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Evaluation of immune factors of blood serum after pulpectomy of dog's teeth using two root filling material

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ABSTRACT

Background and Purpose: This study aimed to evaluate the immune factors of blood serum (IgG, IgM, and IgE serum levels and complement components C3 and C4 and blood cells after pulpectomy of dog's teeth using two root fillers (ZOE and Chitosan). *Method*: The present study is a semi-experimental study conducted on 40 root canals in two groups (20 channels in 2 dogs for Chitosan and 20 channels equal to 2 dogs for ZOE). The healthy teeth of the first, second and third premolars of the lower and upper jaw (40 canals) in 4 dogs aged 12-18 months were selected for the treatment of pulpectomy. Before treatment and after 28 days, 5 cc of blood was taken from the dogs and the serum immune factors were studied. The mean values obtained from the two groups were compared using T-test. Data analysis was performed using SPSS software version 22. *Findings*: The rate of WBC changes between the two treatment groups with ZOE has increased compared to Chitosan (P = 0.03). The levels of C3 and C4 decreased more due to the use of ZOE than chitosan. The rate of IgG changes with ZOE increased compared to chitosan. The rate of change in IgM and IgE between the two treatments groups with ZOE was not significant compared to Chitosan. IgE levels in the chitosan-treated group did not change significantly (P> 0.9). The rate of IgE changes between the two treatment groups with ZOE and chitosan was not significant (P = 0.65). There was no change in the serum level of factors associated with red blood cells, including, number of red blood cells, HCT, MCV, MCH, and MCHC. The rate of albumin and total protein changes between the two treatment groups with ZOE was not significant compared to chitosan. *Conclusion*: In comparison between two root canal material, ZOE increased white blood cells and immunoglobulins. There was no difference in blood factors and protein levels between the two groups.

Keywords: Immunoglobulins, Complement, Dental pulpectomy, ZOE, Chitosan

1. INTRODUCTION

Pulp therapy is very important for preservation of primary teeth in children (Estrela et al., 2001). Decreased dimensions of the maxillary arch, overgrowth of the opposite tooth, asymmetry of the maxillary arch and chewing impairment and aesthetic are among the consequences of premature loss of primary teeth (Dean, 2016). An important goal of dental pulp treatments is to remove microorganisms from the root canal and prevent subsequent infection (Anderson et al., 1990). Pulpectomy is one of the types of pulp therapy that is indicated for teeth with evidence of irreversible inflammation of the pulp, chronic inflammation or necrosis of the root pulp (Ramar & Mungara, 2010; Meshki et al. 2020). The characteristics of ideal filling material for a primary tooth root canal are the same rate of resorption as the root canal resorption and compatibility with periapical tissues and permanent tooth buds. And if it is pushed over the apex, it will disappear quickly. It has antiseptic properties and easily fill root canals; it should beradiopaque and not changes the colour of the teeth. If necessary, it can be easily removed. Different materials have also been introduced to fill the roots of primary teeth, the most important and common of which is Zinc Oxide Eugenol (ZOE). This oxide is one of the most widely used materials for filling the root canal of primary teeth (Fuks et al., 2019). That is the first filling material for the root canal, which is recommended for primary teeth. It is also the only substance explicitly recommended in clinical guidelines prepared by the American Pediatric Dental Association (AAPD) until 2008 (Abrams, 1995). A dilute mixture of ZOE, a consistency in which the material flows easily, is usually made. The main components of this compound are Zinc Oxide and Eugenol. Eugenol is bactericidal and its effect is enhanced when combined with zinc oxide (Meeker et al., 1986), which alone does not have an inhibitory effect, thus preventing the cellular metabolism of bacteria (Anderson et al., 1990). Although it does not adhere to the structure of the tooth and can flood the surface of the tooth if it has the right viscosity (Abrams, 1995). Zinc Oxide Eugenol can be prepared as a concentrated or dilute mixture. This feature allows it to be used in a variety of ways to fill the root canal (Sakaguchi & Powers, 2012; Ahmed, 2019). The disadvantages of this matrial are as follows: Slow absorption (Nurko'i & Garcia-Godoy, 1999), irritation of periapical tissues (Erausquin & Muruzábal, 1967), Bone necrosis and cementum (Spedding, 1985), changing the direction of the permanent replacement teeth (Hendry et al., 1982). A new substance recently proposed for the treatment of root canals is chitosan, which is derived from the deacetylation of chitin and has good antimicrobial, biocompatible and anti-inflammatory properties (Sun et al., 2006). After cellulose, chitin (the main part of crustaceans) is the second most basic biological substance in nature that can act as an economic and ecological resource. Chitosan, a cationic polysaccharide, has an extraordinary anti-toxicity and biological compatibility. It has a positive charge and can bind to the negative charge of bacterial or fungal surfaces and destroy them by increasing its permeability. Also, it is effective against a wide range of bacteria and fungi, and possibly against viruses and tumour cells. It has anti-inflammatory effects and improves the immune response and tissue regeneration (Sun et al., 2006; Huang et al., 2016). Babashahi and his colleagues in a study that evaluated the filling of canals with 3% nano-chitosan compared to ZOE and Metapex with rotary methods compared with manual, showed that the POV of chitosan was different from the other two. The

average POV for the manual method was 91.46% and for the rotary method, it was 88.51%. So there was no significant difference. The space before filling the channel for the manual method was 3.89 mm^3 and for the rotary method was 3.26 mm^3, which showed a significant difference. Therefore, the effect of the type of material on POV is contrary to the effect of root length and channel formation methods (Babashahi et al., 2019). This study aimed to evaluate the immunological factors (Measurement of serum IgG, IgM, and IgE serum levels and complement components C3 and C4) of blood serum and blood cells after pulpectomy of dog teeth using two root fillers (ZOE and chitosan).

2. MATERIALS AND METHODS

The present study is a semi-experimental study performed on 40 root canals in two groups of 20 teeth (20 channels in 2 dogs for Chitosan and 20 channels equal to 2 dogs for ZOE dough). The study duration was 3 months, started from October 2019 to December 2019. The criteria for entering the study were dogs that were systematically perfectly healthy and had all of their premolar teeth present and free of decay and fracture. Dogs that were not systemically healthy and lacked premolar teeth were excluded from the study. To make 3% chitosan, 20 g of acetic acid solution (England-BDH) 1% v/v was mixed with 20 mg of chitosan powder (SIGMA, America) with a viscosity of less than 25 cp. The resulting mixture is kept in a closed container for 24 hours with the aim of complete hydration of chitosan. 0.2 g of triphenylphosphine (TPP) solution (United States-Acros Company) is weighed and brought to 20 g with distilled water. TPP is used to form nanoparticles. 10 grams of TPP 0.1% solution (v/v) was weighed to prepare chitosan nanoparticles. 20 g of 0.1% chitosan solution (v/v) was placed in a container with a magnetic stirrer at 1400 rpm while a magnet was inside the container. A 0.1% drip solution was added to the 0.1% chitosan solution to obtain a dark-coloured suspension, which is the same as the chitosan-TPP complex. Then barium sulfate (Pars peyvand, Iran) was used to create opacity in nanoparticles. 30 g of the TPP-chitosan suspension obtained from the third step was weighed and poured into a container. It then weighs 10% of the weight of the suspension, about 3 grams of barium sulfate. Slightly remove the solution and add the drops to the barium sulfate to form uniform dough. The residual chitosan-TPP suspension is then added to the resulting mixture. 0.9 g of chitosan powder (SIGMA, America) with medium viscosity 500 cp was added to the suspension slowly to form a paste in the final composition. After complete stirring, the gel was rested for 24 hours for complete hydration. After 24 hours, the nano-chitosan in 3% chitosan gel is ready to fill the roots. In the next step, a canine extraction deciduous tooth was selected as a pilot for pulp treatment. The access hole was prepared with a diamond bur. The canal was cleaned with K-file (Mani-Korea) with numbers 20, 25, 30 and 35. After each filing, it was rinsed with normal saline. Then the prepared root was filled with chitosan by injection. Radiography was then taken. Chitosan filled the canal but the opacity of obturation was not as well as ZOE.

The animals were selected according to the instructions of the Medical Ethics Committee on the use and care of laboratory animals. Dogs were the proposed animal for this study because, among carnivores, they had a similar human structure to the simple brachydont. But other animals (rodents) have hypsodont teeth. Also, among carnivores, long-term dogs are easier to maintain, while working with dogs is easier than cats (Lawson et al., 1960). The animals studied were four male dogs, ranging in age from 12 to 18 months, of a native breed that had previously been licensed by the Veterinary Ethics Committee (IR.AJUMS.ABHC.REC.1398.061). The animals were kept individually in two groups as 2 dogs with ZOE pulpectomy treatment and 2 dogs treated with chitosan pulpectomy at the dog storage facility individually and were fed standardized food and water. The dogs were prepared from dog breeding center and after entering the center, they were examined by a veterinarian and if the dog's health was confirmed and they had the conditions to enter the research, they were admitted to the quarantine unit. Combination vaccines were then administered to them according to standard protocols. Antibacterial treatment was also performed on the animals and they were kept in quarantine for ten days. Then, they were re-examined and, if confirmed, entered the nests of the main animals and were included in the plan (During the ten days of quarantine, they were examined twice by a veterinarian). The day before the main operation, radiography of the upper and lower jaws was taken to ensure the health of the tooth's roots. Then, the healthy premolar teeth of the first, second and third blades of the lower and upper jaws (40 canals) in four 12-18-month-old dogs with Iranian mixed breed were selected for the treatment of pulpectomy. The dog's first premolar teeth had one channel and the dog's second and third premolars had two channels each. 20 canals of the 6 upper and lower right premolar teeth (10 root canals per dog) were filled in each of the first two dogs with Chitosan nanoparticle paste. Also, the 6 teeth of the upper and lower right premolar (10 root canals) were filled with ZOE in each of the second two dogs. To perform a pulpectomy and continue filling in each animal, food abstinence was given for 24 hours. After bringing the animal to the operating room, 0.1 mg/kg body weight of the animal was injected into the midazolam to sedate the animal to access the cephalic vein and implant an angiocatheter in it. After 20 minutes, angiocatheter was implanted in the cephalic vein of one of the anterior limbs and at the same time, using ketamine anaesthesia (10 mg/kg), diazepam (1 mg/kg) mixed in one a syringe was injected into a cephalic vein. At the beginning of the study, 5 ml of blood was extracted from the animal through the angiocatheter located in the cephalic vein to obtain the blood film and the factors considered in this study at

zero time (time before pulpectomy and filling) from each animal as the control group. After isolation with rubber dam and disinfection with chlorhexidine 2% of the access, the hole was embedded from the occlusal surface, and the length of operation was estimated to be 2 mm shorter than the X-ray epoxy with K-file size 20. The teeth were filing in the usual way up to file number 40. After each filing, the channels were washed with normal saline with a minimum volume of 3.6 ml. If there is debris in the channels, the filing will continue after the last wash. After washing, the channels were dried with sterile paper and then the root canals were filled by injection technique (Figure 1). All teeth were filled with amalgam. To evaluate the quality and extent of the filling, a graph was prepared from all channels. In case of overfill; those teeth were removed from the study. After 28 days, 5 cc of blood was taken from the dogs in the experimental group and the serum levels of immune factors were studied. After completing these steps, blood serum IgG, IgM, and IgE serum levels were measured by SRID (Single Radial Immunodiffusion). The amount of each immunoglobulin is measured by a separate kit, and each kit is in the form of a plate containing a homogeneous mixture of agar gel with an antibody against an immunoglobulin or complement. The plate contains 12 holes that were poured into each hole in the appropriate amount and concentration of the patient's serum according to the kit instructions. And after the incubation period, due to the antigen-antibody reaction, visible ring-shaped sediment was seen around the hole. The diameter of the sedimentary ring was proportional to the antibody titer of the serum. Using standard samples consisting of 2 samples with a predetermined concentration, after reading the diameter of their sedimentary ring, the diameter of the diameter-concentration ratio curve was drawn and using this curve, other immunoglobulins were evaluated. According to Biogen's guidelines, using the Hamilton syringe, 6 microliters of serum or standard was poured into each hole in the IgM kit, and 2 microliters of serum or 11 times diluted standard was poured into each hole in the IgG and IgE kit. After 48 to 72 hours, the incubation time was read using a sedimentary eye magnifying glass and the square of the number was drawn on the curve. The desired immunoglobulins were measured in both case and control groups and the mean values obtained from the two groups were compared using T-test. To measure CH50, red blood cells with EDTA buffer were used. The lysis percentage was measured using an Agilent spectrophotometer. C3 and C4 measurements were performed using nephelometry method. In this method, C3 and C4 in the serum sample were formed separately with anti-C3 and anti-C4 antibodies in the solution and caused the solution to become cloudy. In nephelometry, a single monochromatic light was emitted at a wavelength of 400 to 840 nm, which, after colliding with the antibody and antigen complex, dispersed, which was directly proportional to the amount of C3 and C4. For calibration and control of the above tests, kits made by binding Site Company of England were used. To measure haematological parameters including haemoglobin (HGB) by cyanmethemoglobin method and to measure hematocrit (HTC) by microhematocrit method and general counting of red blood cells (RBC) and white blood cells (WBC) by the manual method using neubauer hemocytometer slide. The values of Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were calculated using the existing standard formulas and the total serum protein was measured using the ELISA (enzyme-linked immunosorbent assay) method. Data analysis was performed using SPSS 22 editing software. Mann–Whitney test was used to compare ranked quality variables in two groups, and Fisher's exact test was used to compare qualitative variables.

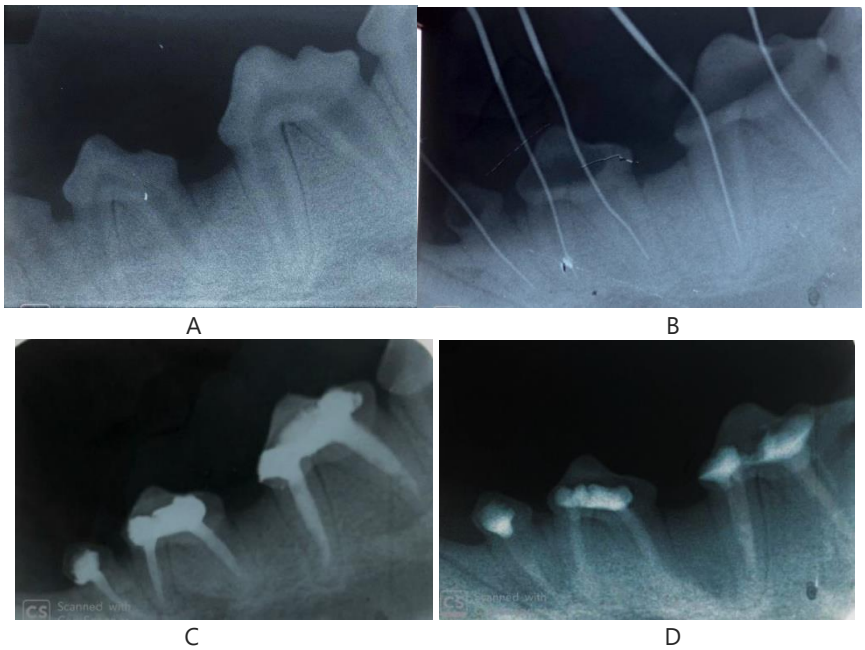

Figure 1 A) initial radiography B) determination of working length C) Pulpectomy with ZOE D) pulpectomy with Chitosan

3. FINDINGS

Results from CBC for groups treated with ZOE and chitosan

Comparing the rate of MCV changes between the two treatment groups with ZOE and chitosan was not significant (P = 0.9) (Figure 2). The rate of MCH changes between the two treatment groups with ZOE and chitosan was not significant (P> 9) (Figure 3). According to Figure 4, the rate of MCHC changes between the two treatment groups with ZOE and chitosan was not significant (P = 0.16). As shown in Figure 5, the amount of HGB in the ZOE-treated group decreased compared to the previous one but was not statistically significant (P = 0.1). The amount of HGB in the chitosan-treated group increased compared to the previous one but was not statistically significant (P = 0.1). The rate of HGB changes between the two treatment groups with ZOE decreased compared to chitosan and did not show a statistically significant difference (P = 0.04). According to Figure 6, the rate of HCT changes between the two treatment groups with ZOE and chitosan was not significant (P = 0.4). The rate of RBC changes between the two treatment groups with ZOE and chitosan was not significant (P = 0.3) (Figure 7). By examining the amount of WBC, the amount of WBC in the group treated with ZOE has increased compared to before (P = 0.02). The amount of WBC in the chitosan-treated group increased compared to the previous one, but the increase was not significant (P = 0.06). The rate of WBC changes between the two treatment groups with ZOE has increased compared to chitosan (P = 0.03) (Figure 8).

Results from biochemical testing for groups treated with ZOE

The rate of albumin changes between the two treatment groups with ZOE was not significant compared to chitosan (P = 0.58) (Figure 9). The total protein changes between the two treatment groups with ZOE and chitosan were not significant (P = 0.36) (Figure 10).

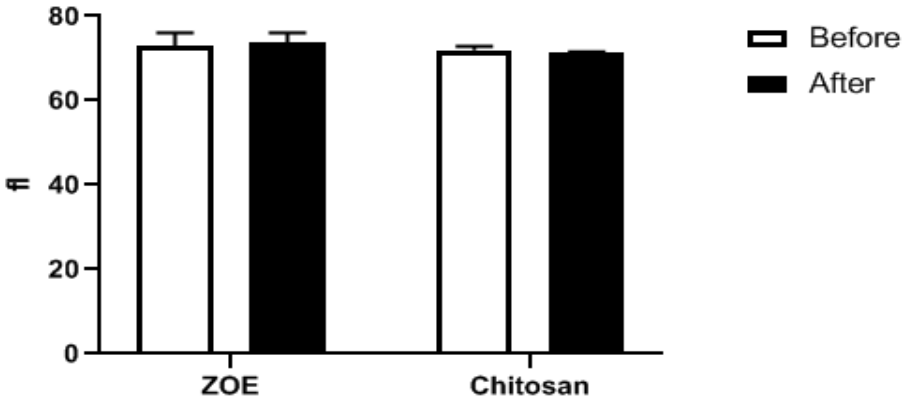

Figure 2 Comparison of MCV levels in ZOE and chitosan

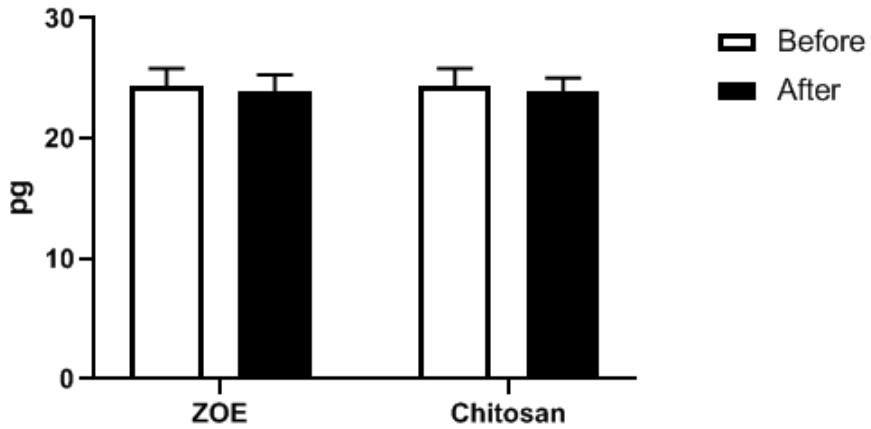

Figure 3 Comparison of MCH levels in ZOE and chitosan

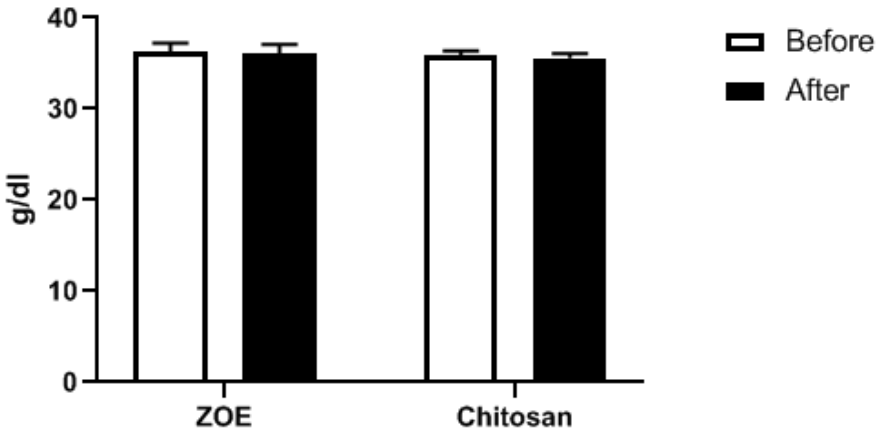

Figure 4 Comparison of MCHC levels in ZOE and chitosan

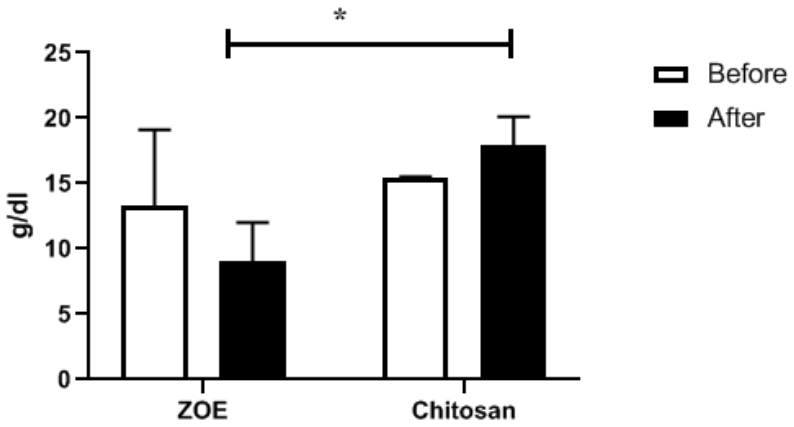

Figure 5 Comparison of HGB levels in ZOE and chitosan

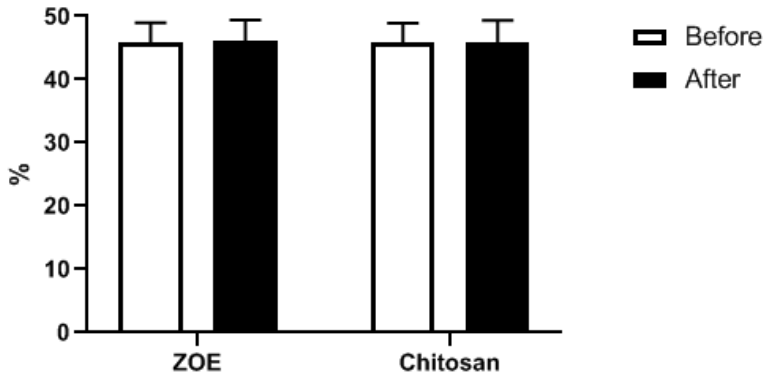

Figure 6 Comparison of HCT in ZOE and chitosan

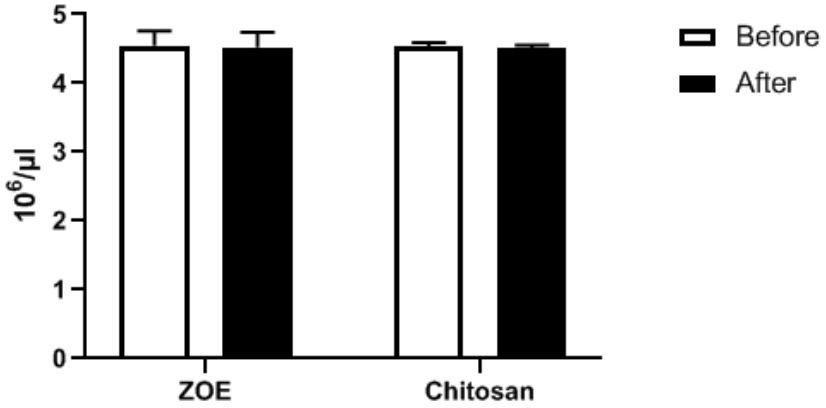

Figure 7 Comparison of RBC levels in ZOE and chitosan

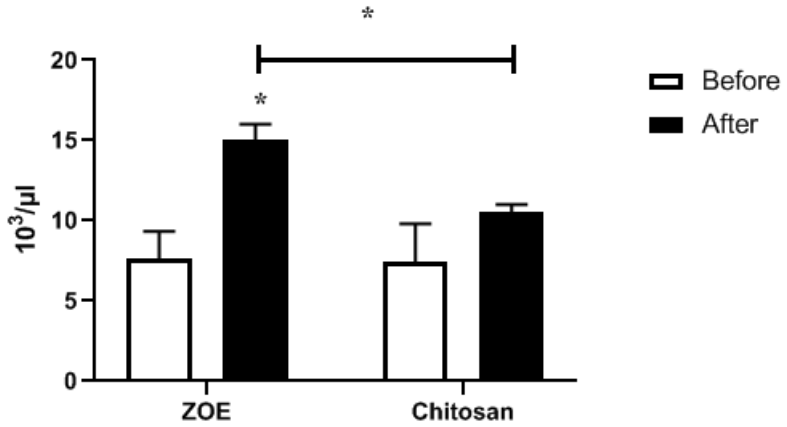

Figure 8 Comparison of WBC levels in ZOE and chitosan

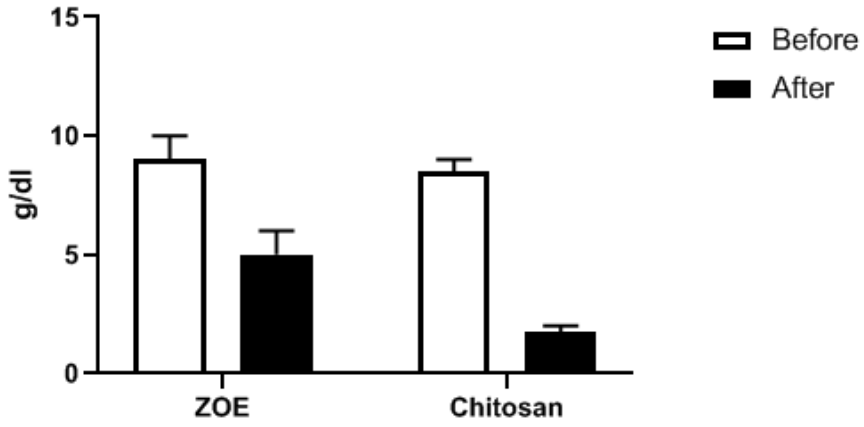

Figure 9 Comparison Albumin levels in ZOE and chitosan

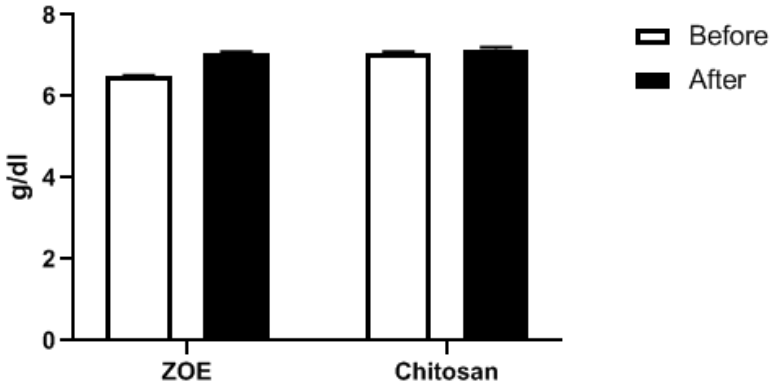

Figure 10 Comparison of total protein levels in ZOE and chitosan

Immunology test results for groups treated with ZOE and chitosan

According to Figure 11, the rate of C3 changes between the two treatment groups with ZOE decreased compared to chitosan (P = 0.05). Also, the rate of C4 changes between the two treatment groups with ZOE decreased compared to chitosan (P <0.0001) (Figure 12). The level of IgG in the ZOE-treated group has increased (P = 0.021). Also, this rate has increased in the group treated with chitosan (P = 0.021).

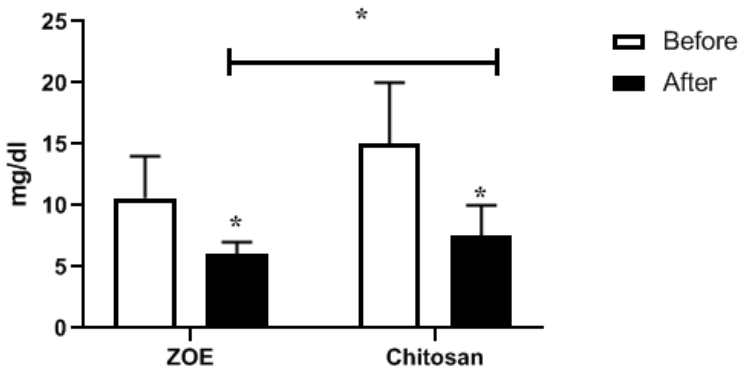

Figure 11 Comparison of C3 levels in ZOE and chitosan

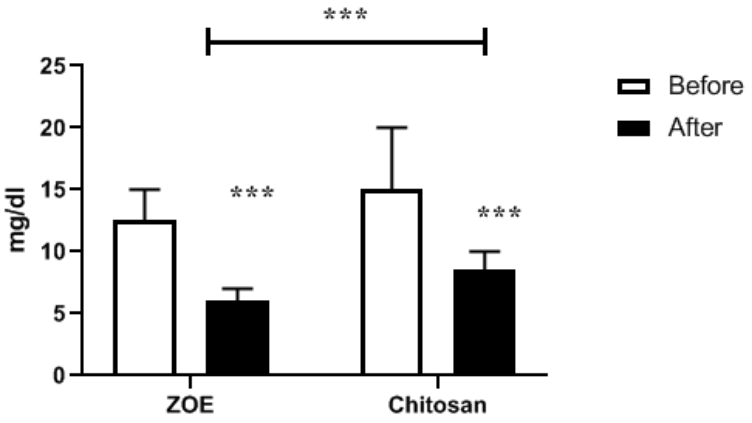

Figure 12 Comparison of C4 levels in ZOE and chitosan

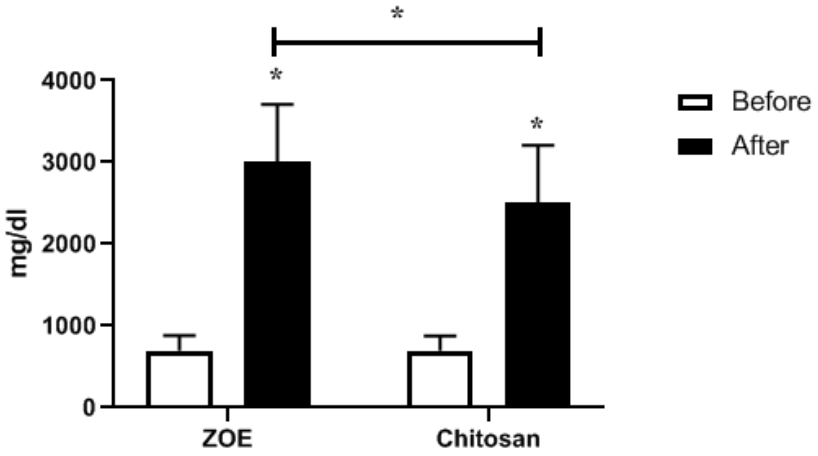

Figure 13 Comparison of IgG levels in ZOE and chitosan

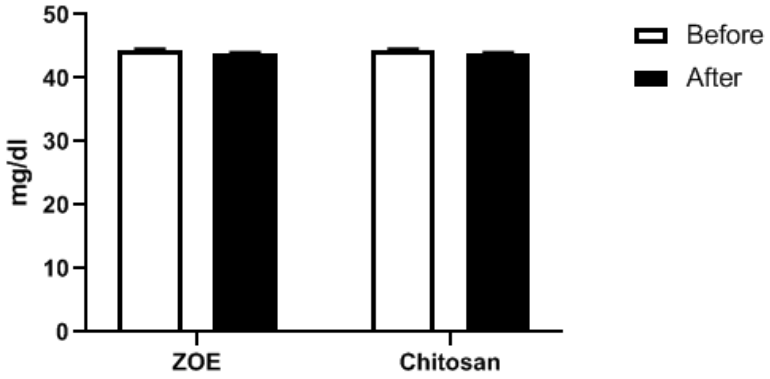

Figure 14 Comparison of IgA levels in ZOE and chitosan

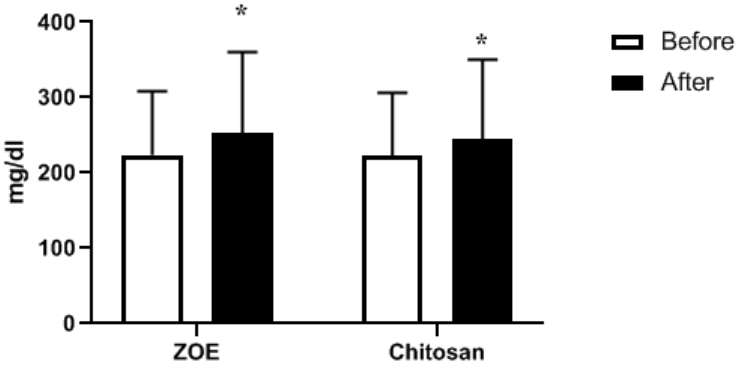

Figure 15 Comparison of IgM levels in ZOE and chitosan

As a result, the rate of IgG changes between the two treatment groups with ZOE has increased compared to chitosan (P = 0.021) (Figure 13). The rate of IgA changes between the two treatment groups with ZOE and chitosan was not significant (P> 09) (Figure 14). According to Figure 15, the amount of IgM in the group treated with ZOE has increased compared to before (P = 0.04). The rate of IgM in the chitosan-treated group has also increased but not significantly (P = 0.06). The rate of change in IgM between the two treatment groups with ZOE was not significant compared to chitosan (p = 0.18). As Figure 16 shows, the level of IgE in the ZOE-

treated group did not change significantly (P> 0.9). IgE levels in the chitosan-treated group did not change significantly (P> 0.9). The rate of IgE changes between the two treatment groups with ZOE and chitosan was not significant (P = 0.65).

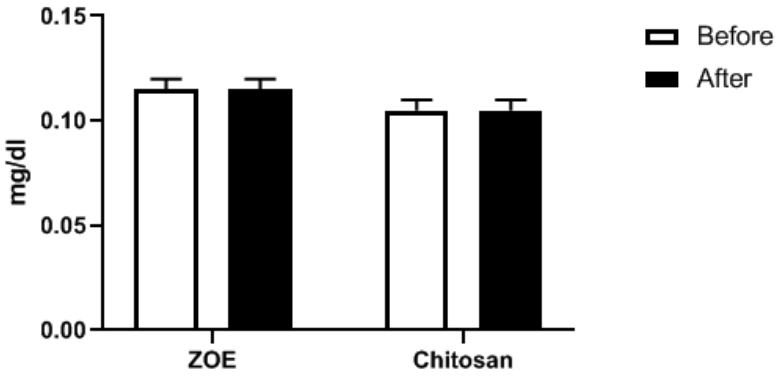

Figure 16 Comparison of IgE levels in ZOE and chitosan

4. DISCUSSION

The present study aimed to determine and compare the following cases before and after pulpectomy with ZOE and chitosan: Comparison of IgG, IgM and IgE serum levels, Determination and comparison of serum levels of C3 and C4 complement components, Determination and comparison of haemoglobin, MCV, MCHC, MCH, WBC and RBC, Determination and comparison of total protein, Alb, HCT. In general, evaluation of serum immunological factors was performed after pulpectomy using two root canal materials. According to the results of this study, the levels of C3 and C4 decreased more due to the use of ZOE than chitosan. Chitosan has been able to improve humoral immunity. Decreased activity of components of the complement system may be due to the following: Microbial infections, Autoimmune diseases, including SLE and rheumatoid arthritis, hereditary angioedema, acquired angioedema, Various types of kidney disease, including glomerulonephritis, lupus nephritis, membranous nephritis, IgA nephropathy, Rejection of kidney transplantation, cirrhosis, hepatitis, malnutrition, toxic septicemia, shock, serum sickness (complex immune disease). The protein levels of the complement system decrease during acute or chronic inflammation and return to normal after the inflammation has resolved (Bellanti, 2013). The complement system is an important component of the natural immune system, which plays a very important role in defending against microorganisms. This system can be traced in the bloodstream during fetal growth and development and before the emergence of IgM antibodies. The emergence of complement components before IgM synthesis may indicate that before the development of antibodies, complement and exogenous cells (phagocytes) play a major role in protecting individuals (Bellanti, 2013). Gland showed that ZOE has antibacterial properties, but the use of chitosan reduces the drug's bacterial resistance and is stronger than ZOE, which happens by changing the activity of the complement system (Ramar & Mungara, 2010). Chitosan is effective in reducing inflammation and helping the complement system due to its antibacterial properties. Takagi said chitosan has high bending strength, resistance to moisture and antibacterial properties (Takagi et al., 2003). Chitosan accelerates the healing of infections due to its durable coating, water absorption, biocompatibility and high oxygen permeability (Fuks, 2000).

Previous studies have confirmed the antioxidant, anti-inflammatory and antibacterial properties of chitosan in the treatment of infections in various areas, which is consistent with the present study (Dai et al., 2011; Sarhan & Azzazy, 2015). In the present study, the rate of IgG changes between the two treatment groups with ZOE increased compared to chitosan. The rate of change in IgM and IgE between the two treatment groups with ZOE was not significant compared to Chitosan. IgG increases in inflammatory and tumour diseases and can activate complement and leukocytes. For example, in Lichen Planus disease, G-type autoantibodies are formed against the basal layer, which destroys the tissue by activating leukocytes (Modre et al., 1999). The results of a study by Ghaliani et al. showed that Aphthous stomatitis increased IgA, IgM and did not change IgG and decreased C3 and C4 levels in people's blood serum (Sklavounou et al., 1983). In inflammatory conditions, the body responds by increasing IgM and IgG, and IgE only increases in allergic conditions (Schroeder & Cavacini, 2010). In this study, the number of red blood cells between the two groups of chitosan and ZOE did not differ. This is because red blood cells are reduced in systemic diseases such as anaemia and hematolytic diseases, and inflammatory processes do not affect the number of red blood cells unless they are chronic. MCV has not

changed in this study. MCH levels refer to the mean haemoglobin found in red blood cells (Gokhale et al., 2010). Pulpectomy treatment does not affect the level of red blood cells and related blood factors. The use of ZOE and chitosan did not affect red blood cell counts. Factors associated with red blood cells, including HCT, MCV, MCH, and MCHC, also change in patients with diabetes (Alamri et al., 2019). However, because the animals used in this study were healthy and did not have any disease, there was no change in the serum level of these factors. In this study, the rate of albumin and total protein changes between the two treatment groups with ZOE was not significant compared to chitosan. The most common cause of albumin depletion is acute and chronic inflammation. This effect is due to the increase in consumption by cells and the reduction of its synthesis and the entry of this protein into the extravascular space. In cases of decreased albumin, other plasma proteins are increased to compensate for albumin deficiency (Henskens et al., 1996). In the present study, the number of white blood cells in animals treated with pulpectomy with ZOE increased more than that of chitosan. White blood cells, including lymphocytes, respond to the destruction of foreign particles and infectious agents by producing antibodies. When the body is in a state of acute inflammation, the immune system is highly active in defensive response to this inflammation, which increases white blood cells and increases the production of immunoglobulins. That's why a 2002 study by Jamshidi et al. showed that a decrease in the number of white blood cells in people with periodontal disease caused a systematic spread of opportunistic infections (Jamshidi et al., 2002).

One of the limitations of this study is the following: Difficult to find perfectly healthy dogs, Chitosan preparation suitable for filling teeth, Lack of studies and determination of the protocol required for the use of chitosan, Problem radiography due to the shallow depth of the animal's mouth, The need for animals to leave the study with calcific channels, Precise quarantine to reduce animal mortality. According to the results, chitosan can be used for filling canal teeth by changing the structure and conducting further studies. Achieving a decisive result is also required.

5. CONCLUSION

Compared to the two fillers in the dental canal, ZOE causes more inflammation and increases white blood cells and immunoglobulins. There was no difference in blood factor and protein levels between the two groups. The levels of albumin, C3, and C4 in the ZOE group were lower than in chitosan. The levels of IgG, IgM, and IgE in the ZOE group were higher than those of chitosan. The levels of MCHC, MCH, MCV, HCT, RBC and total protein did not differ between ZOE and chitosan. According to the results, the rate of immune stimulation and post-pulpectomy inflammation by ZOE appears to be higher than that of chitosan. Finally, due to less inflammation and changes in structure and more studies, chitosan can be recommended to fill the root canal of deciduous teeth. It is recommended that more studies be done on chitosan as a root filler and compare with other substances such as vitapex.

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Ethical approval

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Data availability

All data associated with this study are present in the paper.

Peer-review

External peer-review was done through double-blind method.

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