflammatory cells were predominantly observed. The tissue was in an initial state of organization with presence of few fibroblasts and connective fibers (Figures 3B). On the 10th day, connective tissue with fibers and few fibroblasts was observed and a fibrous capsule tissue was present. Macrophages and giant cells with material in their cytoplasm were also observed (Figures 3C).On 30th day chronic inflammatory reaction with mild amount of muscle necrosis and moderate amount of fibrosis and foreign body reaction was seen. Tissue reaction showed a transition stage to develop into granuloma (Figure 3D).

3.4 Distilled water

On the 1st day mild inflammatory reaction with tissue distension was observed (Figure 4A), by 30th day inflammatory reaction subsided completely (Figure 4D)

IV. Evaluation Periods

4.1 Day 1

All the inflammatory cells except for oedema have significant association with the material used. Oedema,could more be due to the surgical trauma than due to injection of material and is found more with the Tubliseal and the variation is statistically significant at 0.05 level(Table 1).Comparison of acute inflammation at day 1 based on the material used is given in Table1. Severity of PMNLs is more in Sealapex (3 severe and1 moderate, mean score 2.2), followed by Tubliseal (mean score 1.4) and least in Distilled water (4 mild, mean score=0.8). The Kruskal Wallis Test ($p>0.05$) shows that the variation in severity of PMNLs among the four materials is not statistically significant (Table 2).

The severity of eosinophils is also found slightly high among Sealapex, but the variation of severity in eosinophils found in the materials is not statistically significant ($p>0.05$).

When considering the severity in lymphocytes, it is high (mean= 1.2) in Sealapex (4 mild and 1 moderate) and no inflammation found among Tubliseal and I - mild inflammation found in Diaket and Distilled water. The Kruskal Wallis Test ($p<0.01$) shows that the material used is significantly associated with the level

of inflammation in lymphocytes. The pair wise comparison shows that the inflammation in lymphocytes is significantly high in Sealapex when compared with all the other three materials ($p<0.05$).

4.2 Day 5

The distribution of the scores attributed to the materials is given on Table 3. None of the neither acute nor chronic inflammatory cells showed any significance in relation to the experimental materials. Edema is the only significant tissue reaction during this period ($p<.05$), (Table 4) was more in relation to Diaket than Tubliseal and was insignificant ($p>.05$) in relation to other two test materials.

4.3 Day 10

The distribution of the scores attributed to the materials is given on Table 5.significant amount of lymphocytes were seen in association with all the test materials ($p=.014$). In all test materials including control, statistically significant ($p<.05$) amount of lymphocytes were found. The lymphocytic proliferation was seen highest in case of Diaket when compared to other two materials (Table 6).Edema is still persistent and evident in this time period with all materials and it is most severe with Diaket and Tuliseal ($p<.05$).Another tissue reaction that is significant is muscle necrosis ($p=.013$), it is relatively more in Sealapex ($p<.05$) than in other two test samples.

4.4 Day 30

At 30th day both acute and chronic cells is seen to negligible level and tissue has regained its normal health or healing has started (Table 7).Only tissue reaction that is evident is muscle necrosis ($p=.018$) Muscle necrosis is significant in Diaket and Tubliseal ($p<.05$).

V. Discussion

Several studies have evaluated sealer cytotoxicity using in vitro cell culture assay, implantation into muscle [3] and periradicular response [4]. In vivo tests are based on clinical and histological evaluation of tissue responses.

In the present study, materials were injected subcutaneously into the tissue in a controlled manner [5]. All sealers used in this study were aggressive to the subcutaneous tissue in the beginning of the experiment. The inflammatory reaction, however, become milder on the 30th day [6] .A stronger action of the sealers in the beginning and annulling of the inflammatory response over time have been reported elsewhere [7].

On the 1st day, the reaction observed to all sealers was more likely due to the surgical trauma rather than caused by the materials' toxicity [6, 7]. However, it allowed evaluating the behavior of the materials along the experimental time and during the natural skin healing process as the initial period [8, 9, and 10]. At this time, the tissue was disorganized and infiltrated with neutrophils, which is consistent with the findings of other studies [11, 12].

On the 5th day, the tissue was disorganized and infiltrated with neutrophils, which is consistent with the findings of other studies[13].A combination of acute and chronic inflammatory cells were seen[14,15]. On the 10th day, the tissue was more organized in all sealer groups and was infiltrated with chronic cells, such as macrophages, lymphocytes and plasma cells. Fibrous capsule formation was observed only with Diaket [16,17,18] and Tubliseal[19,20,21].

On the 30th day, although the tissue inflammatory reaction to all sealers was milder than that observed on the 1st day after injection, it was still present. Surrounding Tubliseal [22], a persistent inflammatory response was observed, which has already been reported. This could be attributed to eugenol release from this material whose eugenol content is high right after mixing, but decreases with time [24, 25]. Zinc-oxide-and-eugenol-based sealers have residual eugenol after mixing. As previously stated, this residual eugenol (>5%) is sufficient to cause an inflammation. In the present study freshly mixed Diaket was placed directly into the tissue [26, 27].At the end of 30 days, histopathological observation exhibited chronic inflammatory reaction with mild amount of necrosis and foreign body reaction. The cytotoxicity did not decrease proportional to their setting time as does that of zinc oxide eugenol based sealer tested under the same parameter[28,29,].Therefore the possibility that these type of sealers could irritate periapical tissue for a longer period of time should be considered [30,31]Inflammatory reactions associated with seal apex is in equal magnitude as that of other sealers in the initial stage[32,33,34],but by around 10th day inflammatory cells subsided drastically and a healing potential has become active by around 30 days[35,36].

In view of the methodological differences among in vivo investigations, it is difficult to compare directly our results to those of previous studies [37, 38]. Further research should be conducted to contribute to the development of a root canal sealer that fulfills all properties of an ideal material.

VI. Figures And Tables

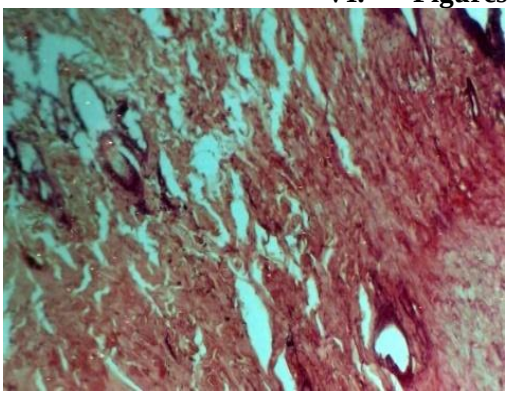

Fig 1A

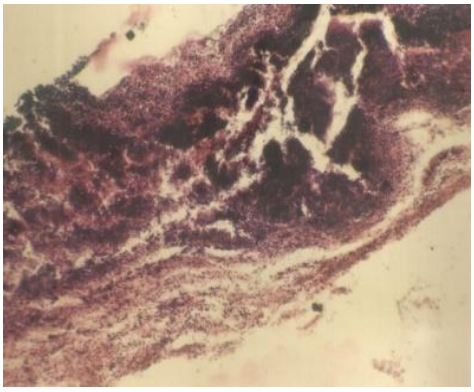

Fig 1 B

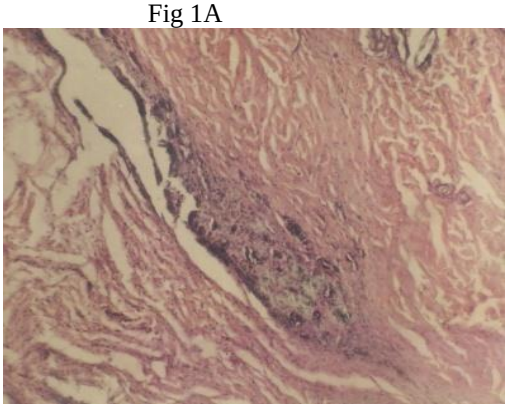

Fig 1C

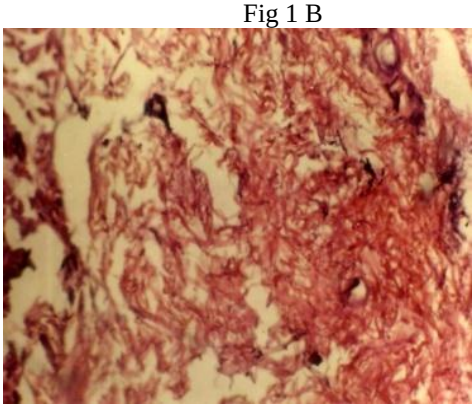

Fig 1D

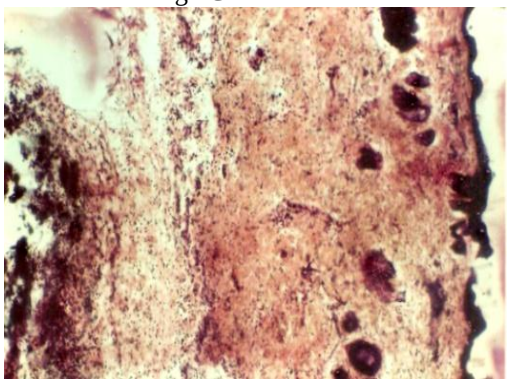

Fig 2A

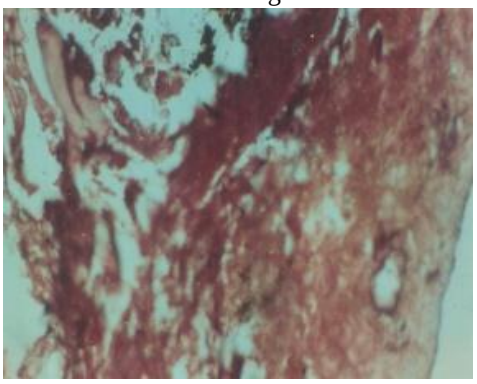

Fig 2B

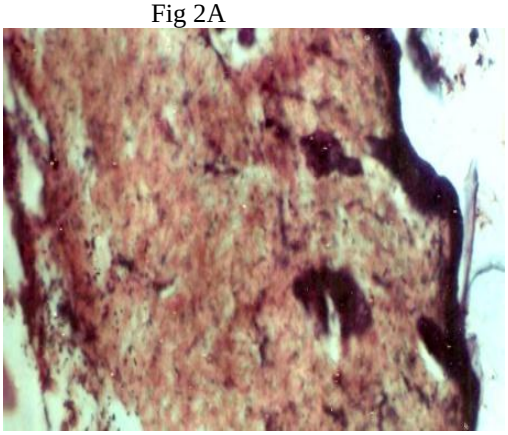

Fig 2C

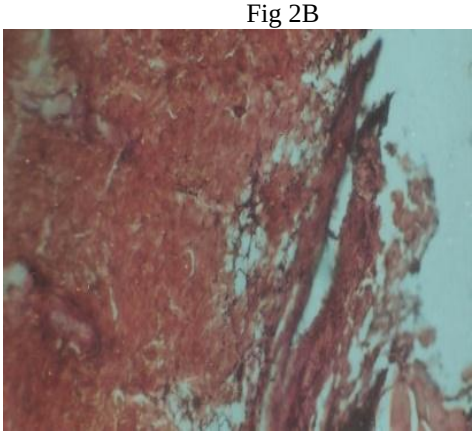

Fig 2D

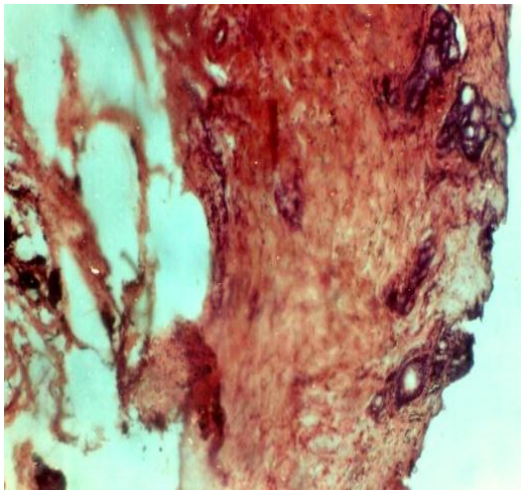

Fig 3A

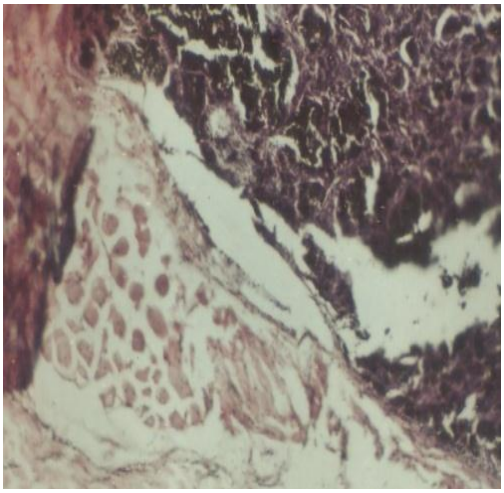

Fig 3B

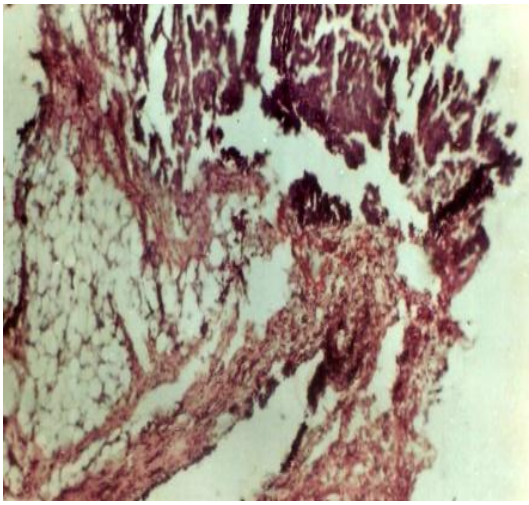

Fig 3C

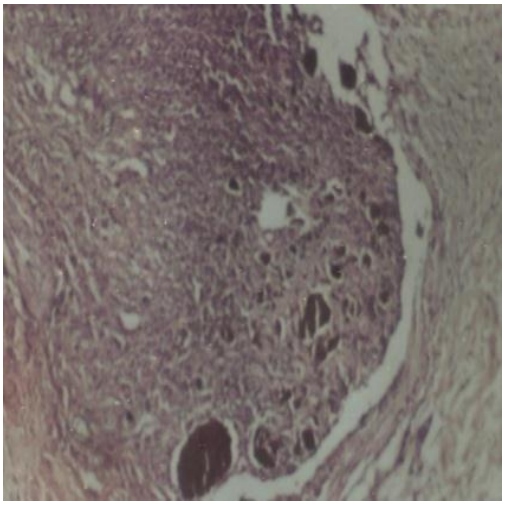

Fig3D

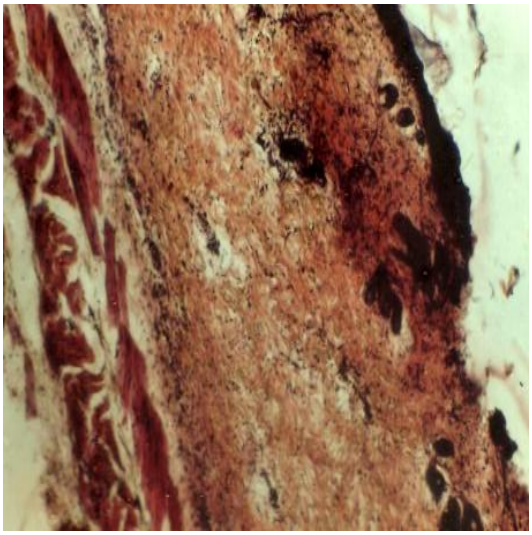

Fig 4A

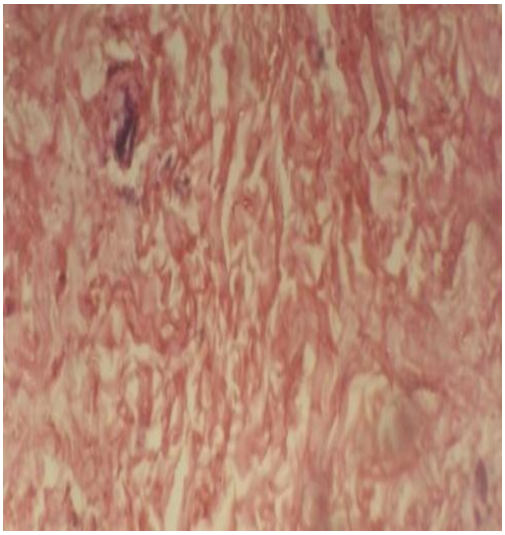

Fig 4D

TABLE.1 Comparison of acute inflammation among the material used at day1

Inflamation	Severity	Sealapex	Diaket	Tubliseal	Distilled water	Z#	p
PMNLs	Nil	1	1	1	1	3.59	0.166
	Mild	0	3	1	4		
	Moderate	1	1	3	0		
	Severe	3	0	0	0		
	Mean ± SD	2.2 ± 1.3	1 ± 0.71	1.4 ± 0.89	0.8 ± 0.45		
Eosinophils	Nil	1	4	4	5	5.12	0.077
	Mild	3	1	1	0		
	Moderate	1	0	0	0		
	Mean ± SD	1 ± 0.71	0.2 ± 0.45	0.2 ± 0.45	0 ± 0		
lymphocytes	Nil	0	4	5	4	10.91**	0.004
	Mild	4	1	0	1		
	Moderate	1	0	0	0		
	Mean ± SD	1.2 ± 0.45	0.2 ± 0.45	0 ± 0	0.2 ± 0.45		

Kruskal Wallis Test

Lymphocytes : SealapexvsDiaket Z= 2.4, $p<0.05$ (Mann-Whitney U test)
SealapexvsTubliseal Z= 2.9, $p<0.01$
Sealapexvs Distilled water Z= 2.4, $p<0.05$

TABLE 2. Comparison of chronic inflammation among the material used at day1

Inflamation	Severity	Sealapex	Diaket	Tubliseal	Distilled water	Z#	p
Plasma cell	Nil	5	4	4	5	1.08	0.584
	Mild	0	1	1	0		
	Mean ± SD	0 ± 0	0.2 ± 0.45	0.2 ± 0.45	0 ± 0		
Histiocytes	Nil	4	4	5	4	1.08	0.584
	Mild	1	1	0	1		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0 ± 0	0.2 ± 0.45		
Giant cells	Nil	4	4	5	4	1.08	0.584
	Mild	1	1	0	1		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0 ± 0	0.2 ± 0.45		
Oedema	Nil	5	2	1	5	7.22*	0.027
	Mild	0	3	1	0		
	Moderate	0	0	3	0		
	Mean ± SD	0 ± 0	0.6 ± 0.55	1.4 ± 0.89	0 ± 0		
Granulation tissue	Nil	4	4	4	4	0	1.000
	Mild	1	1	1	1		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0.2 ± 0.45	0.2 ± 0.45		
Muscle necrosis	Nil	4	4	4	4	0	1.000
	Mild	1	1	1	1		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0.2 ± 0.45	0.2 ± 0.45		
Fibrosis	Nil	4	5	4	4	1.08	0.584
	Mild	1	0	1	1		
	Mean ± SD	0.2 ± 0.45	0 ± 0	0.2 ± 0.45	0.2 ± 0.45		
Foreign body reaction	Nil	4	4	4	5	0	1.000
	Mild	1	1	1	0		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0.2 ± 0.45	0 ± 0		

Kruskal Wallis Test

TABLE 3 Comparison of acute inflammation among the material used at day 5

Inflamation	Severity	Sealapex	Diaket	Tubliseal	Distilled water	Z#	p
PMNLs	Nil	1	1	1	4	4.36	0.113
	Mild	0	4	1	1		
	Moderate	1	0	3	0		
	Severe	3	0	0	0		
	Mean ± SD	2.2 ± 1.3	0.8 ± 0.45	1.4 ± 0.89	0.2 ± 0.45		
Eosinophils	Nil	5	4	5	4	2	0.368
	Mild	0	1	0	1		
	Mean ± SD	0 ± 0	0.2 ± 0.45	0 ± 0	0.2 ± 0.45		
Lymphocytes	Nil	1	1	1	5	0	1.000
	Mild	4	4	4	0		
	Mean ± SD	0.8 ± 0.45	0.8 ± 0.45	0.8 ± 0.45	0 ± 0		

Kruskal Wallis Test

TABLE 4 Comparison of acute and chronic inflammation among the material used at day 5

Inflamation	Severity	Sealapex	Diaket	Tubliseal	Distilled water	Z#	p
Plasma cell	Nil	5	5	5	5	0	1.000
Histiocytes	Nil	4	4	5	4	1.08	0.584
	Mild	1	1	0	1		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0 ± 0	0.2 ± 0.45		
Giant cells	Nil	5	4	4	4	1.08	0.584
	Mild	0	1	1	1		
	Mean ± SD	0 ± 0	0.2 ± 0.45	0.2 ± 0.45	0.2 ± 0.45		
Oedema	Nil	4	1	5	4	7.28*	0.026
	Mild	1	4	0	1		
	Mean ± SD	0.2 ± 0.45	0.8 ± 0.45	0 ± 0	0.2 ± 0.45		
Granulation tissue	Nil	4	4	4	5	0	1.000
	Mild	1	1	1	0		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0.2 ± 0.45	0 ± 0		
Muscle necrosis	Nil	1	1	1	5	0	1.000
	Mild	4	4	4	0		
	Mean ± SD	0.8 ± 0.45	0.8 ± 0.45	0.8 ± 0.45	0 ± 0		
Fibrosis	Nil	4	2	1	4	5.32	0.070
	Mild	1	3	1	1		
	Moderate	0	0	3	0		
	Mean ± SD	0.2 ± 0.45	0.6 ± 0.55	1.4 ± 0.89	0.2 ± 0.45		
Foreign body reaction	Nil	4	4	4	4	0	1.000
	Mild	1	1	1	0		
	Moderate	0	0	0	1		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0.2 ± 0.45	0.4 ± 0.89		

Kruskal Wallis Test

Oedema :DiaketVsTubliseal Z= 2.5, p<0.05

TABLE 5 Comparison of acute inflammation among the material used at day 10

Inflamation	Severity	Sealapex	Diaket	Tubliseal	Distilled water	Z#	p
PMNLs	Nil	4	4	3	4	0.64	0.727
	Mild	1	1	2	1		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0.4 ± 0.55	0.2 ± 0.45		
Eosinophils	Nil	4	4	4	5	0	1.000
	Mild	1	1	1	0		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0.2 ± 0.45	0 ± 0		
Lymphocytes	Nil	1	0	1	4	8.47*	0.014
	Mild	0	0	1	1		
	Moderate	4	1	3	0		
	Severe	0	4	0	0		
	Mean ± SD	1.6 ± 0.89	2.8 ± 0.45	1.4 ± 0.89	0.2 ± 0.45		

Kruskal Wallis Test

Lymphocytes : SealapexvsDiaket Z= 2.4, p<0.05
Sealapexvs Distilled water Z= 2.2, p<0.01
DiaketVsTubliseal Z= 2.5, p<0.05
DiaketVs Distilled wate Z= 2.8, p<0.01
TubliseaVs Distilled wate Z= 2.0, p<0.05

TABLE 6 Comparison of acute and chronic inflammation among the material used at day 10

Inflamation	Severity	Sealapex	Diaket	Tubliseal	Distilled water	Z#	p
Plasma cell	Nil	1	1	2	5	1.14	0.565
	Mild	3	4	3	0		
	Moderate	1	0	0	0		
	Mean ± SD	1 ± 0.71	0.8 ± 0.45	0.6 ± 0.55	0 ± 0		
Histiocytes	Nil	1	1	1	4	0	1.000
	Mild	4	4	4	1		
	Mean ± SD	0.8 ± 0.45	0.8 ± 0.45	0.8 ± 0.45	0.2 ± 0.45		
Giant cells	Nil	2	1	1	5	0.64	0.727
	Mild	3	4	4	0		
	Mean ± SD	0.6 ± 0.55	0.8 ± 0.45	0.8 ± 0.45	0 ± 0		
Oedema	Nil	5	0	0	4	11.54**	0.003
	Mild	0	4	4	1		
	Moderate	0	1	1	0		
	Mean ± SD	0 ± 0	1.2 ± 0.45	1.2 ± 0.45	0.2 ± 0.45		

Granulation tissue	Nil	0	1	1	1	0.78	0.679
	Mild	1	1	1	4		
	Moderate	4	3	3	0		
	Mean ± SD	1.8 ± 0.45	1.4 ± 0.89	1.4 ± 0.89	0.8 ± 0.45		
Muscle necrosis	Nil	0	1	1	4	8.63*	0.013
	Mild	1	4	4	1		
	Moderate	4	0	0	0		
	Mean ± SD	1.8 ± 0.45	0.8 ± 0.45	0.8 ± 0.45	0.2 ± 0.45		
Fibrosis	Nil	4	1	2	4	3.5	0.174
	Mild	1	4	3	1		
	Mean ± SD	0.2 ± 0.45	0.8 ± 0.45	0.6 ± 0.55	0.2 ± 0.45		
Foreign body reaction	Nil	0	0	0	5	0	1.000
	Mild	4	4	4	0		
	Moderate	1	1	1	0		
	Mean ± SD	1.2 ± 0.45	1.2 ± 0.45	1.2 ± 0.45	0 ± 0		

Kruskal Wallis Test

Oedema : Sealapex vs Diaket Z= 2.9, p<0.01
Sealapex vs Tubliseal Z= 2.9, p<0.01
Diaket Vs Tubliseal Z= 2.4, p<0.05
Tubliseal Vs Distilled water Z= 2.4, p<0.05

Muscle necrosis : Sealapex vs Diaket Z= 2.4, p<0.05
Sealapex vs Tubliseal Z= 2.4, p<0.05
Sealapex vs Distilled water Z= 2.7, p<0.01

TABLE 7 Comparison of acute inflammation among the material used at day 30

Inflamation	Severity	Sealapex	Diaket	Tubliseal	Distilled water	Z#	p
PMNLs	Nil	4	4	4	5	0	1.000
	Mild	1	1	1	0		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0.2 ± 0.45	0 ± 0		
Eosinophils	Nil	5	3	5	4	4.31	0.116
	Mild	0	2	0	1		
	Mean ± SD	0 ± 0	0.4 ± 0.55	0 ± 0	0.2 ± 0.45		
Lymphocytes	Nil	1	0	1	5	3.65	0.161
	Mild	1	1	0	0		
	Moderate	3	1	4	0		
	Severe	0	3	0	0		
	Mean ± SD	1.4 ± 0.89	2.4 ± 0.89	1.6 ± 0.89	0 ± 0		

Kruskal Wallis Test

Table Comparison of acute and chronic inflammation among the material used at day 30

Inflamation	Severity	Sealapex	Diaket	Tubliseal	Distilled water	Z#	p
Plasma cell	Nil	1	1	2	4	0.64	0.727
	Mild	4	4	3	1		
	Mean ± SD	0.8 ± 0.45	0.8 ± 0.45	0.6 ± 0.55	0.2 ± 0.45		
Histiocytes	Nil	1	2	1	5	0.64	0.727
	Mild	4	3	4	0		
	Mean ± SD	0.8 ± 0.45	0.6 ± 0.55	0.8 ± 0.45	0 ± 0		
Giant cells	Nil	1	1	1	4	0	1.000
	Mild	4	4	4	1		
	Mean ± SD	0.8 ± 0.45	0.8 ± 0.45	0.8 ± 0.45	0.2 ± 0.45		
Oedema	Nil	5	5	5	4	0	1.000
	Mild	0	0	0	1		
	Mean ± SD	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.45		
Granulation tissue	Nil	4	4	4	1	0	1.000
	Mild	1	1	1	4		
	Mean ± SD	0.2 ± 0.45	0.2 ± 0.45	0.2 ± 0.45	0.8 ± 0.45		
Muscle necrosis	Nil	5	1	1	5	8*	0.018
	Mild	0	4	4	0		
	Mean ± SD	0 ± 0	0.8 ± 0.45	0.8 ± 0.45	0 ± 0		
Fibrosis	Nil	1	0	1	1	5.55	0.062
	Mild	4	1	1	4		
	Moderate	0	4	3	0		
	Mean ± SD	0.8 ± 0.45	1.8 ± 0.45	1.4 ± 0.89	0.8 ± 0.45		
Foreign body reaction	Nil	1	1	1	5	4.65	0.098
	Mild	4	0	0	0		
	Moderate	0	4	4	0		

	Mean ± SD	0.8 ± 0.45	1.6 ± 0.89	1.6 ± 0.89	0 ± 0		

Kruskal Wallis Test

Muscle necrosis : SealapexvsDiaket Z= 2.5, p<0.05
SealapexvsTubliseal Z= 2.5, p<0.05
DiaketVs Distilled wate Z= 2.5, p<0.05
TublisealVs Distilled wate Z= 2.5, p<0.05

VII. Conclusion

Under the tested conditions, it may be concluded that the sealers had a similar pattern of irritation, which was more severe in the beginning and milder with time, in such a way that all sealers showed a persistent mild reaction. Sealapex yielded better tissue organization than Diaket and Tubliseal. Diaket showed a persistent chronic inflammation till a period of 30 days, while for .Tubliseal the tissue reaction became negligible at the end of study period.

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