Evaluation of Propolis from Kurdistan region as a new resinous sealer in root canal obturation-part I biocompatibility study

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ABSTRACT

Background: Many materials were proposed as root canal obturating materials but the biocompatibility issue remains to be a critical one. Propolis has been used as a therapeutic agent since the time of Hippocrates. It is known that propolis exhibits some pharmacological activities, such as antibacterial, antiviral, antifungal and anti inflammatory activity.

Materials and methods: Eighteen albino rats were used in the study and divided randomly into three groups of 6 animals for each group. Each group was scheduled to be sacrificed at different time periods, which were three days, one week and three weeks. Propolis and ZOE sealer implants of 4mm in diameter and 0.5 gm in weight were implanted in the dorsal side of the rats. At the end of the implantation, the rats were scarified and the histopathological picture was made to the implantation site.

Results: Zinc oxide eugenol sealer showed severe inflammation after 3 days of implantation whci subsided after 7 days. After 21 days, moderate inflammatory reaction was evident. Propolis presented moderate reaction after 3 and 7 days but with presence of signs of collagen fiber formation. After 21 days, connective tissue capsule was present.

Conclusion: Propolis presented better biocompatibility than zinc oxide eugenol sealer.

Key words: Propolis, biocompatibility. (J Bagh Coll Dentistry 2013; 25(3):8-13).

INTRODUCTION

Root canal treatment aims to eliminate infection of the root canal and to completely fill the canal space in order to prevent apical and coronal penetration of liquids and microorganisms. Various methods have been proposed for root canal filling. The most frequently used methods use semisolid materials such as gutta-percha in combination with a root canal sealer or paste [(1)]. Biological compatibility of root canal sealers is of importance as these materials come into contact with periapical tissues including fibroblasts. The tissue response to these materials may influence the final outcome of root canal treatment [(2)]. Several different methods have been described for assessing tissue toxicity. One of them evaluates the biocompatibility of endodontic sealers in subcutaneous tissue of rats using by implantation of polyethylene tubes filled with the material to be tested. This method is simple and easy to be reproduced and standardized [(3, 4)].

Propolis, or 'bee glue', is a complex resinous mixture of plant-derived products gathered, modified and used by bees as a general purpose sealer, draught excluder and antibiotic in their hives.

Propolis typically consists of waxes, resins, water, inorganics, phenolics and essential oils [(5)]. Propolis has been used as a therapeutic agent by the world population since the time of Hippocrates. It is known that the ethanol extract of propolis (EEP) exhibits some pharmacological activities, such as antibacterial, antiviral, antifungal, anti inflammatory, anesthetic and cytostatic properties [(6,7)].

Few studies have been conducted, mainly on animals and to a lesser extent on humans, to investigate the use of propolis in different dental fields [(8-10)]. The aim of this study was to estimate biocompatibility of ethanolic extract of propolis collected from Iraqi Kurdistan region to be used as endodontic sealer.

MATERIALS AND METHODS

In this study 18 male, six week old albino rats were used to evaluate subcutaneous biocompatibility of Propolis as endodontic sealer compared with commercially available ZOE sealer. Their weights ranged from 150-200 grams. They were kept in the animal house of college of Medicine / Hawler Medical University. The room temperature was maintained at 25^{o}C. A 12 hr light/dark cycle was set. Rodent food rich in nutrient and tap water were used as bedding.

The animals were divided randomly into three groups of 6 animals for each group. Each group was scheduled to be sacrificed at different time

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periods, which were three days, one week and three weeks.

Anesthesia and surgical procedure

The surgical procedure was done under general anesthetic drug by intraperitoneal injection of 50mg/Kg (B.W.) Ketamine Hydrochloride. Autoclave was used to sterilize all instruments with a pressure of 15 lb/sq in above atmospheric pressure to obtain a temperature of 121°C for 15 minutes.

The dorsal skin was shaved, disinfected with 5% tincture of iodine, and small incisions, approximately 15 mm long, were made with a number ten blade, in both sides of the dorsum. Two separate pockets were created by blunt dissection to implant the test materials in subcutaneous tissue to a depth of 15 mm to avoid interference of suture and the healing process of the skin wound.

Propolis and ZOE sealer implants 4mm in diameter and 0.5 gm in weight were prepared and sterilized by ultraviolet radiation (over night). The Propolis was implanted in the left pocket while the ZOE sealer was implanted in right pocket of each rat (Figure 1).

Figure 1: Implantation of the test material.

After implantation, margins of the wound were joined and closed with interrupted suture (4-0 black silk sutures) distant from the material for a perfect cooptation. After suturing, asepsis was performed again.

Post-operatively, the animal was kept under observation till recovery from anesthesia. Later on, no re-operations were required and no wound dehiscence occurred. Sutures on the dorsal surface of the third group which were to be scarified after three weeks were lifted after seven days.

2. Termination

At the end of the experimental periods (3, 7 and 21 days), the animals were sacrificed by anesthetic overdose (chloroform) in glass jar. The skin overlaying the implants was shaved and a standard quadrangular incision was performed 10 mm away from the suture line and down to the subcutaneous tissue. Then, an incision was made parallel to the skin surface. The implants with surrounding tissue were removed from the rats, immersed in 10 % formalin solution and fixed for 24 hours (Figure 2).

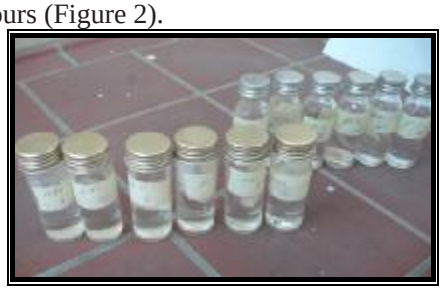

Figure 2: Fixation of the implants in 10% formalin.

3. Histotechnical procedures

After fixation, the tissues were processed by paraffin embedding and then longitudinally sectioned through the implants. Serial sections approximately 5µm thick were obtained from each specimen. The specimens were placed on microscopic slides. After proper attachment, the paraffin was removed by means of xylene and the sections were dehydrated with decreasing concentrations of ethanol and deionized water. Finally the sections were stained with hematoxylin and eosin (Figure 3).

Figure 3: The prepared slides of the implanted tissues.

The histological sections were analyzed at different magnifications (40X and 400X) under a digital biological microscope, noting tissue reactions on the sealer–connective tissue interface. All specimens underwent blinded examination by a single examiner who did not know which sealer or which period was being examined.

The criteria of inflammation were estimated by counting the number of inflammatory cells using eye piece graticule. Inflammatory cells were counted in thirteen squares on the grid in an N letter pattern (Figure 4). The severity of

inflammation was scored into the following grades according to the highest number which may be counted into the following grades:-

- No inflammation 0-20 (Normally found inflammatory cells).
- Mild 21-111 (increasing by 90).
- Moderate 112-202
- Sever 203-292

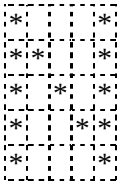

Figure 4: Eye piece graticule.

RESULTS

Results of zinc oxide eugenol sealer implantation

Three days after implantation, panoramic view of the experimental area shows a hole that represents the exact position of the tested material which has been degraded during slide preparation. Remnants of tested material can be seen on the periphery of the hole which is followed by region of sever tissue deformation, losing of normal architectures of the connective tissue and heavy infiltration of inflammatory cells (Figure 5,6).

The higher magnification shows sever inflammatory reaction (no. of 286 cells) with complete invasion of collagen fibers with inflammatory cells and many dilated blood vessels that contain RBC and inflammatory cells (Figure 7).

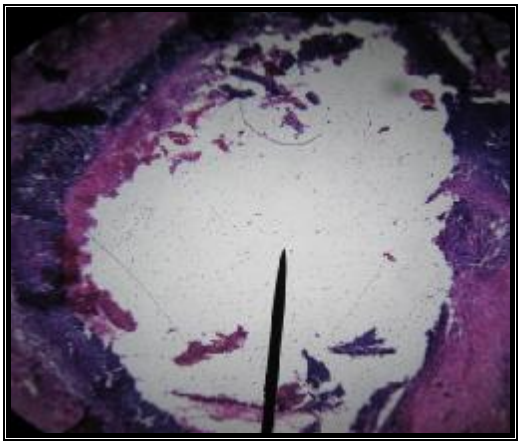

Figure 5: Panoramic view of three day ZOE implantation hole representing the exact position of the tested material. (H&Ex25)

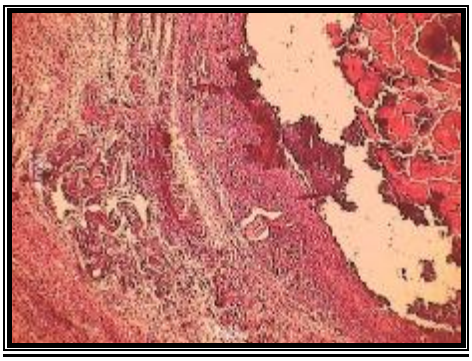

Figure 6: Three days ZOE implant showing sever inflammatory cell infiltration and dilated blood vessels. (H&E x 40).

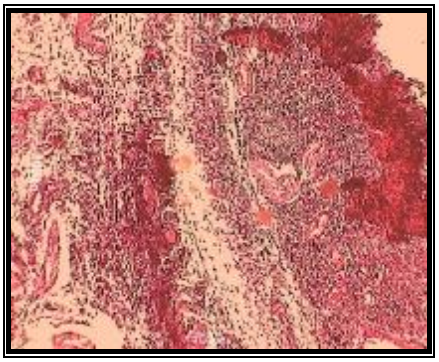

Figure 7: Three days of ZOE implanted with sever inflammatory cell infiltration and dilated blood vessels. (H&E x 100).

Seven days after implantation, although the hole created by the insertion of the test material was reduced but the area still shows sever inflammation with the heavy inflammatory cells, focus of necrosis and complete hylanization with complete loss of the tissue architectures (Figure 8). The degree of inflammation gradually reduced as we move away from tested position; many dilated blood vessels are seen and the region filled with multinuclear macrophages.

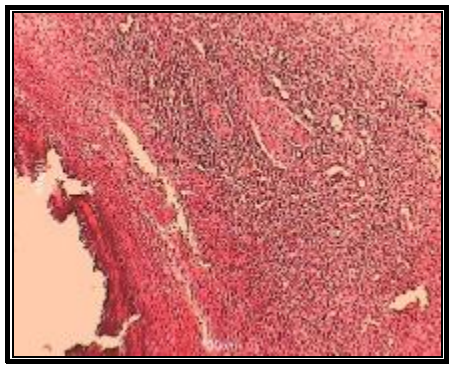

Figure 8: Seven days ZOE implant showing the area still shows sever inflammation with the heavy inflammatory cells (H&E x 100).

The hole created by the tested material was still present twenty one days following the implantation indicating the material still not resorbed. The inflammatory reaction became moderate (number of inflammatory cells 195). The periphery of the region shows highest intensity inflammatory reaction which diminishes as we move away together with high number of fibroblast and many newly formed collagen fibers. Angioblast can also be seen with budding of new blood vessels that indicates the repair and healing process. (Figure 9,10).

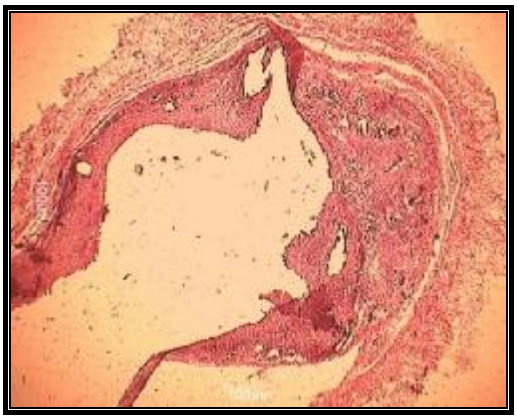

Figure 9: Twenty one days after implanting ZOE (H&E x 40).

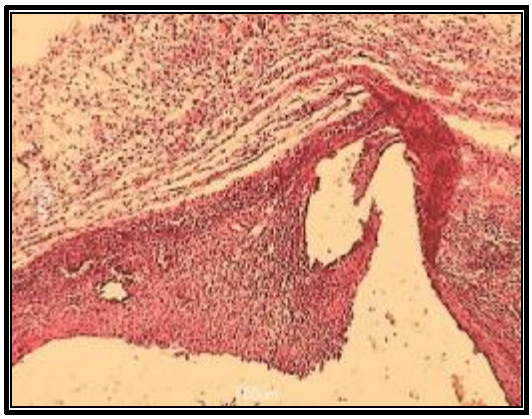

Figure 10: Twenty one days after implanting ZOE showing moderate inflammatory reaction. (H&E x 100)

Results of Propolis implantation

The panoramic view of the area of material insertion shows some trace amount of inserted material which was encircled by fibrous capsule that isolated the tested material from the affected area which is infiltrated by inflammatory cells and shows many congested blood vessels (Figure 11)
Increasing the power of magnification and by counting the number of inflammatory cells, the affected region exhibits moderate inflammatory reaction and the region mostly infiltrated by polymorphonucleocytes (PMNL) which also infiltrate the congested blood vessels in the region. Although the inflammatory reaction is still present next to the capsule, the area shows some regions of normal architectures with presence of collagen fiber (Figure 12).

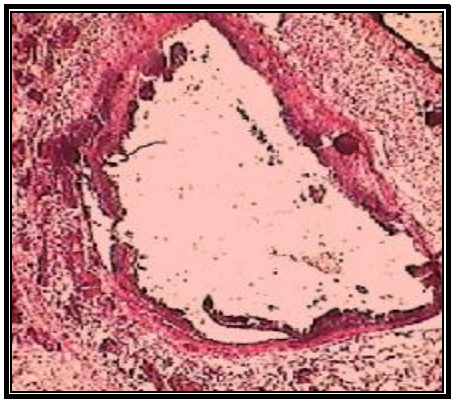

Figure 11: Three days Propolis implant encircled with fibrous capsule. (H&E x 40).

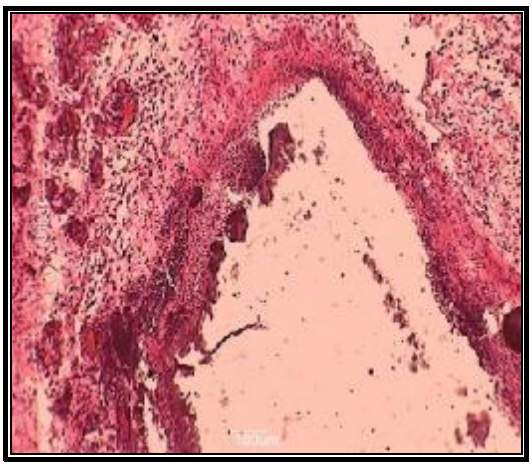

Figure 12: Three days Propolis implant. (H&E x 100).

Examining the section of seven day implant of Propolis revealed that the fibrous capsule encircling the tested material becomes more obvious with many collagen fibers that arranged in radial direction and isolating the tested material from affected region. In spite of moderate inflammatory reaction still seen with the infiltration of many macrophages, many fibroblasts are seen together with lot of normal bundles of collagen fibers (Figure 13, 14).
Histological findings after twenty one day implantation of Propolis shows that connective tissue capsule is still present and seems to be consist of many thin layers of connective tissue.

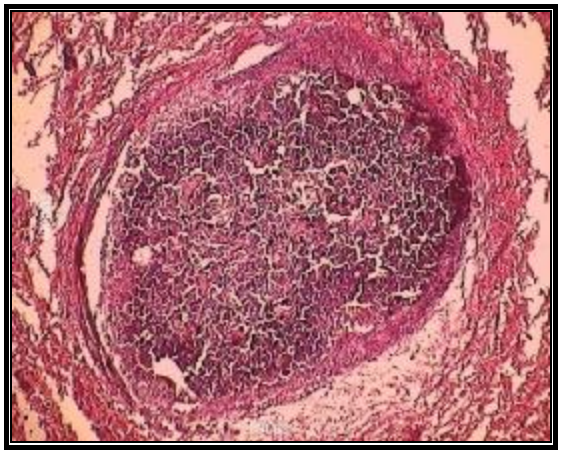

Figure 13: Seven days Propolis implant more obvious fibrous capsule. (H&E x 40).

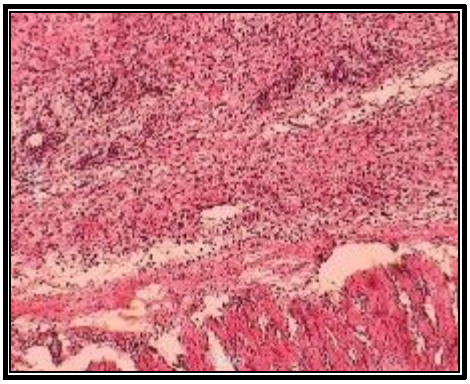

Figure 14: Seven days Propolis implant with moderate inflammatory reaction (H&E x 100).

The region next to the capsule shows moderate inflammatory reaction although it's obviously less than previous group. Thicker normal collagen fiber can be seen in the region as a result of fibroblastic proliferation together with angioblastic proliferation that results in the budding of new blood vessels (Figure 15).

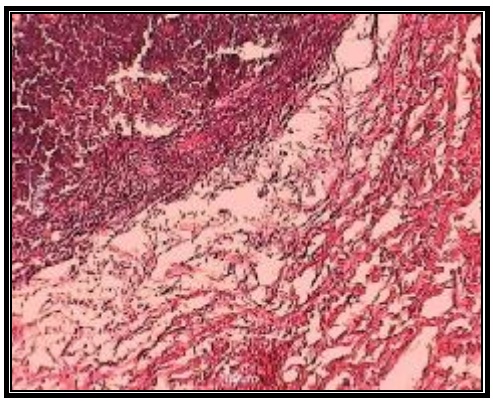

Figure 15: Twenty one days after implanting Propolis showing budding of new blood vessels (H&E x 100).

DISCUSSION

Results of this study revealed the three days control specimens (zinc oxide eugenol) with sever inflammatory reaction with complete invasion of collagen fibers with inflammatory cells and dilated blood vessels. On the other hand, the experimental specimens (Propolis) revealed fibrous capsule that encircled and isolated the tested material with moderate inflammatory reaction mostly infiltration by polymorphonuceocytes (PMNC) with congested blood vessels. The area next to the capsule showed also some regions of normal architectures with presence of collagen fibers. The reaction observed to the three days specimens may be more likely due to the surgical trauma rather than caused by the materials' toxicity. However, it allowed evaluating the behavior of the materials along the experimental time and during the natural skin healing process as the initial period. At this time, the tissue was disorganized and infiltrated with neutrophils, which is consistent with the findings of the study by Gomes-Filho *et al* [(11)].

On the seventh day, the areas of zinc oxide eugenol implantation still showed sever inflammation although the hole created by the insertion of the tested material was reduced. But the degree of inflammation was gradually reduced moving away from the tested position with presence of many dilated blood vessels and multinuclear macrophages. When the Propolis specimens scrutinize after seven days, the fibrous capsule was more obvious with moderate inflammatory reaction with infiltration of many macrophages and lot of fibroblasts.

Observing both specimens after three weeks revealed mild inflammatory reaction (number of inflammatory cells=195), although the periphery of the region implanted by zinc oxide eugenol showed higher intensity inflammatory reaction with high number of fibroblast and newly formed collagen fibers and new blood vessels away from periphery which indicates healing process. The connective tissue capsule around Propolis was still present with moderate inflammatory reaction in the region next to it although it was obviously less than the previous group with fibroblast proliferation together with budding of new blood vessels showing phenomena of healing.

Results obtained from this study regarding zinc oxide eugenol coincide with Gomes-Filho *et al* [(11)]; Economides *et al* (12) and Zafalon *et al* [(13)]. The reasonable explanation for this result may be that Tubliseal is a typical zinc oxide-eugenol sealer. Its' irritative ability could be attributed primarily to eugenol and secondarily to the zinc

ions that it contains. It should be noted that free eugenol is still present even after the sealer has been set and is available for release over an extended period.

End results regarding Propolis implantation comes in agreement with many studies as Castaldo and Capasso [14]; Farrĕ *et al* [15]; Orsi *et al* [16] and Al- Nema *et al* [10] since these studies have the same opinion that the activity of Propolis is dependant upon the chemical composition of Propolis samples collected in different geographical areas as well as the method of extraction. Some of these components may act synergistically. The exact mechanism underlying the positive effects of Propolis and its components is not fully understood and requires further experimental studies [14, 17].

According to the conditions of this study the Propolis extract that was tested may be considered as biocompatible.

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